Development of Glass Reinforced Hydroxyapatite for Hard Tissue Surgery

Thesis submitted by
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"...All we know is still infinitely less than all that still remains unknown..."

William Harvey (1628)
Dedicated to my parents
Abstract

Hydroxyapatite (HA) is of great interest as a material for surgical implants due to its biocompatibility with living tissue, however, synthetic HA is limited in its use as a biomaterial, this is primarily due to its low load bearing capacity and is reflected by the relatively low mechanical properties compared to bone. The aim of this project is to improve the mechanical properties of synthetic hydroxyapatite by optimising the processing method and also by incorporating a phosphate glass as a sintering aid to form Glass Reinforced Hydroxyapatite (GR-HA).

Phosphate glasses were introduced into hydroxyapatite at 2.5wt% and 5wt% additions, during the milling process and subsequently sintered to 1200°C or above. The materials were characterized in terms of density, phase analysis, porosity, grain size, flexural strength, hardness, flexural modulus and fracture toughness.

Mean values ranging from 96.43 to 142.59MPa were obtained for the flexural strength of a GR-HA containing a 2.5wt% calcium phosphate glass with a 15mol% composition of CaF₂. This composite therefore exhibits strength values that are comparable to those recorded for cortical bone (50-150MPa). HA alone, however, exhibited much lower flexural strength values ranging from 42 to 53MPa. The increased mechanical properties were attributed to the presence of residual stress introduced due to HA decomposing to β-tricalcium phosphate (β-TCP) and in some cases α-tricalcium phosphate (α-TCP).

Hot pressing was also employed as an alternative processing method that could potentially enhance the mechanical properties. This method gave flexural strength values of 79 and 92MPa for HA and GR-HA respectively at a sintering temperature of 1100°C. Sintering at temperatures above 1100°C did not improve the mechanical properties any further even though densification occurred at a lower temperature. This could be attributed to other factors such as grain growth.

In vitro studies examined the effects of surface chemistry on cell attachment, cell spreading and proliferation, differentiation and function of an osteoblast-like cell line cultured on the surface of HA and GR-HA. The results suggest that the presence of phosphate glasses provides a suitable chemical composition which up-regulates the expression of proteins such as bone sialoprotein (BSP), osteonectin (ON), fibronectin (FN) and collagen I (COL I) compared to HA alone. Compared to the control, both HA and GR-HA did not show a high level of cell proliferation.
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1 Introduction
The desire to develop materials that can be applied to serve as a means of replacing damaged or diseased body parts has become an increasingly important area of medicine.¹

Current research is essentially focused towards developing implant materials that carry out their function without toxicity or foreign body reaction, and then are completely resorbed and replaced by new tissue. So, in ideal terms an implant material must maintain long term mechanical as well as chemical stability.¹

It has recently come to light that ceramics, in particular hydroxyapatite, are ideal materials for implantation. This is mainly attributed to their chemical properties, however, the development of a ceramic material which can perform its mechanical function successfully has not, as yet, been fully realised.¹

Hydroxyapatite (HA) is the main inorganic phase constituent of bone and has the approximate chemical composition $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Furthermore, structural and chemical analyses indicate that the ionic substitutions occur within the HA lattice under physiological conditions.²,³

Because synthetic hydroxyapatite has a chemical similarity with biological hydroxyapatite, it seems reasonable, therefore, to suggest that its application as an implant material may have the potential capability of undergoing bonding osteogenesis and maintain long term chemical stability in vivo.³
A variety of hydroxyapatite forms can be readily manufactured to suit the specific requirements of an implant material. These include dense hydroxyapatite, potentially ideal with regards to implants of high load bearing capacity; granular hydroxyapatite, can be fitted to a desired shape or space, and can be applied in dental restorations and alveolar ridge augmentation; porous hydroxyapatite, designed for bone ingrowth; and hydroxyapatite coatings, to provide a separating region between the metal implant and bone where the implant can perform its function as a high impact absorbing material and also prevent the metal reacting and migrating into neighbouring tissues, and where the coating can bind directly to the bone.¹

Recent developments aiming towards strengthening hydroxyapatite have involved the introduction of phosphate based glasses. These glasses have been introduced as a sintering aid, in which the primary aim is to enhance densification and reduce porosity through liquid phase sintering. However, x-ray analysis has shown that the introduction of phosphate based glasses increases reactivity, resulting in the degradation of HA to β-TCP and α-TCP secondary phases. This is believed to occur through chemical interactions, or ionic substitutions to be more precise, between the glass and HA. This in some instances has proved to enhance the mechanical properties of the composite material, in particular the flexural strength.⁴

In addition, the substitutions that occur within the HA matrix as a result of incorporating phosphate glasses may also potentially provide a means of supplementing the necessary trace ions that bone cells require for regeneration.⁴
The main aim of this study was to develop a glass reinforced hydroxyapatite composite with optimal mechanical properties, paying particular attention to flexural strength. A variety of parameters were taken into consideration during processing, such as glass weight additions to hydroxyapatite powder, looking at different compositions of glass, using different solvents with the added introduction of a dispersing agent, experimenting with different powder to solvent milling ratios and hot pressing. Furthermore, additional work was performed to assess the material’s biocompatibility through cell culture. A number of techniques were applied for the characterisation of these materials, such as x-ray diffraction (XRD), scanning electron microscopy (SEM), fourier transform infrared (FT-IR) and transmission electron microscopy (TEM).
2 Literature Review
2.1 Physiopathology of bone

2.1.1 Connective tissue

There are three main types of connective tissue, (1) dense, (2) loose and (3) fatty. Dense connective tissue is made up of large numbers of collagen fibres and is found in structures such as ligaments, tendons, aponeuroses, fasciae, fibrous capsules, etc. Loose connective tissue contains fewer collagen fibres relative to dense connective tissue but contains rather more ground substance and tissue fluid. Fatty connective tissue can be distinguished from the other types of connective tissue by the build up of fat cells.$^5$

2.1.2 Fibres

There are three main types of fibres that constitute connective tissue: (a) Collagen, (b) Elastic and (c) Reticular.$^5$

2.1.2.1 Collagen fibres

Collagen fibres can be found in all connective tissues, such as bone and cartilage, and is the most abundant protein in the body. Collagen is present in the organic phase of bone and it is a highly adaptable protein, which produces different types of collagen according to the sequence of amino acids in its molecular structure. Collagen type I is the most abundant form of collagen of the fifteen collagen types found in mature bone.$^5$

In bone and cartilage the collagen fibres are readily identified as a spread of loose connective tissue where they regularly form bundles of interlacing fine parallel fibrils. However, collagen bundles in dense connective tissue are present in larger numbers and
are more closely packed. An important property that collagen fibres possess is their great tensile strength. The collagen fibres tend not to exhibit any signs of extension upon sudden strain, but break rather than stretch when the limits of their tensile strength are reached. This is a characteristic phenomenon seen in tendons.\textsuperscript{5}

The formation of collagen is controlled by the activity of fibroblasts, a connective tissue cell and vitamin C. An inhibitory factor that affects the formation of collagen is the steroid hormone cortisone and some related compounds. However, with enhanced activity of fibroblasts in certain circumstances, there is a higher rate of formation of collagen.\textsuperscript{5}

2.1.2.2 Elastic fibres

Elastic fibres are long single fibres, which are attached to the dermis and epidermis (subcutaneous tissue) and are primarily responsible for some of the elasticity shown in skin. With age the elasticity diminishes, for example if one was to pinch a fold of skin on the back of the hand of a young person it would snap back into position immediately, whereas in older people the skin would take longer to subside.\textsuperscript{5}

2.1.2.3 Reticular fibres

Reticular fibres are a network of thin branching fibres that are abundant in blood-forming organs and lymph glands but not so well marked in subcutaneous tissue.\textsuperscript{5}
2.1.3 Bone structure

The human skeleton, which provides the framework of the body, can be categorised into the axial skeleton, which consists of the vertebrae, skull, ribs, sternum, and hyoid bone, and the appendicular skeleton, which comprises the bones of the upper and lower limbs and the pelvis. Every bone consists of a medullary cavity containing cancellous (trabecular, spongy) bone, which is surrounded by a bone cortex composed of compact (dense) bone. Surrounding the cortex is the periosteum, which is a dense fibrous tissue membrane. However the periosteum does not cover the articular surfaces as these are covered with cartilage. The marrow spaces between the bone trabeculae are filled with adipocytes and haematopoietic tissue and this tissue is responsible for the production of erythrocytes and leucocytes, which are an important part of the extensive vascular network contained within cortical and cancellous bone. 6

Long bone can be categorised into the following regions: (1) the epiphysis, this is the part of the ends of bones which are surrounded by articular cartilage; (2) diaphysis or shaft; (3) metaphysis, this is the region which lies between the epiphysis and the diaphysis; (4) growth plate (physis), this is a cartilaginous region that separates the epiphysis from the metaphysis and is an area of continuous endochondral ossification in an actively growing bone. 6

2.1.4 Bone composition

Bone is a specialised connective tissue composed of an extracellular matrix that is part organic and part inorganic within which lie bone cells. 35 per cent constitutes the organic
part of bone and of that, 95 per cent is made up of mainly type I collagen fibres, the rest consists of non-collagen proteins of bone, such as osteocalcin, osteonectin and osteopontin, plasma proteins, lipids, and glycosaminoglycans. The inorganic mineral component of bone makes up 65 per cent of the bone matrix, and consists of crystalline salts, that are mainly calcium and phosphate based. A well known form of crystalline salt that is a constituent of bone is calcium hydroxyapatite \([(Ca)\textsubscript{10}(PO\textsubscript{4})\textsubscript{6}(OH)\textsubscript{2}]\). \(^{6-9}\)

2.1.5 Lamellar bone

The matrix of lamellar bone can be distinguished from other forms of bone due to the manner in which it is organised. The matrix of lamellar bone is made up of bundles of thin collagen fibres.\(^{10}\) It is organised in such a way that each bundle is laid down approximately at right angles to adjacent bundles. This structural organisation gives this type of bone a characteristically high density of collagen, in fact lamellar bone has the highest density of collagen per unit volume of tissue. Both cortical and cancellous bone in a normal mature skeleton is composed almost entirely of lamellar bone.\(^6\)

Immature bone, otherwise termed woven bone, is initially formed in the developing skeleton and can also form in a variety of pathological conditions such as rapid bone formation. Mechanically, woven bone is not as strong as lamellar bone. The nature in which the matrix is organised distinguishes woven bone by the presence of bundles of randomly arranged short, thick collagen fibres.\(^6\)
2.1.6 Cortical bone

The cortex of cortical bone surrounding the medulla (cancellous bone) consists almost entirely of lamellar compact bone. Cortical bone consists of central Haversian canals that are surrounded concentrically by lamellae (Figure 1). Haversian canals are lined with osteoblastic cells (cells that produce the organic matrix of bone and are responsible for mineralisation) and also contain blood vessels. Collectively the concentric lamellae and the Haversian canals form long cylindrical columns in the long axis of bone and these are therefore termed Haversian systems or osteons. Between each Haversian system there are small segments of interstitial lamellar bone, which fill the spaces between concentric lamellae. Additionally, the periosteal and endosteal surfaces of bones contain circumferential lamellae, which are oriented around the long axis of the bone. 8,11-14

![Schematic representation of the structure of bone.](image)

**Figure 1.** Structure of bone. 5
2.1.7 Periosteum

The periosteum is a section which covers the outer surfaces of most bones and is comprised of a layer of dense fibrous tissue. In children the periosteum is thick and is loosely attached to the cortex, in adults however, it is more adherent. The outer layer of the periosteum consists of a layer of collagen fibres which are continuous with those of the joint capsule, ligaments, and tendons. The inner cellular layer contains a layer of osteoprogenitor cells that are flattened and spindle shaped when inactive, but differentiate into osteablasts which subsequently lay down the bone matrix on the underlying cortical bone surface during growth or repair.⁶

2.1.8 Cancellous bone

Cancellous bone can be found between the cortex of bone, a region known as the medulla. It is made up of a trabecular network of plates and bars of bone. The trabecular network in mature bone contains lamellae of mineralised collagen fibres oriented in parallel along the long axis of a bone trabecula. Haversian systems are not usually contained within cancellous bone trabeculae, this is due to the trabeculae being relatively thin. Nevertheless, trabeculae are surrounded by vessels within haematopoietic or fatty marrow which is responsible for vascularisation. The marrow fills the cavities that separate the bone trabeculae which are lined by a continuous layer of flattened bone lining cells or plump osteoblasts. These bone lining cells make up the endosteum which covers the surface of bone trabeculae, the inner (medullary) surface of the cortex, and Haversian canals in bone.⁶
2.1.9 Cement lines

Cement lines mark the boundary between discrete areas of bone regeneration, these lines are found in both cortical and cancellous bone. If there is an increase in the number of cement lines occurring in cortical and cancellous bone then this would indicate an increase in bone regeneration. Some areas of bone exhibit uninterrupted straight cement lines that are indicative of normal remodelling, however areas of bone showing indented lines are a result of bone regeneration after extensive osteoclastic resorption.\(^6\)

2.1.10 Bone cells

The bone cells that are responsible for bone formation are those belonging to the osteoblast lineage (osteoblasts, bone lining cells, osteocytes). These osteoprogenitor cells are derived from the mesenchymal stromal stem cell. Progenitor cells, that are responsible for the production of other connective tissue cells, such as adipocytes, chondrocytes, fibroblasts are also derived from the mesenchymal stromal stem cell. Osteoclast cells are accountable for bone resorption, these are derived from the pluripoietic haematopoietic stem cell, a cell capable of differentiating into other peripheral blood cells. During prominent bone remodelling, where the activity and formation of cells belonging to the osteoblast and osteoclast lineage occur, both osteoblasts and osteoclasts are present and their activity are closely linked.\(^6,7,15\)
2.1.10.1 Bone lining cells

The development of the organic matrix of bone and its subsequent mineralisation can be attributed to osteoblasts. These cells can be described as rounded or polygonal mononuclear cells. Furthermore, osteoblast cells that have been entombed in the mineralised matrix of bone which they have formed are termed osteocytes. These cells are located in the lacunae and have densely stained nuclei and obscure cytoplasm. In immature bone the osteocytes lie in large round lacunae and are more closely packed than in lamellar bone where they lie in oval or flattened lacunae and are oriented in the direction of collagen fibres. The importance of the remodelling process of bone is attributed to the communication of osteoblasts, osteocytes and osteoclasts via their extracellular processes and also through strain-related influences on osteocytes. Furthermore, changes in strain which are short durations of dynamic, unusual strain are responsible for inducing adaptive remodelling. In addition, the transduction of mechanical stimuli into cellular activity is believed to be the result of the direct deformation of the cells themselves, changes in intralacunar pressure, and the electrical potentials produced from the flow of charged fluid through the extracellular spaces. Therefore through this stimulus, the osteocytes communicate with the osteoblasts that lie on the surface of the matrix to initiate regenerative bone growth.\(^6\)

2.1.11 Intramembranous ossification

Intramembranous ossification and subsequent bone formation occurs through the proliferation and differentiation of mesenchymal cells within mesenchymal connective tissue. Mesenchymal cells proliferate and differentiate into osteoblasts that form osteoid which is then rapidly mineralised to woven bone (immature bone), this is then remodelled
to form the lamellar bone which is present in the mature skeleton. The process of bone growth through intramembranous ossification can be described as appositional growth, this involves osteoblasts laying down the bone matrix on an already existing bone surface resulting in an increase in bone width. Mineralisation of osteoid on the underlying cortex by osteoprogenitor cells in the periostuem occurs very rapidly. In contrast, mineralisation in Haversian systems occur at a much lower rate.\textsuperscript{6,15}

2.1.12 Endochondral ossification

Endochondral ossification is the process of bone growth in length. The formation of bone in long bones, vertabrae, and bones of the skull occur via continuously enlarging cartilage. In long bones endochondral ossification appears in the middle of the shafts, this is evident from the seventh week of intrauterine life, with the emergence of primary centres of ossification within the cartilage of bones. Cartilage cells that are present in the middle of the shafts become hypertrophied and later die which give way to the calcification of the cartilage matrix. Mesenchymal cells which lie in the perichondrium undergo proliferation and subsequent vascularisation, to produce the periosteum from which osteoblasts differentiate to form the osteoid, followed by mineralisation to woven bone. This process continues towards the centre of the cartilaginous area resulting in the deposition of bony matrix on the scaffold of calcified cartilage.\textsuperscript{6,15}

The longitudinal growth of bones occurs in a region of bone known as the growth plate or physis which lies between the bony epiphysis and the bony metaphysis. The thickness of the growth plate remains constant with mineralisation of cartilage (formed by
chondrocytes) on the metaphyseal side of the growth plate to form metaphyseal bone. Consequently, the length of the metaphyseal and diaphyseal regions of bone increase. 6,15
2.2  Mechanical properties of bone

2.2.1  Strength and stiffness of a structure

The determination of the mechanical properties of bone, for example the shaft of a femoral bone, requires a load-deformation test. The type of tests carried out that attempt to mimic the type of loading that occur in a real skeletal system, involve tension, compression, torsion, or bending. In a load-deformation graph, the extent of deformation can be measured against the applied load. 14

A typical load-deformation graph (Figure 2) for the compressive strength of a femoral shaft exhibits the following characteristics: 10

1. Proportional region; Where the load and deformation are linearly proportional
2. Proportional limit; After this point the material is permanently deformed
3. Elastic deformation; bone can return to its original shape when load is removed. This occurs prior to the yield point
4. Plastic region; At the yield point the material begins to exhibit plastic behaviour
5. Ultimate load; at this point the material fails
The definition of strength of a material can be subject to debate. In some cases strength can be considered as the load at the yield, or, as the point of failure. However, strength is more frequently referred to as the load at the point of failure.\textsuperscript{16}

The slope of the proportional region of the load-deformation curve represents the \textit{stiffness} or \textit{rigidity} of the structure. This can be defined as the load required to deform it by a given amount. The ease by which the structure can be deformed is known as \textit{compliance}, which is taken to be the inverse of stiffness.\textsuperscript{16}

\textit{Toughness} is another important mechanical property, which can be determined by measuring the area under the load-deformation curve. This measures the amount of energy or work needed to yield (area beneath yield point) or fracture (area beneath the failure point) the structure.\textsuperscript{16}
Stress is a property which can be defined as the force per unit area. However, there is a more general definition of stress. Consider a point in the structure, and imagine that point to be a cube with six faces (Figure 3). For each face there is one perpendicular (normal) load applied and also two other parallel or shear loads. In total there are three loads acting upon a face, which if divided by the area of the face become the three stresses associated with the direction of that face. If every component force has an equal but opposite force acting upon it, for sake of stability, then there must be $3 \times 3 = 9$ stress components at each point of a loaded material. This therefore means that three out of the nine are normal stress components, and the other six are shear stresses. However, some of the shear stress components are equal to each other, reducing the total amount to six stress components, of which three are normal and three are shear. The units of stress are usually measured in Pascals (Pa) or N.m$^{-2}$.16

Figure 3. Diagram displaying stresses associated with each face.16
Strain is a measure of deformation of a material or a fractional change in dimension of a loaded body. Normal strain is considered to be a fractional change in length of a structure, attributed to tensile or compressive stress, and shear strain is thought to be an angular displacement brought about by shear stress and can be obtained by dividing the shear displacement by the width of the shear specimen (Figure 4).\textsuperscript{16}

\[ e = \frac{y - y_0}{y_0} \quad \text{Normal strain} \]

\[ e = \frac{X}{Y} = \tan \Lambda \equiv \Lambda \quad \text{Shear strain} \]

Figure 4. Diagram showing normal and shear strain.\textsuperscript{16}

It is important to note that the location of this cube in a structure is a crucial aspect in defining the type of stresses that it experiences, however, orientation also plays an important role. It has been mentioned that each face of a cube will experience normal and shear stresses, but in certain orientations the magnitude of these will undoubtedly vary.\textsuperscript{16}
A stress value for compression tests on a bone shaft can be obtained from a load-deformation curve by dividing the load by the cross-sectional area of the bone cortex. A strain value can be obtained by dividing the fractional change in length by the original length of the shaft segment. Therefore, the Young's modulus or elastic modulus is calculated by dividing stress by strain.\(^\text{16}\)

### 2.2.2 Osteonal remodeling of compact bone

It has been well documented that the flexural fatigue strength and resistance to tension for bone is reduced due to osteonal remodeling. Other mechanical properties that are also affected are compression, shear, and bending strengths.\(^\text{17}\)

Studies made on the effects of osteons on the mechanical properties of non-human bone have shown that completely remodelled bone was 35% weaker than primary bone.\(^\text{18}\) This is attributed to the replacement of highly mineralized bone matrix with less calcified material in recently formed haversian systems. Additionally the presence of newly formed haversian systems and subsequent rise of new haversian canals introduces porosity into the structure. Tension and compression loading of haversian and primary cow bone showed a 20% decrease in strength for both modes of testing as a result of osteonal remodeling.\(^\text{14}\) However, no change was observed for the elastic modulus. This was substantiated by additional data which showed again a decrease in strength in tension and also a decrease in the shear loading mode; 11% in tension loading and 9% in shear loading.
Further investigations have found that the same effect was observed on the three-point bending properties of bovine femur and the compressive properties of porcine mandible. Another factor is known to contribute to the weakened strength in bone as a result of osteonal remodelling. The cement line, which lies between osteons, has been shown to decrease strength in bovine bone with respect to both monotonic tension and flexural fatigue. Results showed a 17% decrease in the original tensile strength of decalcified osteonal bone, however, with decalcification of the osteonal wall segments the decline in strength is even greater. This process is commonly termed hypercalcification.\textsuperscript{16}

Cortical bone, when subjected to tension or compression loading, undergoes plastic behavior. The plastic deformation that occurs in bone is at its greatest when the load is applied parallel to the direction of orientation of the collagen fibers. The amount of plastic deformation that is observed in osteonal bone in relation to primary bone is very much greater. This reinforces the speculation that cement line slippage or yielding is accountable for the plastic ductility seen in osteonal bone.\textsuperscript{8,11-14}

Tensile strength and stiffness test that were carried out by Vincentelli and Grigorov on primary bone near the periosteal and endosteal surfaces of human tibia and similarly with Haversian systems in the cortex of the same bone showed lower strength and stiffness values for Haversian bone compared to primary bone (Table 1).\textsuperscript{19}
Viscoelasticity is another property that is intrinsic in cortical bone, it is defined as the rate at which the material deforms. The construction of bone and the kind of structure it adopts is said to be associated with the presence of osteons, as was mentioned earlier. Their contribution to the viscoelastic behavior comes in various forms such as the presence of osteoid in varying amounts, the fluid flow through haversian canals, canaliculi, or the interstitial spaces in the bone matrix, and the presence of cement lines.

Studies were made by McElhaney measuring the rate of deformation of bovine femoral bone over a variety of deformation speeds (Table 2). What was concluded was that cortical bone becomes stronger, stiffer, and more brittle with increasing strain rate. Specimens which tended to fail under loading at high strain rates were characteristic of failure along the cement lines, whereas those which were loaded under low strain rates were characteristic of failure along the shear planes passing through the osteons. However, there was no quantification of the histological structure to support this even further.

Table 1. Table comparing strength and stiffness values for primary and haversian bone.

<table>
<thead>
<tr>
<th></th>
<th>Strength (MN.m$^2$)</th>
<th>Stiffness (GN.m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary bone</td>
<td>161 ± 11</td>
<td>19.4 ± 2.4</td>
</tr>
<tr>
<td>Haversian bone</td>
<td>130 ± 14</td>
<td>17.6 ± 2.0</td>
</tr>
</tbody>
</table>
The presence of porosity in the structure of bone is another factor that is known to weaken the strength of bone. Porosity can be defined as the ratio of void volume to total volume. It may also be said that the volumetric porosity is equivalent to the porosity measured in a two-dimensional cross section of bone. The size of voids within the structure of bone can vary between a few micrometres to several hundred micrometres in diameter. Canaliculi and osteocyte lacunae are the smallest in size, however, these are not considered to have a great effect on the mechanical properties of bone, because the distribution is uniform throughout all kinds of bones.

The greatest effects on the mechanical properties of cortical bone are associated with the haversian canals and related resorption cavities and vascular channels. The marrow spaces between trabeculae are responsible for affecting the mechanical properties observed in trabecular bone.

<table>
<thead>
<tr>
<th>Strain rate (sec^{-1})</th>
<th>Ultimate compressive Stress (MN.m^{-2})</th>
<th>Young's modulus (GN.m^{-2})</th>
<th>Maximum strain %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001</td>
<td>176</td>
<td>18.6</td>
<td>1.88</td>
</tr>
<tr>
<td>0.01</td>
<td>207</td>
<td>20.0</td>
<td>1.82</td>
</tr>
<tr>
<td>0.1</td>
<td>231</td>
<td>24.1</td>
<td>1.75</td>
</tr>
<tr>
<td>1.0</td>
<td>252</td>
<td>27.6</td>
<td>1.25</td>
</tr>
<tr>
<td>300.00</td>
<td>283</td>
<td>33.1</td>
<td>1.00</td>
</tr>
<tr>
<td>1500.00</td>
<td>365</td>
<td>42.1</td>
<td>0.90</td>
</tr>
</tbody>
</table>

Table 2. Table showing changes in mechanical properties as a function of strain rate.\(^\text{20}\)
The following graph shows the relationship between the ultimate stress and porosity for cortical bone specimens (Figure 5). The dashed line represents the linear regression for trabecular bone. The curves for cortical and trabecular bone are markedly different. The line representing trabecular bone suggests that most of the material is void space, whereas the curve line suggests that cortical bone consists mainly of a solid matrix.\textsuperscript{22}

The mineral content of bone has a significant bearing on the outcome of the mechanical properties. For instance, Amprino postulated that there was a clear relationship between the extent of mineralisation and increasing hardness, and found that the hardness of cortical bone tested longitudinally was 20-25\% greater compared to those tested transversely.\textsuperscript{23} This relationship was substantiated by Weaver, who showed that the hardness varied from one type of bone to another in the same skeleton, but did not vary much between the similar bones in different skeletons.\textsuperscript{24}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Fig5.png}
\caption{Graph showing changes in ultimate stress with change in porosity.\textsuperscript{22}}
\end{figure}
Studies to determine the age-dependent strength characteristics of human bone tissue were found to be anomalous. Melick and Miller concluded that the decline in tensile strength of the adult human femoral cortex, when tested at a low strain rate, was approximately 4% per decade.\textsuperscript{12} Hazama found that a similar rate of decay with age occurred for shear strength for subjects between twenty to thirty-nine and sixty to eighty years of age.\textsuperscript{21} Contrastingly, Evans and Lebow, and Sedin and Hirsch found no decrease in strength with age in the data acquired for bending tests on fresh embalmed femoral samples.\textsuperscript{13,26}

2.2.3 Fatigue crack propagation in bone

Fatigue testing involves cyclic loading of a material, in which the material undergoes progressive failure. A test involving compact tension bovine specimens has been used with pre-machined cracks to measure the crack growth initiated by cyclic loading.\textsuperscript{17} These cracks were machined parallel to the long axis of the bone, or otherwise referred to as the longitudinal axis, and perpendicular or transverse to the axis of the bone. An initial load of 100N was applied to the specimen and then cycled sinusoidally between 50 and 150N. A crack propagation gauge was used to monitor the initiation and the subsequent propagation of the material.
The rate of crack propagation \( \frac{da}{dn} \) can be expressed in terms of a power law, and is dependent on the cyclic stress intensity at the crack tip \( \Delta K \):

\[
\frac{da}{dn} = c \Delta K^m
\]

\textit{Equation 1}

Where,

\( \Delta K = K_{\text{max}} - K_{\text{min}} \) = stress intensity factor range (MN.m\(^{3/2}\))

\( c \) and \( m \) are both constants

The mechanism of crack growth for this test can be best illustrated schematically exhibiting the relationship of \( \frac{da}{dn} \) as a function of \( \Delta K \) (Figure 6).\(^{17}\) These terms are expressed logarithmically:

\[
\log \frac{da}{dn} = \log c + m \log \Delta K
\]

\textit{Equation 2}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{diagram.png}
\caption{Logarithmic relationship for \( \frac{da}{dn} \) and \( \Delta K \).\(^{17}\)}
\end{figure}
This graph can be split up into three regions, with the linear logarithmic region depicted as region 2. Furthermore, the calculation of the critical stress intensity factor $K_c$ can be obtained from region 3, this is where $\Delta_{\text{max}}$ approaches to $K_c$. This can be expressed in the following equation:

$$K_c = \frac{P}{BW^{1/2}} \left[ 29.6 \left( \frac{a}{W} \right)^{1/2} - 185.5 \left( \frac{a}{W} \right)^{3/2} + 655.7 \left( \frac{a}{W} \right)^{5/2} - 1017 \left( \frac{a}{W} \right)^{7/2} + 638.9 \left( \frac{a}{W} \right)^{9/2} \right]$$

Equation 3

where,

- $P$: load (N)
- $B$: thickness (m)
- $W$: width (m)
- $a$: crack length (m)

The comparison of longitudinal and transverse crack growth can be displayed in Figure 7. This graph shows the relationship between crack length ($a$) and number of cycles to failure. The two curves of longitudinal and transverse crack growth show a lower rate of crack growth, higher critical stress intensity value and higher fatigue life for transverse crack growth relative to longitudinal crack growth.

![Graph comparing longitudinal and transverse crack growth.](image)

Figure 7. Graph comparing longitudinal and transverse crack growth.¹⁷
The following table compares the parameters for longitudinal and transverse crack growth:\(^{17}\)

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Fatigue life (cycle)</th>
<th>(K_c) (MN.m(^{3/2}))</th>
<th>(c)</th>
<th>(m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longitudinal</td>
<td>1300</td>
<td>2.26</td>
<td>2.18</td>
<td>0.57</td>
</tr>
<tr>
<td>Transverse</td>
<td>3400</td>
<td>2.98</td>
<td>3.4</td>
<td>1.27</td>
</tr>
</tbody>
</table>

**Table 3.** Longitudinal and transverse crack growth.\(^{17}\)

The comparison of fatigue parameters given in the table, illustrates the difference in fatigue behaviour of longitudinal versus transverse crack propagation in bone. This is a clear indication of how the anisotropic properties of bone play a role in determining the manner in which bone propagates under cyclic loading.\(^{17}\)

2.2.4 Anisotropy

Definition: 'Phenomenon whereby any property of a substance depends on the direction relative to some defined axes in the material.'

The anisotropy of bone is related to the replacement of highly mineralised bone matrix with less calcified material, increasing cortical porosity, altering collagen orientation, and by the introduction of cement line interfaces which have unique mechanical properties.

The arrangement of collagen fibres and mineral crystals in cortical bone is highly ordered with the mineralised collagen fibre matrix orientated approximately parallel to the long axis of bone.\(^{27}\) The manner in which the collagen fibres are orientated can account for the differences observed in the mechanical properties associated with the longitudinal, radial,
and circumferential directions. Plexiform and circumferential lamellar forms of primary bone, as well as osteonal bone, all appear to be organized differently. While heavily remodelled bone is intrinsically isotropic in the transverse direction, plexiform and circumferential lamellar bone, however differ in the radial and circumferential directions.

Contributions made by Reilly and Burstein in determining the anisotropic effects by load-deformation tests have produced data that compare the elastic moduli and ultimate stress in tension and compression in different directions for human haversian bone, bovine haversian bone and primary bone belonging to the femur (Table 4). What the results show is a general decrease in tensile strength and elastic moduli for human and bovine haversian bone relative to primary bone, in both longitudinal and transverse directions.$^{14}$
## Elastic moduli

<table>
<thead>
<tr>
<th></th>
<th>Tension</th>
<th></th>
<th>Compression</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Long</td>
<td>Trans</td>
<td>Long</td>
<td>Trans</td>
</tr>
<tr>
<td>Human haversion</td>
<td>17.9 ± 9</td>
<td>10.1 ± 2.4</td>
<td>18.2 ± 0.9</td>
<td>11.7 ± 1.0</td>
</tr>
<tr>
<td>(ages 41 to 71)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine haversion</td>
<td>23.1 ± 3.2</td>
<td>10.4 ± 1.6</td>
<td>22.3 ± 4.6</td>
<td>10.1 ± 1.8</td>
</tr>
<tr>
<td>Primary</td>
<td>26.5 ± 5.4</td>
<td>11.0 ± 0.2</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

## Ultimate Stress

<table>
<thead>
<tr>
<th></th>
<th>Tension</th>
<th></th>
<th>Compression</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Long</td>
<td>Trans</td>
<td>Long</td>
<td>Trans</td>
</tr>
<tr>
<td>Human haversion</td>
<td>135 ± 16</td>
<td>53 ± 11</td>
<td>105 ± 17</td>
<td>131 ± 21</td>
</tr>
<tr>
<td>(ages 23 to 63)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine haversion</td>
<td>150 ± 11</td>
<td>49 ± 7</td>
<td>272 ± 3</td>
<td>146 ± 32</td>
</tr>
<tr>
<td>Primary</td>
<td>167 ± 9</td>
<td>55 ± 9</td>
<td>—</td>
<td>—</td>
</tr>
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</table>

Table 4. Table comparing the elastic moduli and ultimate stress in tension and compression in different directions for different bone.14
In fact there are similarities in the elastic moduli and tensile strength for human and bovine haversian bone, in the transverse direction, but the cow bone seems to have higher values for both properties in the longitudinal direction. The ratios for the elastic moduli for longitudinal and transverse tension, are similar in compression for all three types of bone, but the elastic anisotropy ratios for bovine haversian bone is greater than human haversian bone because the human bone is less stiff in the longitudinal direction. Both cow and human bone display higher strength values in compression compared to tension for both directions. The strength anisotropy for bovine bone is greater than in human bone, for both tension and compression, but the anisotropy for both cow and human bone is more pronounced in tension than compression. In some aspects haversian bovine bone is similar to human haversian bone and can be used as a suitable model, but haversian cow bone is essentially more isotropic and does appear to be stronger and stiffer in the longitudinal direction.

The data presented by Reilly and Burstein infers that the osteonal remodeling occurring in human bone has two important effects which make the whole bone transversely isotropic rather than orthotropic. Firstly, secondary osteons diminish the tensile strength and stiffness more longitudinally than transversely, this is evident when comparing human to bovine haversian bone and suggests that it is due to the more extensive intracortical remodelling of human bone. Secondly, they assist the transformation of bone from a orthotropic material to a more transversely isotropic material with respect to strength.

Authors have reported a difference in tensile properties of bone. The conditions in which the specimens were tested was an important factor in determining the tensile strength, since
they can be tested in either dry or wet conditions. Results undertaken by Dempster and Coleman show that there is a large difference in tensile strength values for rewetted dry bone between longitudinal and transverse directions, as opposed to Sweeney, who reported no difference under wet conditions. This phenomenon occurs as a result of initiation of cracks in cortical bone along the cement lines between osteons after drying. The cracks would therefore give rise to stress concentrations, which lower the strength of dry bone. Therefore, it is the effect of stress concentration which is responsible for the marked differences in tensile strength seen in dry bone for loads applied in longitudinal and transverse directions.
2.3 Apatites

2.3.1 Introduction

The importance of apatites to biologists, mineralogists, and inorganic and industrial chemists cannot be emphasised enough. The fact that apatites form the mineral component of bone and teeth is a valid enough reason to consider their true significance in biomedical applications. It is due to their biocompatible nature that these materials are constantly used for bone replacement or for coatings of bone prostheses. The involvement of apatites and related calcium phosphates in the mineralisation processes and pathological calcifications is attributed to the versatility of these minerals in accepting a large variety of substitutional ions. 7,30-51

The structure and chemistry of the apatites and related calcium phosphates has only recently been, to some extent, fully understood. The basic apatite structure was determined in the early half of the 20th century, however, the structure of the other calcium phosphates has come under some considerable scrutiny. The location of the carbonate group within the structure of biological, mineral and synthetic apatites was also a major debate at the time. Furthermore, the idea that apatites could be precipitated from solution to give Ca/P ratios ranging from 1.6667 to 1.5 without any apparent changes in the x-ray diffraction pattern was also ambiguous.

Analytical tools such as single crystal X-ray diffraction have been utilised to determine the structural aspects and crystal chemistry of the apatites and other calcium phosphates. However, due to the lack of suitable single crystals, the application of infra-red and nuclear
magnetic spectroscopy have provided other ways of understanding the relationship between carbonate and apatites and the variation in the Ca/P ratios of precipitated apatites.

2.3.2 The chemistry of the calcium orthophosphates

The calcium orthophosphates are salts of tribasic phosphoric acid, $\text{H}_3\text{PO}_4$. The conditions in which the calcium orthophosphates contribute to the development of biological systems depend on the compounds that the tribasic phosphoric acid form. $\text{H}_3\text{PO}_4$ can produce compounds that consist of $\text{H}_2\text{PO}_4^-$, $\text{HPO}_4^{2-}$ or $\text{PO}_4^{3-}$ ions. Compounds that contain $\text{H}_3\text{PO}_4^-$ ions are not normally found in biological systems, and this is due to the acidic conditions needed for the formation of these particular compounds. However, the mineral part of bone and teeth contain both $\text{HPO}_4^{2-}$ and $\text{PO}_4^{3-}$ ions. More importantly, calcium phosphates occur as minerals in the apatitic form, for instance hydroxyapatite, where the basic apatitic calcium phosphate can consist of hydrated calcium phosphates that contain $\text{OH}^-$ ions.

Other members of this phosphate family include pyrophosphates ($\text{P}_2\text{O}_7^{2-}$) and polyphosphates that are less significant biologically, however, calcium pyrophosphates are known to occur in some pathological calcifications. Furthermore, the nucleation and crystal growth of calcium phosphates in aqueous systems can be greatly inhibited by the presence of pyrophosphates.

Calcium phosphates dissolve in water with varying difficulty, but all dissolve in acids. They occur as solids and are usually white in appearance. The likely conditions for the
synthesis of calcium phosphates can be depicted by phase diagrams, which display the thermodynamically stable phases. Under any given conditions the resulting phase that actually forms is usually determined by kinetic rather than thermodynamic effects.  

\[
\text{Log}_{10} \text{ Ca conc. (mol.l}^{-1}\text{)}
\]

![Diagram showing solubility isotherms of calcium phosphate phases](image)

**Figure 8.** Solubility isotherms of calcium phosphate phases in the \(\text{Ca(OH)}_2-\text{H}_3\text{PO}_4-\text{H}_2\text{O}\) system at 37°C.  

One example of how these calcium phosphate phases form under varying conditions can be illustrated by the phase diagram for the \(\text{Ca(OH)}_2-\text{H}_3\text{PO}_4-\text{H}_2\text{O}\) system at normal temperatures and pressures (Figure 8) and also with the more acidic and soluble region of the \(\text{CaO-P}_2\text{O}_5-\text{H}_2\text{O}\) system at higher temperatures and/or pressures.  

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We have briefly mentioned the importance of the kinetic factor in the solution chemistry of calcium phosphates, however, matters are further complicated by another factor; the formation of calcium phosphate ion pairs, in particular \([\text{CaHPO}_4]\) and \([\text{CaH}_2\text{PO}_4]^+\). The speciation diagram for the \(\text{Ca(OH)}_2-\text{H}_3\text{PO}_4-\text{H}_2\text{O}\) system illustrates how the concentrations of each of a variety species formed changes as a function of pH at normal temperatures and pressures. It is apparent that if the formation of ion pairs and other complex ions are not taken into consideration, then the resulting solubility product would appear to be dependent on the pH.\(^{30}\)

A number of thermodynamic properties and calculated solubility constants \((K_s)\) at 25°C can be expressed for a variety of calcium phosphates (Table 5). The thermodynamic properties include standard enthalpies of formation \((\Delta_f H^\circ)\), free energies of formation \((\Delta_f G^\circ)\) or Gibbs free energy, entropies \((S^\circ)\) and specific heat capacities \((C_p)\). The values given for the standard enthalpies and Gibbs free energy denote the formation of calcium phosphates from the constituent elements and also assume that the value for the \(H^+\) ions to be zero. The data tabulated also accounts for the standard free energy of solution of constituent ions for the various calcium phosphates. The value given for the standard free energy of solution \((\Delta_f G^\circ)\) is the result of subtracting the standard free energy for the formation of the salt \((\Delta_f G^\circ)\) from the sum of the standard free energies of solution of the constituent ions. Having obtained \((\Delta_f G^\circ)\), the value for the solubility constant \((K_s)\) can therefore be ascertained from the following equation; \(\Delta_f G^0 = -RT\ln K_s\), where \(R\) is given as the molar gas constant \((\text{J.K}^{-1} \cdot \text{mol}^{-1})\) and \(T\) is the absolute temperature \((\text{K})\).\(^{30}\)
<table>
<thead>
<tr>
<th>Compound</th>
<th>$\Delta H^\circ$</th>
<th>$\Delta G^\circ$</th>
<th>$S^\circ$</th>
<th>$C_p$</th>
<th>$K_s$ Calc. Sol.</th>
<th>Product const</th>
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<tr>
<td></td>
<td>kJ.mol$^{-1}$</td>
<td>kJ.mol$^{-1}$</td>
<td>J.mol$^{-1}$ K$^{-1}$</td>
<td>J.mol$^{-1}$ K$^{-1}$</td>
<td></td>
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<tr>
<td>CaHPO$_4$</td>
<td>-1814.39</td>
<td>-1681</td>
<td>111.38</td>
<td>110.04</td>
<td>1.83 x 10$^{-7}$</td>
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</tr>
<tr>
<td>CaHPO$_4$,2H$_2$O</td>
<td>-2403.58</td>
<td>-2154.58</td>
<td>189.45</td>
<td>197.07</td>
<td>2.59 x 10$^{-7}$</td>
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<tr>
<td>Ca$_2$(PO$_4$)$_2$H$_2$O</td>
<td>-12263</td>
<td>-12263</td>
<td>-</td>
<td>-</td>
<td>1.01 x 10$^{-64}$</td>
<td></td>
</tr>
<tr>
<td>$\beta$-Ca$_3$(PO$_4$)$_2$</td>
<td>-4120.8</td>
<td>-3884.7</td>
<td>236.0</td>
<td>227.82</td>
<td>2.07 x 10$^{-33}$</td>
<td></td>
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<tr>
<td>$\alpha$-Ca$_3$(PO$_4$)$_2$</td>
<td>-4109.9</td>
<td>-3875.5</td>
<td>240.91</td>
<td>231.58</td>
<td>8.46 x 10$^{-32}$</td>
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<tr>
<td>Ca$_{10}$(PO$_4$)$_6$(OH)$_2$</td>
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<td>-12677</td>
<td>780.7</td>
<td>769.9</td>
<td>6.62 x 10$^{-126}$</td>
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<tr>
<td>Ca$_{10}$(PO$_4$)$_3$F$_2$</td>
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<td>-12983</td>
<td>775.7</td>
<td>751.9</td>
<td>6.30 x 10$^{-137}$</td>
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<tr>
<td>H$_2$O</td>
<td>-285.83</td>
<td>-237.129</td>
<td>69.91</td>
<td>-</td>
<td>-</td>
<td></td>
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<tr>
<td>Ca$^{2+}$(aq)</td>
<td>-542.83</td>
<td>-553.58</td>
<td>-53.1</td>
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<td>-</td>
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<tr>
<td>OH$^-$ (aq)</td>
<td>-229.994</td>
<td>-157.244</td>
<td>-10.75</td>
<td>-148.5</td>
<td>-</td>
<td></td>
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<tr>
<td>H$_2$PO$_4$ (aq)</td>
<td>-1296.29</td>
<td>-1130.28</td>
<td>+90.4</td>
<td>-</td>
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<tr>
<td>HPO$_4^{2-}$ (aq)</td>
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<td>-1089.15</td>
<td>-33.5</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>PO$_4^{3-}$ (aq)</td>
<td>-1277.4</td>
<td>-1018.7</td>
<td>-222</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>F (aq)</td>
<td>-332.63</td>
<td>-278.79</td>
<td>-13.8</td>
<td>-106.7</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Thermodynamic properties of different calcium phosphates.$^{30}$
2.3.3 The general chemistry and structure of apatites

The general formula associated with apatites is expressed as Ca$_5$(PO$_4$)$_3$X or as Ca$_{10}$(PO$_4$)$_6$X$_2$, where X can represent a number of ions namely F$, \text{Cl}^-$ or OH$^-$ to give apatites of the fluorapatite, chlorapatite and hydroxyapatite form respectively.$^{53}$ These mineral apatites were often confused with other minerals such as aquamarine, crystollite, amethyst, fluorite, schorl etc. and so the name apatite meaning deceiver, derived from the greek word (απατω), is a fitting description for this family of apatites.$^9$

The apatite structure can accommodate a variety of ionic substitutions, which include the partial or full substitution of Ca$^{2+}$ by Ba$^{2+}$, Sr$^{2+}$ or Pb$^{2+}$ ions and also the substitution of PO$_4^{3-}$ by AsO$_4^{3-}$ ions.$^{30}$ These exchanges can result in apatite structures that are commonly referred to as lead minerals such as Pb$_5$(PO$_4$)$_3$Cl (pyromorphite), Pb$_5$(VO$_4$)$_3$Cl (vanadite) and Pb$_5$(AsO$_4$)$_3$Cl (mimetite). Another common feature of the apatites is the occurrence of halide and OH$^-$ ions present in the same structure, these are normally abbreviated as OH,FAp (hydroxyl fluoroapatite), Cl,OHAp (chloro hydroxylapatite) etc. Other substitutions that can occur include coupled substitutions whereby one ion is exchanged for another that has the same sign but differs in charge. An example of this is the coupled substitution of a calcium ion, Ca$^{2+}$ for sodium ion, Na$^+$, accompanied by the substitution of a phosphate ion, PO$_4^{3-}$, by a carbonate ion, CO$_3^{2-}$ to give Ca$_9$Na(PO$_4$)$_3$CO$_3$(X)$_2$. The coupled substitution given above is therefore necessary to ensure that the neutrality of the apatite structure is maintained. This type of substitution can be termed a type B substitution and such structures can be found in biological apatites in particular carbonate apatites (CO$_3$Aps).$^{33,34,52,54}$
Carbonate apatites, which include the minerals francolite, dahllite and the rock phosphates, are an important but controversial group of apatites. Whether it is viewed from a mineralogical, synthetic or biological perspective the exact nature and crystal structure of carbonate substitution into calcium phosphates has stimulated a great debate since the 1930's. The crystal structure and the effects of carbonate substitution into calcium phosphate apatites was not fully investigated until the 1960's. LeGeros had found that the two type of substitutions, namely type A and type B, had induced changes in the lattice parameters in the carbonate hydroxyapatite crystal lattice.

Type A substitution involves the substitution of the OH⁻ ion for a CO₃²⁻ ion. This exchange results in an increase in the a-axis. This is largely due to the fact that the smaller OH⁻ is replaced by a larger and more planar CO₃²⁻ ion. In contrast, what was observed for type B substitution is a reduction in the a-axis, which is attributed to the exchange of large tetrahedral phosphate group, PO₄³⁻, for a comparatively small CO₃²⁻ ion.

The manner in which the carbonate ion substitutes into synthetic apatites appears to differ to that of biological or natural apatites under similar conditions. What is observed in geological mineral apatites is a shorter a-axis compared to stoichiometric HA when formed at high temperatures and pressures, this is associated with B type substitution. In synthetic carbonate apatite, however, the opposite occurs when formed at high temperatures i.e. type A substitution resulting in a longer a-axis.

The comparison of the lattice dimensions that occur between carbonate hydroxyapatite formed by low temperature precipitation reactions and the apatite phases of biological
tissues, such as enamel, also exhibit differences to what is expected. What was confirmed by LeGeros is an increase in the a-axis for enamel containing 2.5wt% of carbonate compared to stoichiometric HA.\textsuperscript{56} The study was carried using x-ray diffraction and the lattice dimensions ascertained for CHA prepared by aqueous precipitation at different reaction temperatures. The resulting single phase apatite structure showed a shrinkage in the a-axis as a function of increasing carbonate content, which was indicative of a B-type substitution. This mode of substitution was substantiated by a linear correlation indicated by a decrease in the phosphate content as the phosphate ions are progressively substituted for carbonate ions.

Precipitation of carbonate hydroxyapatite at 25°C and 37°C were also undertaken to simulate biological conditions and also mirror the type of carbonate substitutions that occur in enamel apatite.\textsuperscript{56} Crystallographic analysis showed that the crystallinity of the samples were quite poor and consequently any variation in the lattice parameters that would take place could not be detected. However, a drop in the phosphate content was measured and so this might suggest that it is the type-B substitution which occurs in this case.

The biological environment provides the perfect conditions for a variety of ionic substitutions to take place. In the mineral phase of biological tissues simultaneous substitutions of ions can occur, and therefore might explain why enamel has an enlarged a-axis. The simultaneous substitution of the OH\textsuperscript{-} ion for Cl\textsuperscript{-} and HPO\textsubscript{4}\textsuperscript{2-} for PO\textsubscript{4}\textsuperscript{3-} inhibits any reduction of the a-axis during carbonate substitution. This is primarily due to the fact that the Cl\textsuperscript{-} ion has a considerably greater ionic radii to OH\textsuperscript{-}, (Cl\textsuperscript{-} : 1.81Å ; OH\textsuperscript{-} : 1.37Å) and also any reduction in the a-axis that occurs as a consequence of substituting phosphate
groups for smaller carbonate groups can be compensated by the inclusion of large \( \text{HPO}_4^{2-} \) groups.\textsuperscript{57}

Observations made by LeGeros to examine the effect of different reaction temperatures on the lattice parameters of CHA were undertaken through x-ray analysis.\textsuperscript{58} It was shown that changes in the lattice dimension, namely the a-axis, could be determined through shifts in the 2\( \theta \) angle for the 300 reflection. The comparison of these shifts against stoichiometric HA produced under similar conditions showed that under the high temperature solid state conditions (1000°C) there was an increase in the a-axis compared to stoichiometric HA, whereas under the low temperature aqueous precipitation conditions (100°C) there was a decrease in the a-axis. In addition, the CHA produced in the solid state gave a peak associated with the \( \alpha \)-TCP phase. It should be noted that the synthesis of CHA for both reaction conditions used similar carbonate content. The x-ray analysis suggests that there are distinct differences in the chemical structure of CHA produced under different reaction temperatures.

The mode of vibration that is associated with the \( \text{CO}_3^{2-} \) ion in infra-red analysis is the \( v_2 \) mode, which can be used to establish the type of carbonate substitution that takes place. The CHA prepared via the solid state method gave a distinct peak at 877cm\(^{-1}\), whereas the low temperature precipitation method gave two peaks at 871cm\(^{-1}\) and 878cm\(^{-1}\) that are associated with type-B carbonate substitution. These two peaks or doublet of peaks represents \( \text{CO}_3^{2-} \) substituting for \( \text{PO}_4^{3-} \) at two different phosphate sites. The \( v_3 \) peak, however, was shifted to higher frequencies in the high temperature apatite compared to the
precipitated and was low in intensity. Similarities between the IR spectrum for human enamel and the low temperature precipitated apatite points towards type-B substitution in enamel apatite.

Francolite is a naturally occurring mineral, however, francolite-like analogues (B-CO$_2$FAp) (B- Type-B carbonate substitution) can be synthesised and have potential medical and dental applications.$^{30,33}$ Fluoride containing ceramics such as fluorapatite (FAp) for instance can be used as implant materials which can prevent caries in teeth by releasing fluoride ions into the oral environment.$^{48}$

Synthetic B- CO$_2$FAp can be prepared by heating precipitated CO$_3$Ap with CaF$_2$ in dry CO$_2$ at 900°C. XRD analysis of lattice parameters showed that the natural francolites exhibit shorter a-axis dimensions ($a = 9.35$, $c = 6.89$ Å) compared to pure FAp, but were similar to those of synthetic B- CO$_2$FAp. In addition, the natural francolites also gave IR spectra that were characteristic of synthetic B- CO$_2$FAp.$^{30}$

Using the same conditions apatites such as A- CO$_3$FAp and FAp can be synthesised by heating A-CO$_3$Ap with CaF$_2$ and OHAp with CaF$_2$. An alternative route for the synthesis of B-CO$_3$FAp was also considered. This involved mixing CaCO$_3$ with pure FAp. However, the effectiveness of this preparation depends on the presence of CaO formed during synthesis. The presence of CaO during the synthesis of FAp from OHAp and CaF$_2$ was found to be greater than that of B-CO$_3$FAp synthesised from FAp and CaCO$_3$. This was owing to differences in their dispersions.$^{30}$
The method for the preparation of FAp performed by heating OHAp and CaF2 at 900°C in dry CO2 displayed changes in the lattice parameters from a = 9.37 and c = 6.89 Å (for pure FAp) to a = 9.35 and c = 6.89 Å, which effectively shows a shrinkage in the a-axis. The FAp displayed similar IR CO3^2- bands to those in Fowey Consols francolite (1453, 1429 and 865 cm^-1). This would indicate that the FAp produced using the aforementioned method is not altogether pure and that an uptake in CO2 is involved resulting in a small quantity (x = 0.3) of CO3^2- ions substituting for PO4^3- in similar sites to those seen in natural francolites, in particular the Fowey Consols francolite. The equation that was proposed for this system can therefore be expressed as follows:

\[ \text{Ca}_{10-x}(\text{PO}_4)_{6-2x}(\text{CO}_3,F)_{2x}F_{2(1-x)} \]

The equation proposed for the reaction between FAp and CaCO3 assumes that there are no adjacent CO3^2- and F^- ions, and can be expressed as:

\[ y\text{Ca}_{10}(\text{PO}_4)_6F_2 + x\text{CaCO}_3 \rightarrow \text{Ca}_{10y+x}(\text{PO}_4)_{6y}(\text{CO}_3)_xF_{2y} \]

where \( y = (6-x)/6 \). This is based on the assumption that there are a total of six PO4^3- and CO3^2- ions per unit cell.

If the maximum CO2 uptake for the reaction corresponds to x = 0.3, then the occupancies of the constituent ions can be given as Ca_{9.8}(PO4)_{5.70}(CO3)_{0.30}F_{1.90}. 

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Substitution of Ca$^{2+}$ ions with monovalent ions such as Na$^+$ is prevalent in natural francolites and is accountable for the presence of a relatively higher content of CO$_2$ compared to synthetic francolites.$^{30}$

Another mode of substitution commonly seen in francolites is AB substitution. AB-CO$_3$F,OHAp involves the substitution of CO$_3^{2-}$ for PO$_4^{3-}$ ions (B-type substitution) as well as CO$_3^{2-}$ ions replacing OH$^-$ ions (A-type substitution).$^{30}$ To limit the amount of F$^-$ ions replacing OH$^-$ ions, the preparation of AB-CO$_3$F,OHAp initially involved a reduced amount of CaF$_2$ heated with OHAp to form F$_2$OHAp and a limiting amount of CaO as a by-product. The two products were then heated in dry CO$_2$ at 900°C to form the AB-CO$_3$F,OHAp. The substitutional behaviour involving CO$_3^{2-}$ ions and the component lattice ions of the apatite indicated that a F$^-$ ion content increase resulted in a decrease in intensity of the IR bands representing CO$_3^{2-}$↔OH$^-$ exchange, and an increase for those representing CO$_3^{2-}$↔PO$_4^{3-}$ exchange.

This can also be reflected by changes in the lattice parameters, where there was a substantial decrease in the a-axis dimension as the F$^-$ ion content increased from no CaF$_2$ added to 127% of stoichiometric CaF$_2$ added.$^{30}$ The values changed from $a = 9.53$ to $a = 9.35\text{Å}$ and $c = 6.86$ to $c = 6.89\text{Å}$, in this instance there did not appear to be a marked difference in the c-axis dimensions. The changes in the IR band frequencies were recorded chronologically as a function of increasing F$^-$ ion content. Variations in the frequency of vibrational modes are indicative of changes in the local environment and consequently the
dipole moment to which a particular ion is assigned. It was shown that the band belonging to both substitutions moved from 1465 cm\(^{-1}\) to 1457 cm\(^{-1}\), furthermore the frequency of the mode \(v_{3a}\) for \(\text{CO}_3^{2-}\) replacing \(\text{PO}_4^{3-}\) ion was detected at 1407 cm\(^{-1}\) for a 29\% addition of stoichiometric \(\text{CaF}_2\) \((a = 9.46, c = 6.88\text{ Å})\) and increased to 1429 cm\(^{-1}\) as the \(\text{F}^-\) ion content increased.

Similarly, changes in the vibrational frequency of the same mode were also recorded for synthetic francolites containing \(\text{Cl}^-\) ions, such as \(\text{B-CO}_3\text{Cl,OHAp}\), instead of \(\text{F}^-\) ions (1405 to \(~1429\text{ cm}^{-1}\)), as well as a reduction in the unit cell volume as \(\text{Cl}^-\) ions are progressively substituted by \(\text{F}^-\) ions.\(^{30}\) The process occurring for this reaction differs somewhat from \(\text{B-CO}_3\text{F,OHAp}\), since this describes changes in the mode for \(\text{CO}_3^{2-}\) replacing \(\text{PO}_4^{3-}\) as a result of \(\text{F}^-\) ions substituting for \(\text{Cl}^-\) ions rather than \(\text{OH}^-\) ions. Taking into consideration the ionic radii of \(\text{Cl}^-\) (1.81 Å) and \(\text{F}^-\) (1.33 Å), it is probably more thermodynamically and sterically favourable for \(\text{F}^-\) ions to substitute for \(\text{Cl}^-\) ions rather than the \(\text{OH}^-\) (1.37 Å) ions.

\(\text{A-CO}_3\text{Aps}\) and \(\text{AB-CO}_3\text{F,OHAp}\)s have shown to differ with respect to \(\text{CO}_3^{2-}\) substitution, however, this behaviour can be further elucidated by the relationship between the loss of degeneracy of the \(v_3\) mode to \(v_{3a}\) and \(v_{3b}\) and the extent of \(\text{CO}_3^{2-}\) substitution.\(^{30}\) It has already been discussed that the \(v_{3a}\) mode shows greater change in frequency with respect to increasing \(\text{CO}_3^{2-}\) substitution for \(\text{AB-CO}_3\text{F,Ap}\). In contrast, for \(\text{A-CO}_3\text{Aps}\) the change in the frequency of the \(v_{3b}\) mode is more prevalent, with the \(v_{3a}\) remaining relatively constant. This is ascribed to the angle between the axis undergoing dimensional change and the transition moments of these modes. As substitution increases in \(\text{A-CO}_3\text{Ap}\) the \(v_{3b}\) decreases.
in frequency. Such substitution would result in an increase in the a-axis dimension, however, this dimensional change does not have a bearing on the transition moments of $v_{3b}$ since these lie closer to the c-axis and would only decrease with an increase in c-axis as a consequence of a cell volume increase. The transition moment of the $v_{3a}$ mode lies closer to the a-axis, hence any dimensional change in that axis would result in a change in frequency for that mode.

Sodium-containing AB-CO$_3$Aps prepared using similar methods as AB-CO$_3$Aps, show a similar charge compensation mechanism as the mineral CO$_3$Aps, which take place without lattice vacancies. The charge compensation mechanism involves a loss of a negative charge as CO$_3^{2-}$ substitutes for PO$_4^{3-}$ and a loss of a positive charge as Na$^+$ substitutes for Ca$^{2+}$. The exchanges occurring for sodium-containing AB-CO$_3$Aps and for mineral CO$_3$Aps can be represented by the following formulae:

\[
\text{Ca}_{10-x-y-z}\text{Na}_x\text{Mg}_y(\text{PO}_4)_{6-x}(\text{CO}_3)_z\text{F}_{0.42z}\text{F}_2 \quad \text{mineral}
\]

\[
\text{Ca}_{10-x}\text{Na}_x(\text{PO}_4)_{6-x}(\text{CO}_3)_x(\text{OH})_2 \quad \text{synthetic (excluding CO}_3^{2-} \text{ replacing OH ions between 0.43 and 0.97 CO}_3^{2-} \text{ ions per unit cell)}
\]

Sodium free AB-CO$_3$Aps, however, do not provide such a high degree of carbonate substitution compared to sodium containing AB-CO$_3$Ap, since AB-CO$_3$Ap require Ca$^{2+}$ and OH$^-$ vacancies to accommodate carbonate substitution and hence can be represented as:

\[
\text{Ca}_{10-2x/3}(\text{PO}_4)_{6-x}(\text{CO}_3)_x(\text{OH})_{2-x/3}
\]
It should be noted that a complex charge compensation mechanism along with OH$^-$ vacancies is seen in precipitated sodium containing CO$_3$Aps and appears to be significantly different to the high temperature sodium containing AB-CO$_3$Aps.$^{30}$

$$\text{Ca}_{10-x}\text{Na}_{2x/3}\{\text{PO}_4\}_{6-x}\{\text{CO}_3\}_x\{\text{H}_2\text{O}\}_x\{\text{OH}\}_{2-x/3}$$

complex charge compensation mechanism accompanied by OH$^-$ vacancies seen in ppted sodium containing CO$_3$Aps

Sodium containing AB-CO$_3$Aps can be prepared at a temperature of 870$^\circ$C by drying CO$_2$ over 95% of sulphuric acid with the addition of Na$_2$CO$_3$ at varying content to form three distinct phases, which have different P/Ca and Na/Ca molar ratios.$^{30}$ The values of these molar ratios were given at 0.600±0.003 and 0; 0.574±0.003 and 0; and 0.530±0.003 and 0.18±0.01 respectively. The first apatite formed (A-CO$_3$Ap) gave lattice parameters $a = 9.539$ and $c = 6.865\, \text{Å}$, a density of 3.16gcm$^{-3}$, a carbonate content of 4.7wt%, and unit cell contents of Ca$_{10.01}$PO$_4$$_{6.03}$OH$_{0.32}$CO$_3$_$0.81$. The sodium containing AB-CO$_3$Ap though gave a density of 3.01±0.03gcm$^{-3}$, lattice parameters of $a = 9.367±0.003\, \text{Å}$ and $c = 6.934±0.002\, \text{Å}$ and contained the most CO$_3^{2-}$ (15.4wt%). This gave a formula of:

$$\text{Ca}_{8.50}\text{Na}_{1.57}\{\left(\text{PO}_4\right)_{4.52}\left(\text{CO}_3\right)_{1.48}\}\{\text{OH}\}_{0.10}\{\text{CO}_3\}_{0.97}$$

The IR spectra for a variety of AB-CO$_3$Ap with different Na/Ca ratios were taken.$^{30}$ The bands representing CO$_3^{2-}$ ions replacing PO$_4^{3-}$ were recorded around 1452, 1415 and 873cm$^{-1}$. Additional bands at 1549, 1472 and 880cm$^{-1}$ that were assigned to CO$_3^{2-}$ ions replacing OH$^-$ were detected for low Na/Ca ratios. Although the analysis indicated that a
substantial number of \( \text{CO}_3^{2-} \) ions were located at c-axis sites, the IR spectrum for high Na/Ca ratios didn't exhibit the bands associated with \( \text{CO}_3^{2-} \) ions in these sites. This suggests that the environment of the c-axis sites in which \( \text{CO}_3^{2-} \) occupy for high sodium containing AB-CO_3Ap and pure A-CO_3Ap must differ.
2.4 Hydroxyapatite

2.4.1 Crystallographic structure

Crystallographic structures of crystals can be categorized depending on the type of symmetry inherent to them. These structures can therefore be categorized according to their space group. Hydroxyapatite, in particular calcium hydroxyapatite, has a definite crystallographic structure and composition, and has an intrinsic space group (P63/m) which is based on a repeating unit cell (Figure 9). The unit cell of hydroxyapatite consists of a six fold c-axis perpendicular to three equivalent a-axes (a1,a2,a3) at angles 120° to each other. The groups that make up the closely packed hexagonal unit cell are Ca2+, PO43−, and OH groups.31,47,53,59-62

![Diagram illustrating the unit cell of hydroxyapatite](image_url)
The exact atomic positions of these atoms can be located by x,y,z coordinates within the unit cell. From the ten calcium atoms in the structure, two occupy a position $z = 0$ and two at $z = 0.5$, hence these four calcium atoms belong to the Ca(I) subset. The other six form a triangular arrangement perpendicular to the c-axis, a set of three located at $z = 0.25$ and another set of three at $z = 0.75$, with each arrangement surrounding an OH$^-$ group in the same plane, one OH$^-$ located at $z = 0.25$ and the other at $z = 0.75$.

The six PO$_4^{3-}$ groups belong to a tetrahedral symmetry and are responsible for the stability of the apatite structure. These groups occupy the positions $z = 0.25$ and $z = 0.75$. The oxygens bonded to the phosphorous atoms in the phosphate groups are also labelled, these are denoted by O$_{I}$, O$_{II}$ and two O$_{III}$. The atomic arrangements that the atoms adopt in hydroxyapatite are similar to those seen in F-Apatite, Ca$_{10}$(PO$_4$)$_3$F$_2$, and Cl-Apatite, Ca$_{10}$(PO$_4$)$_3$Cl$_2$, but the OH$^-$ atomic positions in this instance are described for F$^-$ and Cl$^-$ ions that substitute for the OH$^-$ groups.

Changes in the properties of Ca-hydroxyapatite can occur as a result of ionic substitutions of Ca$^{2+}$, PO$_4^{3-}$ and OH$^-$ groups within the apatite structure. The changes in properties include lattice parameters, morphology and solubility, however, they can take place without significantly altering the hexagonal symmetry. It has, though, been reported that the symmetry changes from hexagonal to monoclinic occur upon Cl$^-$ substitution for OH$^-$ and this is in fact a loss in symmetry which is thought to reflect the alternating positions of Cl$^-$ atoms and an expansion of the cell in the b-axis.
The solubility properties of apatites can give an indication of the crystallinity, stability and crystal size of a particular apatite. Fluoroapatite for instance, exhibits a higher degree of the aforementioned properties, and consequently are less soluble, compared to fluoride free apatites and biological apatites. This can be attributed to a contraction in the a-axis without any change in the c-axis, and arises from substitution of $F^-$ for $OH^-$.\textsuperscript{53}

The effect on lattice dimensions and hence the crystal properties within Ca-HA by type A and type B carbonate substitution as well as coupled substitutions such as $CO_3^{2-}$ for $PO_4^{3-}$ and $Na^+$ for $Ca^{2+}$ within Ca-hydroxyapatite have already been previously mentioned in the carbonate apatite section.

Differences in lattice parameters can also be observed for Ca-hydroxyapatite that undergoes cationic substitution. Ions such as strontium ($Sr^{2+}$), magnesium ($Mg^{2+}$), barium ($Ba^{2+}$), lead ($Pb^{2+}$), etc. substitute for $Ca^{2+}$ in the crystal lattice. The extent to which these changes take place reflect the size and amount of the substituting ions. Crystallinity, thermal stability and dissolution properties would consequently alter as cationic substitutions take place, for instance the extent of dissolution would increase for apatites with the substitution of $Sr^{2+}$ or $Mg^{2+}$ for $Ca^{2+}$.\textsuperscript{36,38,41,49,51} Furthermore, the presence two substituents such as $Mg^{2+}$ and $CO_3^{2-}$ would have synergistic effects on crystallinity and dissolution properties of synthetic apatites. Alternatively, antagonistic effects can also be seen with the presence of two substituents such as magnesium and fluoride or carbonate and fluoride, with the fluoride effect being the greatest.\textsuperscript{53}
The type of substitutions that have been mentioned are important in understanding how the change in properties of the apatite relate to the way HA behaves as a biomaterial, in particular the manner that HA interacts with bone mineral of the bone-biomaterial interface. HA can be used for clinical applications such as bone repair, augmentation, substitution and coatings of metals used as dental and orthopaedic implants.$^{53}$

2.4.2 Hydroxyapatite preparation

The preparation of HA can be carried out via a number of methods, which include hydrothermal conversions, solid state reactions, precipitation and hydrolysis.$^{60}$ However, under aqueous conditions such as precipitation or hydrolysis the apatite formed is usually calcium deficient giving a lower Ca/P molar ratio compared to the stoichiometric value of pure HA of 1.67. In contrast, precipitation can also result in a higher Ca/P molar ratio when the reaction is carried out under very basic conditions, and will produce HA containing carbonate.$^{60}$

2.4.2.1 Precipitation method

$$10 \text{Ca(OH)}_2 + 3\text{H}_3\text{(PO}_4\text{)}_2 \rightarrow \text{Ca}_{10}\text{(PO}_4\text{)}_6\text{(OH)}_2$$

The reaction described above is, commercially, the most commonly used precipitation method.$^{63}$ The reaction which is based on the Rathje method involves a stirring suspension of calcium hydroxide, Ca(OH)$_2$ in water into which phosphoric acid, H$_3$PO$_4$, is added dropwise.$^{53,64}$
\[10 \text{Ca(NO}_3\text{)}_2 + 6(\text{NH}_4\text{)}_2\text{HPO}_4 + 2\text{NH}_4\text{OH} \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2\]

The alternative precipitation route above which is based on the Hayek and Newesley method is sensitive to the concentrations of the reactants and pH of the reaction, which consequently has an effect on the product formed upon sintering of the precipitated apatite. In order to avoid the formation of octacalcium phosphate and subsequent conversion to β-TCP, the pH of the reaction needs to be controlled. This can be done by the addition of ammonium hydroxide (\(\text{NH}_4\text{OH}\)) to both solutions of calcium nitrate, \(\text{Ca(NO}_3\text{)}_2\), and ammonium phosphate (\(\text{NH}_4\text{)}_2\text{HPO}_4\) to give a significantly high pH level, typically between 10-12.

2.4.2.2 Hydrolysis method

Acid calcium phosphates can be used as reagents for the preparation of apatite. The hydrolysis of acid calcium phosphates in ammonium, sodium or potassium hydroxide, carbonate, fluoride or chloride solutions is an alternative method for the preparation of apatite. The calcium reagents can involve dicalcium phosphate dihydrate, DCPD, \(\text{CaHPO}_4.2\text{H}_2\text{O}\); octacalcium phosphate, OCP, \(\text{Ca}_8\text{H}_2(\text{PO}_4)_6.5\text{H}_2\text{O}\); or monetite, dicalcium phosphate anhydrous, DCP, \(\text{CaHPO}_4\).^53

\[
\begin{align*}
2\text{NH}_4\text{OH} \\
1. & \quad 10\text{CaCO}_3 + 6(\text{NH}_4\text{)}_2\text{PO}_4 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 10(\text{NH}_4)_2\text{CO}_3 \\
2\text{NaOH} \\
2. & \quad 10\text{CaCO}_3 + 6\text{Na}_2\text{PO}_4 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 10\text{Na}_2\text{CO}_3
\end{align*}
\]
2NH₄F

3. 10CaCO₃ + 6(NH₄)₃PO₄ → Ca₁₀(PO₄)₆F₂ + 10(NH₄)₂CO₃

The hydrolysis of calcium carbonate, CaCO₃, in a solution of ammonium or sodium phosphate can yield apatite, similarly the presence of fluoride solutions can yield F-apatite.

2.4.2.3 Solid state reactions

The preparation of HA can be carried out via solid state reactions which involve pressure and temperatures above 950°C.⁵³

\[
6\text{CaHPO}_4 + 4\text{Ca(OH)}_2 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 6\text{H}_2\text{O} \\
\text{HA}
\]

\[
3\text{Ca}_3(\text{PO}_4)_2 + \text{Ca(OH)}_2 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + \text{H}_2\text{O} \\
\text{HA}
\]

2.4.2.4 Hydrothermal reactions

A temperature of 275°C, and steam pressure of 12,000psi are required to carry out hydrothermal reactions using the reagents depicted in the above solid state reactions. Furthermore, the hydrothermal conversion of reagents such as β-TCP, Ca₃(PO₄)₂, and tetracalcium phosphate, Ca₄P₂O₉ or Ca₄(PO₄)O to HA can also be employed.⁵³

\[
4\text{CaCO}_3 + 6\text{CaHPO}_4 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 6\text{H}_2\text{O} + 4\text{CO}_2
\]
10CaCO₃ + 6(NH₄)₂HPO₄ → Ca₁₀(PO₄)₆(OH)₂ + H₂O + CO₂

Appropriate amounts of CaHPO₄ and (NH₄)₂HPO₄ carried out under the same conditions with CaCO₃ can also convert readily to HA.

2.4.3 Composition of dense HA

The theoretical composition of HA whether it is pure, mineral or commercial will undoubtedly vary depending on the phases that are present when sintered. Pure HA is composed of 39.68wt% Ca; 18.45wt% P; giving a Ca/P weight ratio of 2.151 and molar ratio of 1.6667. Dense HA containing only the apatite phase or mixed with other Ca-P phases can exhibit a variation in the Ca/P ratio when sintered, this can be attributed to the presence of secondary phases such as β-TCP. Other factors such as the composition or calcium deficiency in the material before sintering can also influence the Ca/P ratio.

The presence of β-TCP, tetracalcium phosphate, Ca₄P₂O₉ or Ca₄(PO₄)₂O along with the HA phase in the sintered material is indicative of a Ca/P ratio which is lower than that of just the HA phase (Ca/P=1.67) and is influenced by the sintering conditions and the temperature. Higher Ca/P ratios than 1.67 would indicate the presence of CaO with the HA phase.

"Apatite" preparation (AP) → Ca₁₀(PO₄)₆(OH)₂ + Ca₃(PO₄)₂
Ca-deficient AP >900°C HA β-TCP
\[
\begin{align*}
\beta\text{-TCP} & \rightarrow \alpha\text{-TCP} \\
>1100^\circ\text{C} \\
\end{align*}
\]

\[
\begin{align*}
\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 & \rightarrow 2\text{Ca}_3(\text{PO}_4)_2 + \text{Ca}_4(\text{PO}_4)_2\text{O} \\
\text{HA} & >1300^\circ\text{C} \quad \alpha\text{-TCP} \quad \text{TTCP} \\
\end{align*}
\]

\[
\begin{align*}
\text{Ca}_3(\text{PO}_4)_2 + \text{CaO} & \rightarrow \text{Ca}_4(\text{PO}_4)_2\text{O} \\
\beta\text{-TCP} & >1400^\circ\text{C} \quad \text{TTCP} \\
\end{align*}
\]

The reactions illustrated above are some examples of how HA and other calcium phosphates are formed. The sintering temperatures usually range from 900°C to >1400°C. Calcium phosphates such as β-TCP, can form simply by sintering an apatite to above 900°C whereby decomposition of HA to β-TCP occurs. Above 1100°C inversion of the β-TCP phase to α-TCP takes place.53

The formation of α-TCP and TTCP is yielded when HA is heated to above 1300°C. However, TTCP can also form above 1400°C from β-TCP in the presence of CaO. The presence of HA and CaO above a sintering temperature of 900°C may be attributed to the conditions in which the apatite is prepared, for instance an apatite prepared from a highly alkali solution in the presence of air.53
"Apatite" preparation (AP) → $\text{Ca}_{10}(\text{PO}_4)\text{O}_6(\text{OH})_2$ + CaHPO$_4$

$>900^\circ\text{C}$ HA

CaHPO$_4$ → $\beta$-TCP + Ca$_2$P$_2$O$_7$

$>900^\circ\text{C}$

Dicalcium phosphate anhydrous, CaHPO$_4$, has been reported to form upon sintering apatite above 900°C, however, at these temperatures it is known to be unstable and transforms to $\beta$-TCP and calcium pyrophosphate, Ca$_2$P$_2$O$_7$. In order to minimise the formation of other phases such as $\beta$-TCP and $\alpha$-TCP, TTCP, sintering can be performed under a water vapour pressure of about 500mmHg therefore making HA the more stable phase.$^{53}$

Taking into consideration the reactions discussed, the final composition of dense HA after sintering appears to depend on the sintering temperature, the conditions and Ca/P molar ratio of the apatite preparation prior to sintering.$^{53}$

Characterisation of HA can be carried out using x-ray diffraction. This technique can give an idea of the other phases present in the sintered material, such as $\beta$-TCP, $\alpha$-TCP, TTCP and CaO, in addition, it can also be used to determine the purity and crystallinity of the HA phase.$^{4,53,66}$ Furthermore, unit cell parameters of HA and other phases can also be ascertained through XRD (Figure 10).
The crystallographic properties of powdered HA can be reflected by the intensities and broadening of the peaks in the X-ray pattern. High intensity peaks with small line broadening is characteristic of dense HA powder and is indicative of a highly crystalline material.\textsuperscript{53}

2.4.4 Crystallographic properties

X-Ray diffraction can provide information concerning lattice parameters such as a and c dimensions of a unit cell.\textsuperscript{53} In the case of HA these were found to be 9.422 and 6.881Å for the a and c dimensions respectively. This application can also give the proportion of other phases present with the HA phase, such as $\beta$ and $\alpha$-TCP, TTCP and CaO.\textsuperscript{4,53,66} Infra-red absorption is a technique that can detect vibrational modes corresponding to the energy of a
particular bond such as $\text{OH}^-$ or $\text{PO}_4^{3-}$, and can distinguish between these bonds through peaks that correspond to a characteristic wavenumber.

In physical terms ceramic HA crystals adopt large rhombic shapes that resemble human enamel when sintered at 950°C and are different to smaller acicular apatite crystals before sintering. The presence of lattice defects in the crystal structure of ceramic HA sintered at lower temperatures are known to induce a biological response in vivo compared to those sintered at higher temperatures. This response is related to the type and amount of defects residing in the material. At 950°C HA contains defects that are hexagonal parallelepiped in shape, as well as other types of defects, and are more predominant than those present at 1250°C. It is therefore expected that a material with a greater number of lattice defects would be more reactive.

2.4.5 Infrared

Infrared spectroscopy is a technique that can often be used to compliment x-ray diffraction in the characterisation of hydroxyapatite. The shape and intensity of the absorption bands that arise from the absorption of infrared light by molecules can give a good indication of the crystallinity of the material.

Like x-ray diffraction, infrared can be used to detect additional phases in which modes of vibrations belonging to functional groups inherent in the crystal structure undergo changes in peak shape, intensity and may often involve shifts in vibrational frequency.
The main modes of vibration that hydroxyapatite exhibits are assigned to the hydroxyl stretching mode (~3570 cm\(^{-1}\)), the carbonate \(v_3\) mode (~1648 cm\(^{-1}\)) and the phosphate \(v_3\) modes (~1092 and 1042 cm\(^{-1}\)). Additional infrared modes related to these functional groups are given in Table 6 and are assigned to bending and/or deformation modes.\(^{67-70}\)

Hydroxyapatite can undergo decomposition to secondary phases \(\beta\)-TCP and \(\alpha\)-TCP. This can be detected by changes in peak height and shape of the phosphate \(v_3\) modes accompanied by the loss in intensity of the hydroxyl stretching band.

Infrared spectroscopy can also indicate the presence of substituent ions such as carbonate (\(\text{CO}_3^{2-}\)) and fluoride (\(\text{F}^-\)).\(^{34,68,71}\) It is commonly known that \(\text{CO}_3^{2-}\) substitution can occur either as type A substitution in which \(\text{CO}_3^{2-}\) ions substitute for \(\text{OH}^-\) groups, or type B substitution in which they substitute for \(\text{PO}_4^{3-}\) groups. In the former one is expected to observe an increase in intensity for the vibrational modes related to \(\text{CO}_3^{2-}\) ions in the hydroxyl sites accompanied by a decrease in intensity for the hydroxyl stretch. In the latter one would expect an increase in the vibrational modes related to the \(\text{CO}_3^{2-}\) ions in the phosphate sites accompanied by a decrease in intensity of the phosphate \(v_3\) modes.
<table>
<thead>
<tr>
<th>Peak assignments</th>
<th>Wavenumber (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyl stretch</td>
<td>3568</td>
</tr>
<tr>
<td>Carbonate (v_3)</td>
<td>1650-1300</td>
</tr>
<tr>
<td>-(m: medium intensity)</td>
<td>1648</td>
</tr>
<tr>
<td>-(m)</td>
<td>1454</td>
</tr>
<tr>
<td>-(m)</td>
<td>1419</td>
</tr>
<tr>
<td>-(w: weak intensity)</td>
<td>-</td>
</tr>
<tr>
<td>Phosphate (v_3)</td>
<td>1190-976</td>
</tr>
<tr>
<td>-(vs: very strong intensity)</td>
<td>1092</td>
</tr>
<tr>
<td>-(vs)</td>
<td>1042</td>
</tr>
<tr>
<td>Phosphate (v_1) (m)</td>
<td>962</td>
</tr>
<tr>
<td>Carbonate (v_2) (ms: medium strong)</td>
<td>874</td>
</tr>
<tr>
<td>Phosphate (v_4)</td>
<td>660-520</td>
</tr>
<tr>
<td>-(m)</td>
<td>633</td>
</tr>
<tr>
<td>-(vs)</td>
<td>602</td>
</tr>
<tr>
<td>-(vs)</td>
<td>566</td>
</tr>
<tr>
<td>Phosphate (v_2) (w)</td>
<td>472</td>
</tr>
</tbody>
</table>

Table 6. Infrared peak assignments of hydroxyapatite.\(^{68}\)
2.4.6 Mechanical properties

There are a number of mechanical properties associated with dense HA ceramic that are influenced by certain factors, namely the properties of the apatite powder and the compression and sintering conditions. In general, an increase was reported for the following properties for an increase in sintering temperature from 1150°C to 1350°C; density, grain size, compressive, flexural, torsional and dynamic torsional strengths, and moduli of elasticity in compression and bending.\(^3\,\text{53,72-74}\) Micro-porosity, which is related to density, has an adverse effect on the mechanical properties of dense HA.

The fracture toughness values were also reported and these exhibited an increase when the material was sintered from 1100 to 1150°C, however, there was no significant change between 1150 to 1250°C. Further sintering of the HA ceramic beyond 1250°C gave lower fracture toughness values than HA sintered at 1100°C. A factor which can also cause this decrease in fracture toughness apart from the sintering conditions is the presence of β-TCP within the material. The preparation of the apatite powder can also play an important role in influencing the outcome of the mechanical properties.\(^75,76,77\) Different types of preparation methods can yield powders with varying grain size and composition and has proven to influence fracture toughness. A powder with a small grain size would usually result in the material being more resistant to fracture.

Testing carried out under wet and dry conditions were also reported. These showed a decrease in the flexural strength and fracture toughness in dry conditions compared to wet conditions. Fatigue measurements proved that HA ceramic is an unsuitable material for
implantation, due to its low load bearing capacity and is reflected by the Weibull factor (n=12) obtained under wet conditions (wet condition-condition that simulates the physiological environment).^{53}

<table>
<thead>
<tr>
<th>Material</th>
<th>Strength MPa</th>
<th>Test Method</th>
<th>Fracture Toughness MPa m$^{1/2}$</th>
<th>Young’s Modulus GPa</th>
<th>Hardness Hv</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical bone</td>
<td>50 - 150</td>
<td>Bending</td>
<td>2-12</td>
<td>7-30</td>
<td>-</td>
<td>Hench and Thompson$^{82}$</td>
</tr>
<tr>
<td>Cortical bone</td>
<td>130±14</td>
<td>Tensile</td>
<td>-</td>
<td>17.6±2.0</td>
<td>-</td>
<td>Vincentelli and Grigorov$^{19}$</td>
</tr>
<tr>
<td>Primary bone</td>
<td>161±11</td>
<td>Tensile</td>
<td>-</td>
<td>19.4±2.4</td>
<td>-</td>
<td>Vincentelli and Grigorov$^{19}$</td>
</tr>
<tr>
<td>Cancellous bone</td>
<td>10 - 20</td>
<td>Bending</td>
<td>0.1</td>
<td>0.5 - 0.05</td>
<td>-</td>
<td>Hench and Thompson$^{82}$</td>
</tr>
<tr>
<td>Commercial HA</td>
<td>39 - 43</td>
<td>Tensile</td>
<td>-</td>
<td>11 - 13</td>
<td>450</td>
<td>Denissen et al.$^{100}$</td>
</tr>
<tr>
<td>Precipitated HA</td>
<td>38 – 42</td>
<td>Tensile</td>
<td>-</td>
<td>11 - 13</td>
<td>450</td>
<td>Denissen et al.$^{100}$</td>
</tr>
<tr>
<td>HA - 2.5wt% Bioglass ®</td>
<td>30 - 60</td>
<td>Ring on ring Biaxial Flexure</td>
<td>1.5 - 1.7</td>
<td>89 - 109</td>
<td>-</td>
<td>Santos$^{99}$</td>
</tr>
<tr>
<td>(1200 - 1350°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA - 2.5wt% Phosphate Glass a</td>
<td>36 - 55</td>
<td>Ring on ring Biaxial Flexure</td>
<td>1.1 - 1.5</td>
<td>87 - 113</td>
<td>-</td>
<td>Santos$^{99}$</td>
</tr>
<tr>
<td>(1200 - 1350°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceravital®</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1000 - 150</td>
<td>-</td>
<td>Hench and Thompson$^{82}$</td>
</tr>
<tr>
<td>Cerabone A-W®</td>
<td>680</td>
<td>Bending</td>
<td>2.0</td>
<td>118</td>
<td>680</td>
<td>Hench and Thompson$^{82}$</td>
</tr>
<tr>
<td>HA-2% Phosphate glass b</td>
<td>103 ± 14</td>
<td>Ring on ring Biaxial Flexure</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Knowles et al.$^{137}$</td>
</tr>
</tbody>
</table>

\(^{a}\) Phosphate glass: CaO - 28.21mol%; P_2O_5 - 44.77mol%; Na_2O - 27.02mol%

\(^{b}\) Phosphate glass: Na_2O - 31mol%; P_2O_5 - 45mol%; CaO - 24mol%

Table 7. Mechanical properties of bone and other ceramic materials.

74
Recently developed glass ceramic and glass reinforced hydroxyapatite composites have generally shown an improvement in the mechanical properties compared to hydroxyapatite ceramic. With regards to strength and fracture toughness it is clear that the results obtained display values approaching that of cortical bone (Table 7).

However, the need to develop implant materials with greater strength has resulted in materials with considerably greater Young's modulus values compared to bone. One problem that this may present during implantation is stress shielding, which commonly occurs as a result of a deformation mismatch, i.e. a difference in Young's modulus, between the implant and bone during loading and may cause problems at the bone-implant interface.\textsuperscript{99}

An important aspect of biomaterials is to determine whether or not an implant material is mechanically suitable under physiological conditions. Therefore it is essential to examine the influence of physiological fluids on the strength of an implant.

Work carried out by Thomas et al. showed how the diametral compressive strength of pretreated dense hydroxyapatite displayed little change compared to non-treated hydroxyapatite (Table 8).\textsuperscript{138}
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average strength / MN.m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>117</td>
</tr>
<tr>
<td>Water</td>
<td>108</td>
</tr>
<tr>
<td>Saliva</td>
<td>110</td>
</tr>
<tr>
<td>PECF</td>
<td>116</td>
</tr>
<tr>
<td>Implanted</td>
<td>111</td>
</tr>
<tr>
<td>Autoclaved</td>
<td>112</td>
</tr>
</tbody>
</table>

Table 8. Strength of treated and non-treated hydroxyapatite.\textsuperscript{138}

Santos also examined the effect of immersing hydroxyapatite and HA-glass composites for 84 days in physiological saline solution and de-ionised water. The results showed little change in biaxial strength when both conditions were compared (Table 9).\textsuperscript{99}

<table>
<thead>
<tr>
<th>Material</th>
<th>Water</th>
<th>Physiological solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>26±6</td>
<td>29±7</td>
</tr>
<tr>
<td>HA-3Oxide</td>
<td>63±15</td>
<td>65±17</td>
</tr>
<tr>
<td>HA-Bioglass®</td>
<td>58±16</td>
<td>61±14</td>
</tr>
</tbody>
</table>

Table 9. Biaxial strength of hydroxyapatite immersed for 84 days in water and physiological solution.\textsuperscript{99}

However, De With et al. demonstrated that under wet conditions (distilled water) the bending strength values showed a 75% drop compared to their dry values.\textsuperscript{139}
Further work carried out by Thomas et al. looked at the fatigue properties of dense hydroxyapatite. Static fatigue measurements made on untreated hydroxyapatite samples with an applied stress of 100MN.m$^{-2}$ gave an average failure time of about 7000min. This preliminary study demonstrated that hydroxyapatite gave relatively strong fatigue resistance compared to other ceramics such as alumina.

Work carried out by De With et al. measured the strain-rate dependence of strength for hydroxyapatite ceramic using four cross head speeds (Table 10). However, De With concluded that hydroxyapatite displayed substantial subcritical crack growth under dry conditions and this became more pronounced under wet conditions.

According to De With, the subcritical crack growth which is shown to occur in hydroxyapatite as a result of loading at long durations makes this material mechanically unsuitable as an implant.

<table>
<thead>
<tr>
<th>$v$ (μm sec$^{-1}$)</th>
<th>Dry $\sigma_f$ (MN.m$^{-2}$)</th>
<th>Wet $\sigma_f$ (MN.m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.97 \times 10^1$</td>
<td>96</td>
<td>66</td>
</tr>
<tr>
<td>1.75</td>
<td>94</td>
<td>69</td>
</tr>
<tr>
<td>$2.04 \times 10^1$</td>
<td>101</td>
<td>82</td>
</tr>
<tr>
<td>$1.80 \times 10^2$</td>
<td>124</td>
<td>115</td>
</tr>
</tbody>
</table>

Table 10. Strain-rate dependence of strength for hydroxyapatite ceramic using four cross head speeds under dry and wet conditions.
2.4.7 Surface chemistry

The dissolution properties of dense HA ceramic is important in determining the surface chemistry of the material in relation to biological interactions to the HA implant. When the HA ceramic is subjected to an acid environment partial dissolution occurs on the surface forming a hydrated layer consisting of a number of mineral ions and this behaviour is mirrored by the interaction of biological apatites with biological fluids. This suggests that the composition of the ceramic and the pH of the solution influences the surface chemistry, since a more acidic environment will cause greater dissolution on the ceramic HA surface. Ca$^{2+}$, H$_2$PO$_4^-$, HPO$_4^{2-}$, PO$_4^{3-}$, H$^+$, OH$^-$ and ion pairs CaH$_2$PO$_4^+$ and CaOH$^+$ are the type of species that exist in the hydrated layer.$^{53}$

Surface charges on the HA implant evolve as a result of the electrolytes that exist within biological fluids. Cellular activities involved in bone regeneration on the bone-implant interface are influenced by these surface charges. Surface charges can be expressed by the absolute values of zeta-potential and it has been suggested that differences in these values found for unsintered apatite, Ca-deficient apatite, ceramic HA and stoichiometric HA accounts for the variation in cellular activity on the surface of each material.$^{53}$

2.4.8 Dissolution/precipitation

Observations made on the surface of ceramic HA after implantation in bony sites indicate the presence of a precipitated layer of microcrystals that are characterized as apatites. IR analysis confirmed these to be crystals of carbonate apatite, similarly, these crystals can be found in bone apatite and are closely linked with the organic matrix.$^{53}$
The dissolution-precipitation process involves the release of $\text{Ca}^{2+}$, $\text{HPO}_4^{2-}$ and $\text{PO}_4^{3-}$ ions from the surface of the material through partial dissolution of HA brought about by cellular activity in an acidic environment. There are three main pathways in which carbonate apatite can form:

- Hydrolysis of calcium phosphates (DCPD, OCP, magnesium substituted $\beta$-TCP) by $\text{CO}_3^{2-}$ ions in biological fluid
- Direct formation of carbonate apatite from ions released from partially dissolving ceramic HA ($\text{Ca}^{2+}$, $\text{HPO}_4^{2-}$ and $\text{PO}_4^{3-}$) and biological fluids ($\text{CO}_3^{2-}$ and $\text{Mg}^{2+}$).
- Uptake of calcium ions from serum results in precipitation of carbonate apatite.

It has been shown that the dissolution-precipitation of biphasic materials such as biphasic calcium phosphate is influenced by the ratio of $\beta$-TCP to HA. The $\text{CO}_3$-apatite crystals tended to be more abundant on the surface of the biphasic calcium phosphate with higher $\beta$-TCP/HA ratio. This is due to the greater dissolution of the $\beta$-TCP component of the biphasic material to calcium and phosphate ions.

2.4.9 Tissue response

The biocompatibility of the HA surface allows the cell proliferation and cell attachment of a variety of cell types, these include macrophages, fibroblasts, osteoclasts, osteoblasts and periodontal ligament cells. Dissolution of HA crystals from the surface of the ceramic occurs as a result of cellular interactions. This process is carried out in two ways:
• Intracellularly: Phagocytosis
• Extracellularly: Producing an acid environment for dissolution

Bone cells that attach and proliferate on HA and on bone surfaces do not appear to differentiate between the two surfaces. This suggests that the surface chemistry of HA and bone exhibit similarities.\textsuperscript{53}

The ability of dense HA to promote the attachment and proliferation of matrix-producing bone cells on a CO\textsubscript{3}-apatite surface is indicative of a material which provides the right surface chemistry and surface charges, and in many respects is considered osteoconductive in nature.\textsuperscript{53}
2.5 *Phosphate based glasses.*

Phosphate based glasses can be used for a variety of applications such as glass metal seals, low temperature enamels for metals and optical elements. The variety of applications in which phosphate based glasses feature is owed mainly to their workability. Phosphate based glasses generally have low melting points, low glass transition temperatures and low softening temperatures. However, these glasses tend to have a range of solubility in which the structure of the glass is known to have an influence (Figure 11). This consequently means that they can be applied as materials with low durability.\textsuperscript{78-81}

![Figure 11. Structure of a phosphate based glass.](image)
A variety of glasses with a range in solubility have been developed in order to assess their biocompatibility. It has been reported that too high a solubility is detrimental to cell activity. It was therefore important to find the right glass composition that increases cellular activity. A fixed P\textsubscript{2}O\textsubscript{5} content of 45mol% was found to give a good range of glasses that exhibited good biocompatibility \textit{in vitro}. In addition, the compositions that were formulated with this P\textsubscript{2}O\textsubscript{5} content made them easier to melt and cast.

It has long been established that the solubility is a limiting factor in relation to biocompatibility, however, these glasses can serve as a means of ion release and can be of great benefit as an implant material for hard tissue surgery. Due to the chemical similarity between these materials and bone it is possible through the release of appropriate ions from the glass to regenerate new bone. Potentially, phosphate based glasses can behave as resorbable implant materials that can provide the controlled release of ions into the surrounding tissue which cells require to lay down a new hard tissue matrix that subsequently replaces the glass implant. By experimenting with different glass compositions it is possible to develop an implant material with a solubility that can meet the specific requirements of a hard or soft tissue replacement.

So, bearing in mind the solubility, low melting temperature, low viscosity at very high temperatures and chemical affinity with bone, it can therefore be made possible to incorporate these glasses into HA to produce HA/glass composites. The low viscosity of glasses at high temperatures and low melting temperatures means that they can act as a sintering aid. Their solubility allows the dissolution of ions into the HA matrix which can influence the microstructure and any subsequent phase changes that might occur, and in
addition allows the leaching out of trace elements which may originate from the glass onto the material surface. Hence, the properties mentioned can serve two purposes; one, to improve mechanical properties; two, to improve biocompatibility.
Glass reinforced hydroxyapatite composites (GR-HA) have recently been developed for potential application as implant materials because they are known to have a chemical similarity with living hard tissues. A GR-HA composite can be formulated through the addition of a glass, such as a phosphate-based glass, to HA, and can therefore serve as a means of improving the biocompatibility and strength of the HA. The preparation of glass reinforced hydroxyapatite involves small additions of a glass ceramic powder to HA powder during the milling process. This is then followed by sieving to produce a free flowing powder, pressing and then sintering to form ceramic discs.

GR-HA’s are not only of benefit biologically, but also mechanically. The sintering of HA with the addition of a glass, which can act as a sintering aid, is known to enhance densification and subsequently greatly improve the mechanical strength. The enhanced mechanical properties that GR-HA’s generally exhibit can be explained by the mechanism of sintering that these materials undergo. Liquid-phase sintering, as it is commonly known, is a mechanism that is driven by the presence of a glassy phase, this generates a liquid between the channels of HA particles during sintering thus providing sufficient capillary pressure and surface tension to allow the rearrangement of particles in such a way so as to optimise packing.

Sintering of HA powder in the presence of a glass is known to promote decomposition of the HA phase to secondary phases, such as β-TCP and α-TCP. The extent of
decomposition is dependent on the constituent components present in the glass, the composition of the components present and also the weight addition of the glass powder relative to the HA powder.

Phosphate based glasses for example, can comprise a number of different systems each differing in chemical composition. Components that make up these glasses can include Na₂O, P₂O₅, CaO, MgO, SiO₂, CaF₂, Al₂O₃ etc., in which some components can be formed from the breakdown of precursor materials.⁴⁶

The release of appropriate ions, such as calcium, from an implant material can provide the right chemical environment in which hard tissue cells can regenerate. So potentially, calcium ions can be introduced to a composite in order to improve the biocompatibility and possibly the bioactivity of the material. However, the ratio of CaO to P₂O₅ in a CaO-P₂O₅ glass system, for instance, is an important factor in determining the stability of the HA phase. It has been found that a high Ca/P ratio in a glass causes less decomposition of the HA phase to secondary phases.⁴

A glass composition that contains sodium for instance was found to promote a greater decomposition of HA to secondary phases. Even though the mechanical properties of the GR-HA composite were found to be greater than HA alone they did not altogether prove to be better than soda-free glass composites.⁷⁴

This demonstrates how the mechanical properties depend on the components of the glass, the composition and the subsequent degradation of the HA phase.
Studies have been made that can demonstrate the effects of secondary phase content on the mechanical properties of GR-HA composites (Figure 12). Flexural bend strength measurements that have been carried out for two GR-HA composites, in which one contained a phosphate based glass (composition: Na$_2$O at 31mol%; CaO at 24mol%; P$_2$O$_5$ at 45mol%) at a 2% weight glass addition and the other at 4%, showed a significant difference in values at 1350°C. The results showed that for the GR-HA with a 4 weight% glass addition there was a significant drop in flexural strength at this temperature. The results suggest that this drop was mainly due to the presence of large amounts of α-TCP at this temperature. This, however, did not occur for the GR-HA with a 2% weight addition of glass at this temperature. So at higher sintering temperatures the amount of glass added can have a significant effect on the secondary phase content and consequently on the mechanical properties.

![Figure 12. Flexural bend strength of two GR-HA’s.](image)

Since the flexural strength values for GR-HA’s at both additions showed similar values between 1200 and 1300°C, even though GR-HA with 4% glass gave secondary phases at
these temperatures, suggests that sintering effects were a more predominant factor for the improvement in flexural strength compared to HA. This effect can be shown by the linear shrinkage, which indicates that the inclusion of glass helps to eliminate porosity (Figure 13). The authors also reported that the enhanced mechanical properties exhibited for the GR-HA’s was also due to the inhibition of grain growth as a result of adding the glass. This was shown by the grain size measurements. However, differences in grain size at 1350°C were evident particularly between the GR-HA composites, whereby an increase in grain size was seen for the GR-HA at 4% glass addition. This correlated with the drop in flexural strength at this temperature. The authors concluded that the level of glass addition and also the firing temperature were important factors that influenced the grain size.

![Figure 13. Linear shrinkage of two GR-HA’s.](image)

The microstructure of materials are known to have an influence on the outcome on the hardness and fracture toughness. Results that have been presented by some authors suggest that a combination of residual porosity and secondary tricalcium phosphate phases play a
role in improving the microstructural characteristics of the material and consequently the fracture toughness and hardness.\textsuperscript{87}

The improvement in fracture toughness for the GR-HA composites in part was attributed to the presence of $\beta$-TCP, which is known to be tougher than HA. This was demonstrated by the large increase in fracture toughness values shown for a GR-HA composite at 1300 and 1350°C and correlated with the presence of large amounts of $\beta$-TCP compared to the HA and the other composites at these temperatures.\textsuperscript{87}

The hardness results presented showed that a significant improvement was obtained for a composite between 1250 and 1300°C, where at 1250°C a value well below that of HA was given and at 1300°C the value recorded was significantly higher than HA.\textsuperscript{87} This indicated that the elimination of porosity played a role in improving the hardness of GR-HA composites. However, the porosity of HA and GR-HA composites were similar at these sintering temperatures and so the increase in hardness exhibited for GR-HA composites could also be attributed to the presence of the $\beta$-TCP phase.

X-ray diffraction studies have been carried out investigating the effects of ionic substitutions on the structural properties of HA at the atomic level, this was characterised using Rietveld analysis. In previous studies attempts have been made to introduced ions such as magnesium into the HA lattice via sintering HA in the presence of Mg\textsuperscript{2+}, however, the results suggested that the ions were incorporated into the resulting higher temperature $\beta$-TCP phase and not into the HA structure.\textsuperscript{31}
By contrast, the processing route used to produce GR-HA’s has been investigated and makes use of liquid phase sintering as a method of substituting magnesium ions into the HA crystal lattice via the incorporation of phosphate glasses in the green compact. The results suggested that there were changes to the HA lattice as result of liquid phase sintering. These were ascribed to hydroxyl changes as a result of substituting Ca atoms for Mg ions in the lattice. Changes were measured for distortion, hydroxyl ion occupancies, c and a unit cell dimensions, Ca-OH bond lengths and hydroxyl channel radius.

In addition, the authors suggested that the Mg ions probably had a more significant effect on the β-TCP phase, in particular the stabilisation of the β-TCP to some extent. The results that were presented showed that as the Mg content in the glass increased, the amount of β-TCP formed increased. The stabilisation of the β-TCP phase was ascribed to the substitution of Ca by a smaller Mg ion, and also at an atom position of higher coordination. This substitution is believed to occur because the c and a-axis dimensions were shown to contract in relation to pure β-TCP. However, the results showed that the β-TCP phase was unstable at high temperatures and readily converted to α-TCP. The authors suggested that the changes measured in the structure of the β-TCP phase was probably due to localised Mg concentrations.

The formation of an apatite layer on the surface of GR-HA composites is a characteristic commonly exhibited by these materials when evaluated in vitro. Test carried out showed that on immersion into Hank’s Balanced Salt Solution (HBSS) for periods up to 1, 2, 3 and 4 weeks at 37°C in a nonagitated condition the GR-HA’s formed a needlelike apatite layer.
that covered the whole surface of the material. The extent of apatite layer formation is controlled by the amount of glass addition and also the composition of the glass. These can negate solubility and degradation of the materials, since the solubility is believed to be associated with the presence of β-TCP in the microstructure. The authors reported that the leaching out of Ca\(^{2+}\) and PO\(_4^{3-}\) from the samples could provide an explanation for the formation of the apatite layer. It has been suggested that the mechanism involved an increase in the degree of supersaturation of the surrounding body fluid as a result of these ions leaching out which therefore accelerated the apatite formation. The results generally showed that these materials grew a thicker layer of apatite with respect to immersion time and that the apatite which was formed was calcium deficient. With all these factors taken into consideration it can be said that the GR-HA’s tested showed bioactive behaviour.
Development of Glass Reinforced Hydroxyapatite for Hard Tissue Surgery

George Georgiou

Table 11. Mechanical properties of HA and HA-glass ceramics.

<table>
<thead>
<tr>
<th>Material</th>
<th>Strength MPa</th>
<th>Test Method</th>
<th>Fracture Toughness MPa m$^{1/2}$</th>
<th>Young’s Modulus GPa</th>
<th>Hardness Hv</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>31.4</td>
<td>Ring on ring Biaxial Flexure</td>
<td>0.74 ± 0.2</td>
<td>-</td>
<td>525 ± 18</td>
<td>Tancred et al.</td>
</tr>
<tr>
<td>HA</td>
<td>28 ± 7</td>
<td>Ring on ring Biaxial Flexure</td>
<td>0.57 ± 0.3</td>
<td>89 ± 9</td>
<td>513 ± 52</td>
<td>Santos</td>
</tr>
<tr>
<td>Commercial HA</td>
<td>39 - 43</td>
<td>Tensile</td>
<td>-</td>
<td>11-13</td>
<td>450</td>
<td>Denissen et al.</td>
</tr>
<tr>
<td>Precipitated HA</td>
<td>38 - 42</td>
<td>Tensile</td>
<td>-</td>
<td>11-13</td>
<td>450</td>
<td>Denissen et al.</td>
</tr>
<tr>
<td>Precipitated HA</td>
<td>65 ± 12</td>
<td>4-pt Bending</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Puanjindanetr</td>
</tr>
<tr>
<td>HA-2.5wt% Bioglass®</td>
<td>30-60</td>
<td>Ring on ring Biaxial Flexure</td>
<td>1.5 - 1.7</td>
<td>89 - 109</td>
<td>-</td>
<td>Santos</td>
</tr>
<tr>
<td>HA-5wt% Phosphate glass*</td>
<td>69 ± 21</td>
<td>Ring on ring Biaxial Flexure</td>
<td>1.5 ± 0.2</td>
<td>122 ± 15</td>
<td>-</td>
<td>Santos</td>
</tr>
<tr>
<td>HA-2% Phosphate glass*</td>
<td>103 ± 14</td>
<td>Ring on ring Biaxial Flexure</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Knowles et al.</td>
</tr>
</tbody>
</table>

* Phosphate glass: Na$_2$O – 27.02mol%; P$_2$O$_5$ – 44.77mol%; CaO – 28.21mol%

Previous research carried out shows that the addition of glass to hydroxyapatite enhances the mechanical properties of the material in terms of its strength, Young’s modulus and fracture toughness, and these are summarised in the above table (Table 11).
2.7 Rheology

Ceramic materials for application as implants for bone regeneration purposes can come in various forms. As well as dense blocks, ceramics such as hydroxyapatite can be processed to form porous blocks. This can be done through reticulated foam technology whereby HA slips are used to coat polyurethane reticulated foams and fired to leave porous HA. The optimisation of the HA slip for coating requires a basic understanding of the rheological properties of the ceramic. The main discussion of this section will focus on the various aspects of rheology.89

Rheology is thought of as the study of flow and deformation of matter. The principles involved can be explained by the behaviour of fluid flow and its relationship with viscosity.90

2.7.1 Viscosity

The viscosity of a fluid is a measure of its resistance to flow.91 A fluid is considered a substance consisting of a number of different layers sliding past each other at different velocities during flow. Essentially the viscosity of a particular fluid is a measure of the internal friction or shear between two adjacent component layers.

A common example of the variation in velocities of layers can be described by the flow of a viscous fluid through a pipe. Layers flowing close to the pipe wall will tend to move slower than those at the centre, which move the fastest. This is due to the friction between the wall and the fluid near the wall.
A simple profile can be given to illustrate differences in velocities of a fluid across the pipe (Figure 14).\textsuperscript{91}

![Velocity profile across the pipe](image)

**Figure 14.** Fluid flow across a pipe.\textsuperscript{91}

Viscosity is attributed to molecular motion, which can be described as the transfer of molecules from layers of varying velocities, which as a consequence causes a transfer of momentum between layers. This is the reason why the frictional force between layers occurs.

The difference in velocities across the pipe radius, therefore, can be expressed as the velocity gradient $\frac{dv}{dr}$ where $\frac{d}{dr}$ refers to the change per unit distance.

The tangential stress, $T$, can be described as the viscous force acting per unit area along the boundary of two adjacent fluid layers (Figure 15).\textsuperscript{91}
Figure 15. Diagram illustrating the viscous force acting along the boundary of adjacent layers.\textsuperscript{91}

In a Newtonian fluid the velocity gradient at any point in the fluid across the pipe is proportional to the tangential stress and can be expressed by the following equation:\textsuperscript{92}

\[ T = \frac{F}{A} = \eta \frac{dv}{dr} \]  
\textbf{Equation 4}

Where
- \( T \) : Tangential stress (N.m\(^{-2}\))
- \( \eta \) : Coefficient of viscosity (N.s.m\(^{-2}\)) (constant of proportionality).
- \( \frac{dv}{dr} \) : Velocity gradient (s\(^{-1}\))

The Newtonian relationship between stress and velocity gradient does not hold true for the majority of ceramic materials in suspension. In fact at constant temperature most suspensions show a curve for shear rate against shear stress. This consequently gives a value of \( \eta \) that is not constant but varies as a function of shear rate. Such suspensions can be considered non-Newtonian. Paint for example becomes less viscous the more it is stirred.\textsuperscript{92}
2.7.2 Pseudo-plasticity, Bingham plasticity and dilatancy

Examples of non-Newtonian behaviour are pseudo-plasticity, Bingham plasticity and dilatancy.

Pseudo-plasticity describes systems that give flow curves that pass through the origin and are similar to those expressed for plastics. The difference between pseudo-plasticity and plastic flow is that plastic flow gives a yield value that is indicated by a minimum stress value that intersects the stress axis (Figure 16). A yield value can also be given for suspensions, in particular flocculated suspensions that obey Bingham’s law, however, the shear-stress graph in this case is linear and can be expressed as:

\[ f - f' = \eta_0 D \]  

\textbf{Equation 5}

Where

- \( \eta_0 \) : Constant of absolute viscosity
- \( f' \) : Yield value (N.m\(^{-2}\))
- \( D \) : Shear rate (N.m\(^{2}\).s\(^{-1}\))

Where \( \eta_0 \) is the constant of absolute viscosity and \( f' \) is given as the yield value.

Flocculated systems are commonly known to exhibit this type of behaviour.
Dilatancy is the term that describes systems that have a tendency to increase in viscosity as a function of shear rate and where no yield is given.\(^9^2\)

Dilatancy, although not fully understood, might be attributed to the ‘squeezing out’ of water. This phenomenon is most likely to occur at parts of the suspension that experience the greatest shear and is commonly associated with suspensions containing coarse particles. Alternatively, dilatancy might occur through the formation of large voids when a suspension undergoes shearing. This consequently makes it difficult for water to sufficiently fill these voids and hence might explain why viscosity increases with increasing shear.\(^9^2\)
2.7.3 Thixotropy

Thixotropy is a term that refers to suspensions that break up to form loose structures of low viscosity as shear rate increases, but have a tendency to thicken and increase in viscosity if left unstirred. In many respects this behaviour resembles a hysteresis curve when shear rate is plotted against stress and so thixotropy can be considered a reversible, time dependent property.

The thixotropic property of a suspension can be measured stepwise. Since thixotropy is time dependent, a selection of shear rates can be used with the resulting stress measured 10 seconds after shearing is stopped. This process is carried out from the smallest shear rate in increasing steps to a maximum practical value and then repeated with decreasing shear rates.

Before a suspension is stirred the particles are built up in a kind of three-dimensional network consisting of particle linkages, and accounts for the yield value given when a suspension is initially stirred. Furthermore, the break up of the network structure requires a measurable time. This is indicated by the greater stress needed for this to occur with respect to ‘equilibrium stress’ and suggests this process is not instantaneous. For a decrease in shear, what is usually observed is a downward curve that follows the ‘equilibrium stress’ curve, however, is slightly less in stress which indicates that the structure is attempting to build up rather than remaining broken down (Figure 17).
2.7.4 Measurement of viscosity

There are a number of methods for measuring viscosity, the simplest of which is modelled around determining the rate at which a liquid flows through a tube, with the application of constant pressure.\(^91\)

A relationship between flow and viscosity was given by Poiseuille's equation and can be expressed as:

\[
\eta = \frac{\pi PR^4}{8LQ} \quad \text{Equation 6}
\]

\[
Q = \frac{v}{t} = \frac{\pi PR^4}{8L\eta} \quad \text{Equation 7}
\]

Where

- \(\eta\) : Viscosity
- \(P\) : Applied pressure
- \(R\) : Radius of cross section
- \(L\) : Length of tube
- \(Q\) : \((v / t)\) Rate of flow – volume per second
The rate of flow of a fluid in this model suggests a dependency on applied pressure, tube length, radius of cross section and viscosity. Changing the rate of flow requires a change in pressure or change of tube length. By recording the volume (V) of fluid passing through the tube over time (t), and knowing the pressure applied, radius and length of tube, the viscosity (\(\eta\)) can therefore be determined.

The rotating-cylinder viscometer consists of two concentric cylinders that are immersed in a liquid. The outer cylinder is driven by an electric motor that creates a viscous drag in the liquid as a result of its angular velocity. This in turn exerts a torque on the inner cylinder which is suspended from a light spring and therefore is able to rotate through an angle of twist \(\theta\). A pointer, a component of the inner cylinder, records the angle of twist, which may then be calculated as stress or viscosity. An equation for stress and shear can be given in terms of the parameters associated with this model to calculate the viscosity.

\[
\text{Stress} \quad f = \frac{k\theta}{2\pi R_1^2 L} \quad \text{Equation 8}
\]

\[
\text{Shear rate} \quad D = \frac{2\pi R_1^2 R_2^2 \Omega}{r^2 (R_2^2 - R_1^2)} \quad \text{Equation 9}
\]

Where

- \(\theta\) : Angle of twist
- \(k\) : Spring constant
- \(R_2\) : radius of outer cylinder
- \(R_1\) : radius of inner cylinder
- \(L\) : Length of cylinder
- \(\Omega\) : Viscosity of rotation
- \(r\) : Distance of plane of liquid from centre of suspension
The cone and plate method consists of a plate and a small angle cone which is suspended from a spring and just touches the plate. The liquid is placed on the plate, that rotates at a given speed therefore creating a viscous drag in the liquid which as a consequence exerts a torque \( (k \theta) \) on the small angle cone. As a result the small angle cone twists through an angle \( (\theta) \). The rate of shear of the liquid between the cone and the plate is given as:

\[
D = \frac{\Omega}{\psi} \quad \text{Equation 10}
\]

Where
- \( \Omega \): Velocity of rotation plate
- \( \psi \): Cone angle

Stress \( f \) is expressed as:

\[
f = \frac{3k\theta}{2\pi R^3} \quad \text{Equation 11}
\]

Where
- \( k \): Spring constant
- \( R \): radius of cone

Therefore the viscosity \( \eta \) can be determined from \( f / D \).
2.8 Ceramic processing

2.8.1 Ceramic raw materials

Ceramic raw materials can be categorised into two sections, traditional and modern ceramics. However, before discussing the development of these materials it is also essential to understand and clarify the factors that affect the mechanical properties. It is commonly accepted that the purity, particle size distribution, reactivity, polymorphic form, availability, and costs all contribute in part to the final properties of the material.\(^{90}\)

Throughout several millennia traditional ceramics have been produced from naturally occurring raw materials. From early civilisations pottery was produced from mixing natural clay minerals with water which could be moulded into shapes. This is an indication of the plastic properties resulting from the combination of these two components. The final stage consisted of drying in the sun and firing at high temperatures to harden the ceramic.\(^{90}\)

Clay minerals form over time from the erosion of minerals, such as feldspar (KAlSi\(_3\)O\(_8\)), from igneous rocks such as granite due to environmental effects and deposited into lakes and riverbeds and transformed into clay. Transformation into natural clay minerals in a natural environment takes place through a chemical process whereby feldspar undergoes a reaction with water, dissolved CO\(_2\) and organic acids. The clay minerals that form can therefore be considered hydrated aluminosilicates, these include koalinite [Al\(_2\)(SiO\(_3\))(OH)\(_4\)], hallosite [Al\(_2\)(SiO\(_3\))(OH)\(_4\) · 2H\(_2\)O], pyrophyllite [Al\(_2\)(SiO\(_3\))\(_2\)(OH)\(_2\)], and montmorillonite [Al\(_{1.67}\) (Na, Mg)\(_{0.33}\) (SiO\(_3\))\(_2\)(OH)\(_2\)].\(^{90}\)
2.8.2 Glasses

A glass can be termed a non crystalline ceramic with a very high viscosity and is considered a rigid material in a vitreous state. Advances have been made over the recent past with the development of glasses. The understanding of their properties has created a variety of novel synthetic routes to new high performance materials. The most common glasses are the silicates and the borosilicates.\(^{94}\)

![Diagram of volume changes for supercooled liquids, glasses, and crystalline materials.](image)

**Figure 18.** Comparison of the volume changes for supercooled liquids and glasses with that for a crystalline material.\(^{94}\)

A glass can be formed by cooling the melt at a greater rate than the rate of crystallisation, thus creating a supercooled liquid before passing a glass transition temperature to form a glass (Figure 18).\(^{95}\) For instance, silica quartz can be supercooled to give vitreous silica. X-ray diffraction studies for vitreous silica show a lack of long range order which is indicated
by the lack of diffraction peaks. Other spectroscopic techniques indicate a tetrahedral arrangement of oxygen atoms around silicon. This arrangement can be characteristically seen in quartz. However, the lack of long range order for silica glass (vitreous) can be explained by the variations in Si-O-Si angles.\textsuperscript{96}

Figure 19 depicts a two dimensional structure where the local co-ordination environment is preserved and a two dimensional glass exhibiting a loss in long range order by the variation of the bond angles around oxygen.

\begin{figure}[h]
\centering
\begin{tikzpicture}
\node at (0,0) {\includegraphics[width=\textwidth]{silica_structure.png}};
\node at (2,4) {$n = 12$ Ring};
\node at (4,4) {$n = 10$ Ring};
\node at (-2,3) {$n = 8$ Ring};
\end{tikzpicture}
\caption{Two dimension structure of silica exhibiting a loss in long range order.\textsuperscript{96}}
\end{figure}

The presence of strong covalent Si-O bonds in a three dimensional network, such as in a silicon dioxide melt, means that these bonds do not readily break and reform during cooling therefore facilitating the formation of a glass.
Changes in volume can be associated with vitreous and crystalline materials after they undergo cooling from a molten liquid. An abrupt decrease in volume is expected when a molten material crystallises. A material that undergoes glass formation remains in a liquid state to form a metastable supercooled liquid and then forms a rigid glass below the glass transition temperature $T_g$. The material in this state is usually larger in volume than a crystalline material below $T_g$.

The use of modifiers has been an effective method of lowering the workability of glasses such as vitreous silica which has a high glass transition temperature and is considered a strong glass that can withstand rapid cooling or heating without cracking. Basic oxides such Na$_2$O or CaO can be added to SiO$_2$. This disrupts some of the Si-O-Si linkages by transferring O$^{2-}$ ions to Si(IV) and creating terminal Si-O$^-$ links that associate with either the Na$^+$ or Ca$^{2+}$ ions (Figure 20). Consequently this leads to glasses with lower softening points and so this makes it easier to work at convenient temperatures.\textsuperscript{94}
2.8.3 Sol gels

An alternative route for glass synthesis is the sol-gel process, this can be illustrated by the schematic diagram below (Figure 21):^97

![Schematic diagram for glass synthesis via the sol-gel process.](image)

**Figure 21.** Schematic diagram for glass synthesis via the sol-gel process.\textsuperscript{97}
Crystalline ceramic materials and high surface area compounds such as silica gels can be synthesised via this route. The term sol-gel is a term made up of two steps; the \textit{sol} part meaning solution phase, where a metal oxide (e.g. Tetraethyl orthosilicate) precursor is added followed by hydrolysis with water. The \textit{gel} is formed as a result of hydrolysis. Furthermore, dehydration and sintering of the thick gel yields a compact solid.

An example of a sol-gel process can be illustrated for the preparation of a binary CaO-SiO$_2$ glass (Figure 22).
2.8.4 Some examples of engineering ceramics

2.8.4.1 Aluminium oxide

Aluminium oxide (Al₂O₃) is a naturally occurring mineral which exists in a variety of forms namely rubies, blue sapphires and star sapphires. These forms are based on the corundum lattice structure and contain a number of point defects resulting from impurities within the lattice structure. Electronic transitions occurring from the site of the impurity accounts for the colours associated with these minerals. These occur via the absorption of visible light at the site of the metallic impurity. The presence of chromium for instance accounts for the bright red colour associated with ruby.
The Bayer process is an important process for the production of \( \text{Al}_2\text{O}_3 \) powder, which is used for the manufacture of polycrystalline \( \text{Al}_2\text{O}_3 \) based ceramics. The powder is obtained from the mineral bauxite via this process and involves firstly mixing bauxite with iron hydroxide and other impurities, followed by the selective leaching of the alumina by caustic soda and precipitation of the purified aluminium hydroxide. The final stage involves thermal conversion to \( \text{Al}_2\text{O}_3 \) from aluminium hydroxide.\(^9\)

\( \text{Al}_2\text{O}_3 \) powder is used to manufacture a whole host of ceramic materials for day to day use. These include porcelains, alumina laboratory ware, crucibles, high temperature cements, tiles, armour, medical components, abrasives and refractories.

2.8.4.2 *Silicon carbide powder*

Natural SiC can be found in meteoric iron as small green hexagonal plates and exists as the \( \alpha\)-SiC polymorph.\(^9\) The two polymorphs of SiC, \( \alpha \) and \( \beta \), can be synthesised commercially. The \( \alpha\)-SiC can be formed in large quantities by the Acheson process. This involves mixing \( \text{SiO}_2 \) sand with coke and applying an electric current between two electrodes at opposite ends of a \( \text{SiO}_2/\text{coke} \) mound which resistance heats the coke to approximately 2200°C. This consequently allows the coke to react with \( \text{SiO}_2 \) to produce SiC and CO gas. Once the reaction is completed through further heating, the mound is cooled and the broken up. Intergrown green hexagonal SiC crystals that are low in impurities can be found in the core of the mound. This low impurity product can therefore be suitable for electronic applications. SiC of low purity can be found in the outer layer, and this grade of SiC is suitable for abrasives.
The manufacture of ceramic fibres consisting of beta-silicon carbide can be synthesised from organopolysilanes precursors. Polycarbosilanes can be drawn from a melt and spun into fibres in the first step, followed by a two-stage thermal process in which these pre-ceramic polycarbosilanes are converted into beta-SiC, a material with extremely high tensile strength.90

Other than the applications previously mentioned, SiC are used as high temperature kiln furniture, electrical resistance heating elements, grinding wheels and abrasives, wear resistance applications, incinerator linings and also as highly stressed components in heat engines.90

2.8.4.3 Zirconia

ZrO2 can adopt three distinct crystalline structures; cubic (fluorite structure), tetragonal and monoclinic structures (phases). The difference in crystal symmetry and also the detailed interatomic spacing provide the distinction between the three phases. The transformation of one phase to another occurs by simple atom displacements, and does not involve any change in the numerical coordination of ions.95

Between ~2370°C and the melting point 2680°C, ZrO2 adopts the cubic structure where it is only stable in its pure form at these conditions and is the highest temperature phase for this system.95

Stabilizers, such as CaO, MgO and Y2O3 can be introduced at minimal additions. These allow the cubic phase of zirconia to be preserved to lower temperatures. From the phase
diagram it is clear that the region of thermodynamic stability for cubic ZrO-MgO does not reach room temperature. However, upon cooling to a certain temperature the stability can be retained as a metastable phase.  

Phase transformations can be shown for the ZrO$_2$-MgO system in which the tetragonal to monoclinic transformation occurs at $\sim$1240°C. With regards to structural applications these transformation are considered very important and form the basis for transformation toughening.  

For pure zirconia the transformation from tetragonal to monoclinic phase is also shown to occur at $\sim$1240°C. However, it is often possible to retain the tetragonal phase of lightly doped ZrO$_2$ to room temperature as a metastable phase.  

Tetragonal to monoclinic transformations bring about a change in symmetry and also volume expansion. With regards to toughening and strengthening of materials, this has proved to be beneficial, since the associated energy absorption and volume expansion with respect to the monoclinic phase can be used to hinder crack propagation which can be initiated by the local stress fields that emanate from sharp cracks.
2.8.5 Raw materials selection criteria

2.8.5.1 Purity

Purity has an effect on a number of high temperature properties that include strength, stress rupture life and oxidation resistance.\(^\text{90}\)

The chemistry of the matrix material, nature of impurity and distribution, as well as the condition in which the material is processed (time, temperature, stress, environment) negates the effectiveness of the impurity.\(^\text{90}\)

The presence of calcium in Si\(_3\)N\(_4\) hot pressed with a sintering additive such as MgO is known to be a prime example of how creep resistance is diminished. In this case the presence of calcium at the grain boundaries depresses the softening temperature of the grain boundary glass phase. This consequently reduces the creep resistance of the material by facilitating plastic deformation during heating. In contrast the same material hot pressed with Y\(_2\)O\(_3\) does not exhibit the same behaviour. The calcium impurity in this instance is absorbed into the solid solution by the crystalline structure and does not have a great effect on creep resistance.\(^\text{90}\)

2.8.5.2 Particle size

In order to form maximum packing and uniformity, the particle size distribution must be taken into consideration. Optimal shrinkage and hence reduced porosity through
densification is greatly influenced by achieving maximum particle packing. This can be done by selecting a range of particle sizes.\textsuperscript{77}

In general high porosity and a large grain size usually result from the presence of high porosity in the green compact prior to sintering.

The reactivity of particles during sintering is an aspect of powder processing that involves changes in surface free energies and is dependent on particle size in the green compact. Hence, very small particles have very high surface free energies that drive the sintering process through strong thermodynamic forces that decrease their surface area by bonding together. This allows a greater transport of material to fill the pores between particles and hence greatly reduces porosity.\textsuperscript{90}

2.8.6 Mechanical sizing

There are a number of ways in which sizing of particles can be achieved. These techniques can be can be listed:\textsuperscript{90}

- Screening
- Elution
- Air classification
- Ball milling
- Attrition milling
- Vibratory milling
- Turbomilling
- Fluid energy milling
- Hammer milling
- Roll crushing

An efficient method of particle sizing that is commonly used is screening. For dry ceramics this seems to be an effective way of separating particle sizes. Screening can either involve a single screen with a selective size opening or a range of screens with varying size openings.

A problem that is commonly encountered during screening is agglomeration. This behaviour normally takes place below a sieve opening of 44μm and involves groups of fine particles acting as a single particle that clog the screen and prevent further screening, this can reduce the effectiveness of particle sizing.

2.8.7 Ball milling

Other than the screening method previously discussed, ball milling is a commonly used route for obtaining the desired particle size distribution. This step involves particle size reduction, where the particles are ground in a closed cylindrical container with grinding balls, rods or short cylinders which is continually rotated. In order to obtain effective milling the cylinder is placed horizontally so as to allow the grinding media to cascade. Ceramic particles are effectively broken down into smaller particles through their movement between the grinding media and between the media and the mill wall (Figure 23).
The type of grinding media that can be utilised are WC, steel, ZrO₂, Al₂O₃ and SiO₂. These media have a different specific gravity and it is the specific gravity amongst other factors (relative size and hardness) that dictates the rate of milling. A higher rate of milling can be achieved through a higher specific gravity media and consequently leads to a specific size reduction being reached more quickly.⁹⁰

Milling can be carried out under either wet or dry conditions. Some of the main advantages of wet milling over dry milling is that it can achieve a smaller particle size than dry, higher rotational speeds, a narrower particle size and also allows wet screening of particles through a fine screen. However, the resulting powder after dry milling does not require separation from a liquid.⁹⁰
Overall, the effect of milling achieves a broader particle size distribution compared to screening alone. The particle size distribution can be illustrated as a cumulative mass percent against the equivalent spherical diameter as a function of milling time for silicon powder (Figure 24). 

Cumulative mass percent

Figure 24. Graph showing how particle size is reduced as a function of milling time.
2.8.8 Shape forming processes

There are a number of shape forming processes that can be applied in order to obtain the required ceramic product. These include hot forming techniques as well as the more commonly used cold forming processes.\textsuperscript{99}

The method of shape forming usually involves placing the powder into a die to produce a green compact through the application of pressure. This procedure can fall into a number of categories such as:\textsuperscript{90}

- Uniaxial
- Isostatic
- Hot pressing
- Hot isostatic pressing

It is often common practice to include binders and lubricants in the powder so as to produce a preconsolidated powder that is free-flowing. The advantages of adding these additives is to achieve close packing of powder particles and also to reduce friction and to allow equal pressure to be distributed amongst all regions of the compact.\textsuperscript{90}

Uniaxial pressing involves the application of pressure along a single axis upon a powder that is placed into a rigid die to produce a compact. Mechanical and hydraulic presses are the most commonly used tools used to carry out uniaxial pressing. Even though mechanical presses can be automated and can give a higher production rate, it is often more practical to
make use of hydraulic presses for laboratory purposes where achieving a high production rate is not so critical.  

The mechanics of hydraulic pressure involves the transmission of a given pressure via a fluid against a piston. Acquiring the desired shape and dimensions of the green compact requires an understanding of the nature of the ceramic powder and quantity of powder needed to form that green compact at the necessary pressure.

Uniaxial powder pressing can be conducted either under dry or wet conditions. Dry pressing usually requires the compaction of granulated or spray-dried powder with minimal if not, no moisture. Under compaction the granules are crushed and the resulting particles undergo mechanical redistribution to form a close-packed array. Alternatively pressing under wet conditions involves powder containing 10 to 15% moisture.  

Problems frequently encountered during uniaxial pressing include improper density or size, usually associated with the precompacted powder, die wear, cracking and density variation. An example of how cracking may occur through uniaxial pressing is shown in Figure 25. This shows how cracks initiate at the top edge of the compact as it is ejected from the die.

As soon as the pressure is released from the upper punch, the material rebounds which simultaneously causes friction drag between the material and the die wall and the resulting cracks then manifest from the tensile stress concentrated at the upper edge of the green compact.
Ejection from the die can cause a series of laminar cracks to occur as a result of further rebounding. Rebounding to a larger cross section usually takes place as the material passes above the top of the die. The effect of rebounding can be minimised by the addition of a suitable binder.

Figure 25. Diagrams illustrating how cracks can form during pressing.  

Density variation is another problem associated with uniaxial pressing and can introduce flaws during firing. The density variation can arise from compaction and is probably attributed to the dissipation of pressure through friction between powder particles and also between powder particles and the die wall (Figure 26). This consequently results in a significant part of the compact experiencing a much lower pressure than the pressure applied. This will lead to lower density in these parts and effectively hinder densification during sintering introducing flaws in the final material.
Figure 26. Diagrams illustrating the variation in density for different size dies.  

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The application of pressure in all directions is an effective way of avoiding cracks and non-uniform density. This method is often referred to as isostatic pressing either hot or cold and is an effective means of achieving greater uniformity of compaction and also a greater and more uniform green density.  

2.8.9 Hot pressing

Hot pressing (pressure sintering) is a technique that combines pressing and firing simultaneously. Improved packing by the rearrangement of particles and enhanced particle to particle contact can be achieved via the application of pressure at the sintering temperature. This consequently allows densification to occur at a lower temperature compared to pressure-less sintering.

The advantages of hot pressing can be summarised by the following:

- **Densification time is reduced; reaches densification temperature at a quicker rate**
- **Densification can occur at lower temperatures and thus can inhibit grain growth**
- **Minimises residual porosity**
- **Inhibition of grain growth and minimal porosity usually results in a higher strength material.**
- **There is no need for binders or other additives and so this process can be carried out with a loose powder.**
- **Enhanced high temperature properties can be achieved with the introduction of a reduced amount of sintering aid.**
2.9 **Sintering**

Sintering is the mechanism whereby the consolidation or densification of a body shaped from powder particles occurs. This mechanism greatly influences the final properties of the ceramic such as microstructure, fracture strength etc.\(^1,3,4,35,73,74,101-105\) Porosity is a feature of ceramics that is known to lower the mechanical properties. It is therefore essential that pores between powder particles of a 'green body' are minimised and this can therefore be executed through sintering. As a consequence shrinkage, grain growth and also the development of strong bonds between neighbouring particles is manifested.\(^90\)

For sintering to take place a number of requirements must be met. Firstly, the presence of a material transport mechanism in the form of diffusion and/or viscous flow. Secondly, in order to initiate and sustain this material transport there must be a source of energy. This is provided in the form of heat, capillary pressure associated with surface curvature when two particles come into contact, and also surface tension.

2.9.1 **Stages of sintering**

Sintering can be categorised into three stages, the 1\(^{st}\) stage (initial), the 2\(^{nd}\) stage (intermediate), and the 3\(^{rd}\) stage (final). The initial stage involves the rearrangement of particles that are orientated as such to give a high surface contact followed by bonding at the points of contact between particles leading to initial neck formation, thus creating an area of high energy for material transport (Figure 27).\(^95\)
The intermediate stage involves the growth in the size of the necks between particles which as a consequence reduces porosity and leads to shrinkage (Figure 28). This is the stage where most of the shrinkage takes place and continues in conjunction with grain growth.

Figure 27. Diagram illustrating the first stage in the sintering process.

Figure 28. Diagram illustrating the intermediate stage of the sintering process.
Grain growth can be described as the growth of one particle or grain with the adjacent grain being consumed as the grain boundaries begin to move. This is necessary so as to ensure further neck growth and elimination of porosity and it is dictated by the changes in geometry as this step proceeds. Further sintering results in the isolation of pores. At this point the final removal of pores requires the final stage (Figure 29).

The final removal of pores is dependent on the rate of grain growth. Controlled grain growth and the movement of grain boundaries facilitates the removal of isolated pores and vacancy diffusion (which arise from lattice dislocations) along the grain boundaries. Rapid grain growth, however, results in the isolation of pores within a grain and is due to the grain boundaries moving faster than pores.

The driving force for grain growth is governed by the surface energy. During sintering, curved grain boundaries will tend to minimise their surface area in order to reduce surface free energy simply by increasing their radius of curvature. This is only possible through...
grain growth. This can explain why smaller grains having a smaller radius of curvature and hence higher surface energy have a greater tendency to move, change shape and also become consumed by larger grains.

2.9.2 Mechanisms of sintering

The four types of sintering mechanisms that can take place in ceramics are vapour-phase sintering, solid state sintering, liquid-phase sintering and reactive liquid sintering. The material transport mechanism intrinsic to each type of sintering has to meet a certain criteria. The material transport mechanism for vapour-phase sintering requires a difference in vapour pressure as a function of surface curvature between two particles, a condition that is essential to the driving energy (Figure 30). This transport mechanism is referred to as an evaporation-condensation mechanism.

![Diagram of sintering processes](image)

**Figure 30.** The material transport mechanism for vapour phase sintering.
The material transport between a particle of positive curvature and a particle with a negative curvature is a simple example of how this concept can be explained. A particle with a positive curvature has a higher vapour pressure compared to a particle with negative curvature, hence when in contact this difference in vapour pressure drives the transport of material from the former to the latter particle. This driving force is greatly enhanced for smaller particles with a higher positive radius of curvature.

Vapour-phase sintering alone cannot lead to densification and does not result in shrinkage. Transport of pores to external surfaces does not occur with this mechanism, however, it can alter the shape of pores. Furthermore, bonding between adjacent particles is accomplished providing material strength although permeability is compromised through porosity.

2.9.3 Solid state sintering

The material transport mechanism involved in solid state sintering requires volume diffusion and can be regarded as the movement of atoms or vacancies along a surface or grain boundary or through the volume of the material (Figure 31). In vapour phase sintering the mode of material transport occurs through surface diffusion and does not culminate in shrinkage. Conversely, shrinkage does result from volume diffusion.
The difference in free energy or chemical potential between the free surfaces of particles and the points of contact between neighbouring particles are the conditions that contribute to the driving force for solid-state sintering. This mechanism can be expressed by a formula describing the transport of material by lattice diffusion from the line of contact between two particles to the neck region.

\[
\frac{\Delta L}{L_0} = \left( \frac{K \mu a^3 D^* t}{kT d^n} \right)^m
\]

Equation 12

Where
- \( \Delta L / L_0 \): linear shrinkage
- \( \gamma \): surface energy
- \( a^3 \): atomic volume of the diffusing vacancy
- \( D^* \): self-diffusion coefficient
There are a number of parameters that influence the rate of sintering, most notably the diameter of the particle and also the sintering temperature. This can be substantiated by two graphs that measure the rate of shrinkage as a function of time for four different temperatures (Figure 32). Since particle diameter is inversely proportional to shrinkage according to the equation, it can be said that the sintering of a finer particle size powder occurs more rapidly at lower temperature compared to larger particles.
Figure 32. Graphs showing shrinkage as a function of time for four sintering temperatures. Other factors that can influence the final properties, but are not apparent in the equation, are the homogeneity of particle packing, particle size distribution and particle shape. Elimination of porosity can prove to be difficult during sintering if the uniformity of particle packing is not established. Furthermore, the formation of irregular shaped pores during sintering can be a result of a high concentration of elongated or flattened particles,
therefore making them difficult to remove. Compacts that form large pores and that contain a high volume percentage of porosity develop as a result of the packing efficiency.

Packing efficiency can be influenced by particle size distribution, for instance, if all the particles are of one size, packing efficiency would prove somewhat difficult. A considerable amount of shrinkage can occur in this instance and is accompanied by a significant amount of porosity. This can be avoided if very uniform close packing is achieved during compaction and grain growth occurs during densification. 

Rapid grain growth is a problem often encountered in solid state sintering and is a common feature in pure \( \text{Al}_2\text{O}_3 \). Rapid grain growth in \( \text{Al}_2\text{O}_3 \) involves the movement of grain boundaries at a rate which results in the entrapment of pores within a grain rather than along the grain boundaries. The addition of 0.25wt\% MgO in \( \text{Al}_2\text{O}_3 \) can facilitate the elimination of porosity by slowing the rate of grain growth, thus enabling pores to move along the grain boundaries.

2.9.4 Liquid phase sintering

The presence of a liquid phase at the sintering temperature provides rapid and uniform densification of ceramics. The formation of a liquid phase is strongly dependent on the composition of the starting solids and involves the wetting of particles at the sintering temperature by a viscous liquid.
The rate of liquid phase sintering is determined by particle size, viscosity and surface tension. Capillary pressure and associated surface tensions develop through the presence of the liquid in the narrow channels between particles which subsequently allows the rearrangement of particles, thus achieving better packing. Furthermore, there is an increase in the contact pressure between particles allowing for the dissolution of atoms into the liquid phase and then subsequent re-precipitation (Figure 33). These events collectively assist further shrinkage and densification of ceramics.\textsuperscript{90}

![Solution-reprecipitation](image)

**Figure 33. Solution-reprecipitation.**\textsuperscript{95}

Densification can be further enhanced by the presence of smaller particles that have higher capillary pressure and surface energy due to small radius of curvature, providing a greater chemical potential between the surface of particles compared to the surface of pores and consequently increasing the rate of dissolution / re-precipitation.\textsuperscript{90}
The temperature is also another factor that has a profound effect on the rate of liquid phase sintering. In some instances small increases in temperature can be advantageous in that it enhances the rate of densification as a result of a significant increase in the presence of the liquid. In contrast, exaggerated grain growth can also ensue, which reduces the mechanical strength of the ceramic.\textsuperscript{90}

Variations in microstructure can be observed as a result of liquid phase sintering and are attributed to differences in particle size, the amount of liquid present at the sintering temperature, and the cooling cycle.\textsuperscript{90}

![Figure 34. Different microstructures that may arise from liquid phase sintering.\textsuperscript{90}](image)

- A variation of how the microstructure is affected can arise from sintering of glass particles. This leads to a microstructure containing no grains or grain boundaries, but does leave a few remaining spherical pores (Figure 34; (a)).

- The presence of a large amount of liquid once a composition has undergone densification can lead to the solidification of the liquid to a glass on rapid cooling (Figure 34; (b)).
• There are two similar situations in which there is less liquid and less residual glass present. The first is a coating of the grain boundaries and triple point junctions by a glass of continuous phase. The second situation is where there is only glass present at the triple point junctions and is attributed to a nominal or very small amount of liquid present during sintering (Figure 34; (c) & (d)).

• The formation of a crystallised grain boundary from the crystallisation of the liquid during cooling is yet another example of the variation in microstructure. The presence of glass at the grain boundaries has a profound effect on the mechanical properties of ceramics. The improvements in these properties have been shown to be more marked for a material consisting of a crystallised grain boundary (Figure 34; (e)).

2.9.5 Reactive liquid sintering

The similarity between reactive liquid sintering and liquid phase sintering is that they both provide the same type of driving force required for densification. Reactive liquid sintering though does involve the presence of a liquid during sintering but the liquid in this case undergoes a change in composition or can disappear. Consequently, the material exhibits very good high-temperature properties as a result of the consumption of the liquid phase once it has reacted. 90

Reactive liquid sintering can be achieved by selecting an appropriate composition of starting material that undergo a series of reactions, that include a number of intermediate compounds being liquid, to produce a final stable compound that is solid. An alternative
route for reactive liquid sintering can be done by selecting starting powders that will pass through a liquid phase before forming a solid solution at equilibrium.

An example of structural ceramics that require this type of sintering are SiC and Si$_3$N$_4$. At high temperatures sintering of these materials as a single solid phase can be difficult and so the use of oxide additives to produce silicon oxynitride or oxycarbide glass is preferred.\textsuperscript{90}

2.9.6 Grain growth

Grain growth is an essential part of sintering that occurs during the final stage of sintering and driven by the need to reduce the interfacial energy per unit volume.\textsuperscript{95} Grain growth can proceed in a number of ways. It can either progress as normal grain growth or by discontinuous or abnormal grain growth (Figure 35).

Normal grain growth would involve growth with a relatively narrow grain size distribution which maintains a fixed distribution shape. Abnormal grain growth involves the continuous growth of large grains with the simultaneous consumption of smaller grains.\textsuperscript{95}
Figure 35. Graphs showing normal and discontinuous grain growth.  

Moreover, the necessity to control the final grain size and distribution, which has a bearing on the properties of the ceramic cannot be emphasised enough. The properties that can be obtained from final grain size and distribution are:
1. The strength of the ceramic expressed in terms of Griffith’s fracture law

\[ \sigma_c \propto \frac{1}{\sqrt{c}} \]  

Equation 13

Where

\[ c \] : flaw size - equated to \( R_{g\text{,max}} \) (max grain size)

2. The creep properties

\[ \varepsilon \propto \frac{1}{R_g^m} \]  

Equation 14

Where

\[ m \] : constant between 1 and 3

3. The electrical properties

4. Magnetic properties

The driving force for the grain boundary migration and subsequent grain growth can be defined as the tendency to reduce the boundary energy through a reduction in the grain boundary area.  

\[ \Delta G = \int_{A_1}^{A_2} \gamma \, dA = \gamma (A_2 - A_1) \]  

Equation 15

where

\( \Delta G \) : excess free energy of grain boundaries (Gibbs free energy)
\( \gamma \) : surface energy
\( dA \) : change in grain boundary area

The pressure differences between curved surfaces of grains make this favourable as this allows the reduction in grain boundary area by the migration of boundaries towards their centre of curvature. Since the driving force of grain boundary migration is more
specifically related to the radius of curvature between two adjacent grains, the following
equation can therefore be considered more pertinent.

\[ \Delta P \text{ (driving force)} = \gamma_{gb} \left[ \frac{1}{k_1} + \frac{1}{k_2} \right] \] 
\text{Equation 16}

Where
- \( k_1 \) and \( k_2 \): average radius of curvature of grains 1 and 2
- \( \gamma_{gb} \): interfacial energy of the grain boundary

The velocity or rate of grain boundary migration can be defined as the product of the grain
boundary mobility (\( M_{gb} \)) and the applied driving force from the curvature:

\[ V_{gb} = M_{gb} F_{gb} \] 
\text{Equation 17}

Where
- \( V_{gb} \): rate of grain boundary migration
- \( M_{gb} \): grain boundary mobility
- \( F_{gb} \): applied driving force from curvature

The value of grain boundary mobility is shown to be specific to a particular mechanism of
grain boundary migration.

Grain boundary migration can also be explained in terms of entropy (disorder). A
polycrystal can be heated to a temperature in which a release of grain boundary enthalpy
occurs, and corresponds to the growth of grains to a final grain size. Since a loss in
enthalpy correlates to a release in entropy (\( S_{gb} \)) (from \( G_{gb} = H_{gb} - TS_{gb} \); where \( G_{gb} \) is the
Gibbs free energy, \( H_{gb} \) is the enthalpy and \( T \) is temperature), then it can be said that a
release of boundary entropy occurs (corresponds to a more ordered structure).

Entropy can be expressed as a change in heat capacity with grain size:
The migration and growth of grains can be viewed geometrically. The direction of grain boundary migration is dictated by the radius of curvature of grain boundaries. Grain boundaries that are equal in energy form triple point junctions at angles of $120^\circ$. If this is true for all grains then the grain boundaries would be flat or without curvature and the grain would adopt a six sided geometry. Grain boundaries that are concave usually belong to grains with fewer than six sides and tend to shrink and disappear as grain boundaries migrate toward their centre of curvature. Conversely, grain boundaries which migrate outwards and consequently bring about grain growth are indicative of grains belonging to a geometry of more than six sides and have grain boundaries that are convex (Figure 36).  

\[
\Delta S_{gb} = \int_{\tilde{T}_1}^{\tilde{T}_2} \frac{C_p}{T} dT
\]

Where

- $\Delta S_{gb}$: release in grain boundary entropy
- $C_p$: heat capacity
- $T$: temperature

Figure 36. A schematic representation of the migration and growth of grains.
2.9.7 Particle coarsening (Ostwald ripening)

Particle coarsening occurs in much the same way as grain growth in that it requires a difference in free energy between curved surfaces to drive the process. However, coarsening involves the dissolving of smaller particles in a solid, liquid or vapour medium of appreciable solubility or vapour pressure, whilst the larger ones continue to grow.\textsuperscript{95}

The growth rate of particles in this case is dependent on the radius, which can be shown by the following equation:

\[
\frac{da}{dt} = -\frac{D}{\bar{a}} \left[ \frac{c_0^2 \gamma M}{\rho RT} \frac{1}{a} \left( \frac{1}{a} - \frac{1}{\bar{a}} \right) \right]
\]

Equation 19

Where
- \(a\) : particle radius
- \(\bar{a}\) : average particle radius
- \(D\) : diffusivity in the medium
- \(c_0\) : equilibrium solubility
- \(\gamma\) : surface energy
- \(M\) : molecular weight
- \(\rho\) : density
- \(R\) : molar gas constant
- \(T\) : temperature

Since this process involves particles dissolving and also growing it is worth taking into account their solubility. The following equation, proposed by Thompson and Freundlich, describes the solubility for any given particle of radius \(a\):
\[ c_a = c_0 \exp \left( \frac{2\gamma M}{\rho RTa} \right) \]  

Equation 20

Where

- \( c_0 \) : equilibrium solubility
- \( c_a \) : solubility at radius \( a \)
- \( a \) : particle radius

It is apparent that the exponential term in the solubility equation is a component of the equation for growth rate, therefore the growth rate must vary as a function of solubility. Particles that are smaller than the average particle size give higher solubility, therefore resulting in a large but negative growth rate indicating shrinkage and eventually disappearance. Particles with a radius equal to the average particle size give a growth rate of zero where no concentration gradient exists (a requirement for diffusion of particles through the surrounding medium). Particles with a larger radius than the average would have insignificant solubility, thus expressing a positive growth rate.\(^{95}\)
2.10 Mechanical properties of ceramics

The application of ceramic materials as surgical implants requires an understanding of the mechanical properties. The load a ceramic must withstand under physiological conditions depends on the structural nature and microstructural characteristics inherent to it. It is commonly known that ceramics are hard but brittle materials, properties that are influenced by the nature of the ionic and covalent bonding. In contrast, metals and polymers can exhibit ductile behaviour and differ altogether in their structural nature from ceramics. It is therefore important to address the various physical properties that form the basis of the techniques used for mechanical testing.

2.10.1 Elasticity

Elasticity is a term that refers to the extent of deformation as a consequence of applying a load on a material and reflects the slight change in atomic spacing. The atomic bond strength of the material, the stress, and the temperature influence the deformation of the material. Deformation can also be referred to as strain $\varepsilon$, and the load defined in terms of stress $\sigma$. When stress is applied to a material it undergoes a level of strain up to a point known as the limit of proportionality. Prior to this point the strain is reversible, that is, any deformation in shape and change in atomic spacing that occurs can be reversed thus returning the material to its original state. This behaviour is known as elastic deformation which can be expressed by the following relationship, where $E$ is the modulus of elasticity or Young’s modulus.$^{90,106}$
\[ \sigma = E \varepsilon \]  \hspace{1cm} \textit{(Tensile stress)} \hspace{1cm} \text{Equation 21} \\

Where \\
\( \sigma \) : stress \\
\( E \) : elastic modulus \\
\( \varepsilon \) : strain \\

Similarly, an expression can also be given for shear stress, a form of loading that is applied on a plane of unit area:

\[ \tau = G \gamma \] \hspace{1cm} \text{Equation 22} \\

Where \\
\( \tau \) : shear stress \\
\( G \) : Shear modulus \\
\( \gamma \) : strain \\

Figure 37. Graph showing elasticity on the linear portion of the stress-strain curve.\textsuperscript{90} \\

At certain temperatures ceramics will behave elastically until they reach a level of stress that causes fracture, this is known as brittle fracture (Figure 37). Beyond a certain stress
materials behave plastically, this term refers to materials that undergo permanent deformation and do not return to their original state (Figure 38). This behaviour can also be denoted as plastic deformation or plastic strain and is a characteristic seen in metals.\textsuperscript{90,106} Plastic behaviour can occur in different ways, in aluminium for instance the transition from elastic strain to plastic strain is smooth (Figure 38(a)). However, in other materials the initial plastic strain is discontinuous and can be described as a region beyond the elastic limit where the stress increases the strain until a yield point where the stress drops a little as the sample is stretched slightly (Figure 38(b)). Further stretching increases the stress from a lower yield point.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{plastic_elastic.png}
\caption{Graphs illustrating plastic as well as elastic behaviour.\textsuperscript{90}}
\end{figure}

The brittle behaviour does not hold true for all ceramics. Ceramics having the rock salt structure display plastic deformation, this is primarily due to the dislocations that occur within the cubic structure. At room temperature and under sustained loading ceramics such as LiF, NaCl and MgO belonging to this cubic symmetry undergo plastic deformation.\textsuperscript{90}
2.10.2 Modulus of elasticity

Having established that the modulus of elasticity $E$ represents the proportional region of a stress-strain curve and more specifically the amount of stress $\sigma$ required to produce a unit elastic strain $\varepsilon$: $^{90,106} $

$$E = \frac{\sigma}{\varepsilon}$$  \hspace{1cm} \text{Equation 23}

Where

- $E$: elastic modulus
- $\sigma$: stress
- $\varepsilon$: strain

It is therefore essential to consider the structural aspects, in terms of the atomic bonds, that contribute to the magnitude of the elastic modulus.

Materials that possess a high modulus of elasticity reflect the strength of the atomic bonding. An increase in the inter-atomic spacing requires a greater amount of stress for materials that have strong atomic bonding, hence they exhibit a greater modulus of elasticity. $^{90,106}$ The modulus of elasticity for materials that have weak ionic bonds compared to those with strong covalent bonds differ. NaCl adopts the cubic structure that contains weak ionic bonds, therefore a low modulus of elasticity is expected. This is measured at 44.2GN.m$^{-2}$ for NaCl. Diamond, however, possessing strong covalent bonds has a significantly greater modulus of about 1035GN.m$^{-2}$. This trend is much the same for metals as it is for ceramics or other materials. $^{90}$
Different crystallographic directions have shown to vary in bond strength and hence in modulus of elasticity. Materials possess anisotropy, this is simply a difference in bond strength in a given orientation. An example of this anisotropy can be shown for a single crystal of iron belonging to the body centred cubic structure. The elastic modulus is shown to differ for two planes of orientation, the [111] direction and the [100] direction which display values of 283GN.m$^{-2}$ and 124GN.m$^{-2}$ respectively. The higher value shown in one direction represents those atoms that are most closely packed and also the greater density of the [111] direction compared to the [100] direction.$^{90}$

Poly-crystalline ceramics, though, contain many crystals in random orientation. Polycrystalline ceramics have a single elastic moduli that is obtained from the average of the elastic moduli for various crystallographic directions from individual crystals. However, the anisotropy must be taken into careful consideration when selecting a ceramic because it presents a degree of internal stress to the material and does have an effect on the application of the material.$^{90}$

Thermal expansion, which occurs as a consequence of heating the ceramic, causes a decrease in the modulus of elasticity and corresponds to an increase in the inter-atomic spacing.$^{90}$

Materials consisting of more than one composition have an elastic modulus that is the sum of the moduli of the constituent phases and can be estimated using the law of mixtures.$^{90}$
\[ E = E_a V_a + E_b V_b \]  

Equation 24

Where

- \( E_a \) and \( E_b \) : Elastic moduli of a and b respectively
- \( V_a \) and \( V_b \) : Volume fraction of a and b respectively

Examples of such materials include glass or carbon-reinforced organics and glass-bonded ceramics.

A relationship for the effect of porosity on the elastic moduli can also be given. This assumes that the presence of porosity in a material always gives a decrease in modulus:

\[ E = E_0 (1 - 1.9P + 0.9P^2) \]  

Equation 25

Where

- \( E_0 \) : Elastic modulus of nonporous material
- \( P \) : Volume fraction of pores

2.10.3 Poisson’s ratio

Poisson’s ratio can be defined as the ratio of thickness decrease to the length increase when a tensile load is applied to a material (Figure 39):\(^{90,106}\)

\[ v = -\left[ \frac{\Delta d}{d} \frac{\Delta l}{l} \right] \]  

Equation 26

Where

- \( v \) : Poisson’s ratio
- \( \Delta d / d \) : Change in thickness
- \( \Delta l / l \) : Change in length
Figure 39. Diagram displaying changes in material dimension through tensile loading.

Poisson’s ratio varies for different materials and range from 0.1 to 0.5. Poisson’s ratio can also be expressed as a term in an equation that is related to the Young’s modulus and shear modulus of isotropic and polycrystalline ceramics:

\[ E = 2G \left(1 + \nu \right) \]

Equation 27

Where

- \( E \) : elastic modulus
- \( G \) : shear modulus
- \( \nu \) : Poisson’s ratio
2.10.4 Mechanical strength

There are a variety of ways in which strength can be categorised for both metals and ceramics. It is therefore essential to distinguish and understand the different types of strength and also the strength characteristics of ceramics that form the criteria for establishing a testing method. The various terms for strength can be given as yield strength, tensile strength, compressive strength, flexural strength, ultimate strength, fracture strength, and theoretical strength.\(^\text{90,106}\)

2.10.5 Theoretical strength

The stress needed to break atomic bonds in a structure under tensile loading can be termed the theoretical strength:

\[
\sigma_{\text{th}} = \left( \frac{E\gamma}{a_0} \right)^{\frac{1}{2}}
\]

\text{Equation 28}

Where

- \(\sigma_{\text{th}}\) : theoretical strength
- \(E\) : elastic modulus
- \(a_0\) : inter-atomic spacing
- \(\gamma\) : fracture energy

Most, if not all ceramics exhibit measured strengths that are well below the theoretical strength. This can be attributed to structural flaws and flaws created during ceramic processing. The theoretical strength of a ceramic can be estimated at one tenth to one fifth of the value of the elastic modulus. This range would give a theoretical strength for
aluminium oxide (Al₂O₃), for example, of 38GN.m⁻² to 76GN.m⁻², where the elastic modulus is known to be 380GN.m⁻².

Flaws can arise from different processing methods and the measured strengths differ for each processing method. The theoretical elastic moduli for Al₂O₃ and SiC, for instance, are given as 380 and 440GN.m⁻² respectively. Due to flaws, the measured strengths are expected to be considerably lower compared to the theoretical strengths. It has been shown that the fracture strengths for both materials when processed as fibres and as a polycrystalline yield values at about half and 100th of the theoretical strength respectively.

2.10.6 Flaw size

The occurrence of a flaw in a ceramic material can result in stress concentration and presents itself in a number of ways, such as a crack, pore or inclusion. For non-ductile materials and in particular brittle ceramics the stress concentration at the tip of an elliptical crack can be defined by the following equation:

\[
\frac{\sigma_m}{\sigma_a} = 2 \left( \frac{c}{\rho} \right)^{\frac{1}{2}}
\]

Equation 29

Where

- \(\sigma_m\) : maximum stress
- \(\sigma_a\) : applied stress
- \(2c\) : length of major axis of the crack
- \(\rho\) : radius of the crack tip
Flaw size and strength data for silicon nitride ($\text{Si}_3\text{N}_4$), for instance, can be used as an example to develop an idea of how flaw size effects the stress concentration at the crack tip. If the crack tip radius is assumed to be approximately equal to the atomic spacing $a_0$ ($\sim$2Å), the flaw size $c$ measured at 170µm, and the fracture strength occurs at 150MN.m$^{-2}$, then stress concentration factor is calculated at 1840. This is a relatively large value, which means that even very small flaws present in ceramics are critical and do result in a substantial stress concentration effect.\(^{90}\)

$$\sigma_f = A \left( \frac{E\gamma}{c} \right)^{\frac{1}{2}}$$  \hspace{1cm} \text{Equation 30}

The flaw size can also be used to measure the fracture stress as shown by the Griffith's equation. Where $\sigma_f$ is the fracture stress, $E$ is the elastic modulus, $\gamma$ is the fracture energy, $c$ is the flaw size and $A$ is a constant associated with the specimen and the geometry of the flaw.\(^{90}\)

$$\sigma_f = \frac{Z \left( \frac{2E\gamma}{c} \right)^{\frac{1}{2}}}{Y}$$  \hspace{1cm} \text{Equation 31}

A more general relationship can be derived where $Y$ is a constant associated with flaw depth and test geometry, $Z$ is dependent on the flaw configuration, $c$ is the depth of a surface flaw or in the case of an internal flaw can be taken as half the flaw size. The value of the constant $Z$ can also depend on the flaw shape.\(^{90}\)
The diagram above (Figure 40) illustrates a planar elliptical crack. This can form as a result of machining or can be caused by other factors such as impact, thermal shock or glaze crazing. The constant $Z$ can be expressed as a ratio of the depth of flaw ($c$) to the length of the flaw ($l$).

It is apparent that pores play an important part in determining the extent in which the strength of the material is reduced. This effect is associated with a combination of factors:

1. Pore shape

Pores in ceramics, which evolve as a result of processing, are not entirely spherical, most in fact are irregular in shape. It has therefore been assumed that the stress fracture measured due to a pore is closely correlated to that of an elliptical crack, hence a theoretical value can be reached. In theory, a simple spherical pore should have a lower stress concentration effect compared to a sharp crack. Measurements gathered from four point bending tests for Si$_3$N$_4$ showed this to be true when comparing the measured value against the theoretical
value (based on the Evans and Tappin equation); measured-150MN.m$^{-2}$, theoretical-157MN.m$^{-2}$.

2. Pore-crack combinations

This combination, in simple terms, involves the relationship between pores and cracks at the intersections between a pore and a grain boundary in which cracks can manifest. If a pore has a predominating influence over stress fracture then the critical flaw size can be approximated provided the pore is larger than the grain size of the material. However, if the pore size approaches the grain size then the cracks along the grain boundaries will be the predominating influence, resulting in an effective flaw size greater than that of a pore.

3. Internal pores

The effect of internal flaws on the strength of the material is not fully understood. A single law that unites the effect of surface flaws and those lying further away from the surface cannot be used to approximate a stress fracture value close to the measured value. For surface flaws the ligament theory can be applied to ascertain a theoretical value that is within 10% of the measured value. The dimensions of the critical flaw can be taken as the size of the flaw plus the bridge of material separating the pore from the surface. Furthermore, the stress fracture occurring in this case is associated with those pores residing close to the surface.

Pores that lie progressively further from the surface do not follow the ligament theory. Taking the tensile stress measurements for Si$_3$N$_4$ into consideration, it can be shown that
the tensile stress decreases linearly toward the mid-plane from the surface of the body eventually reaching zero. The peak tensile stress can be said to be equivalent to the measured stress on the surface of the material at the time of fracture. This was measured at 215MN.m$^2$. Corrected stress can be calculated from the linear relationship with respect to pore position, which can be located by the intersecting plane. The corrected stress that corresponds to the plane at the lowest point of the pore was measured at 182MN.m$^2$, whereas the corrected stress at the plane associated with the centre line of the pore was measured at 193MN.m$^2$. This difference demonstrates the linear relationship between stress at the surface and stress at the mid-plane. The calculated fracture stress was measured at 126MN.m$^2$ using the ligament theory. In comparison to the measured values this calculated fracture stress is an unreliable method and shows no close correlation. Alternatively, if $Y$ is taken as 1.77 and the flaw size is assumed to be equal to the pore diameter, then using the Evans and Tappin equation the stress value can be calculated at 182MN.m$^2$. This is a more reliable method as it gives a value that is very close to the corrected value.

4. Pore clusters

This involves the reduction in strength of a material due to the cracking of material bridges that link a group of pores on the surface resulting in the formation of a larger pore. The probability of this occurring is high if the separation of neighbouring pores is within one pore radius.
5. Inclusions

Inclusions are usually introduced into a ceramic material through processing and are often associated with contamination. Differences in the thermal and elastic properties between inclusions and the matrix material contribute to the extent of reduction in strength. The formation of cracks adjacent to inclusions during cooling can be a consequence of differences in the thermal expansion. Furthermore, the formation of cracks when a stress is applied can be associated with the elastic modulus. For a large decrease in strength, the coefficient of thermal expansion and elastic modulus of the inclusion is low compared to the matrix of the material. In contrast, a high thermal expansion coefficient and high elastic modulus results in a smaller reduction in strength. This can be further elucidated by the differences in effective flaw size that manifest in both cases.  

2.10.7 Strength measurement

2.10.7.1 Tensile strength

There are a number of techniques that can be used to measure the strength of a test specimen. However, the tensile strength has its limitations when characterising ceramics. This technique is more commonly used to test ductile metals and can provide information about yield strength, breaking strength and elongation (strain) (Figure 41). Tensile strength can be obtained by dividing the maximum load $P$ by the original cross-sectional area, $A$: 

$$A = \frac{P}{\text{maximum load}}$$
\[
\sigma_t = \frac{P}{A}
\]

\textbf{Equation 32}

Where

- \( \sigma_t \) : tensile strength
- \( P \) : maximum load
- \( A \) : original cross sectional area

For ceramics the maximum load \( P \) corresponds to the stress at fracture for a ceramic. A certain degree of uncertainty in the tensile strength measurement for ceramics is always expected since factors such as misalignment come into play. Misalignment of test specimens can lead to early failure of ceramics due to stress concentration at surface flaws which is brought about by instantaneous bending. It is therefore important that the stress distribution within the test specimen is established. This can be done by using strain gauges. Moreover, if bending does occur then the degree of bending, which does have a significant bearing on the tensile strength measurement, must be ascertained. By considering these two factors together it is possible to gain a more accurate tensile strength measurement.\(^9\)

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure41.png}
\caption{Uniaxial tensile strength.\(^9\)}
\end{figure}
2.10.7.2 Compressive strength

This method of testing is commonly used in ceramics. It involves the measurement of crushing strength of the material. Compared to tensile strength, ceramics exhibit compressive strengths that are much higher, in fact they are estimated to be approximately 15 times greater than tensile values. This phenomenon can be explained. It is due to the nature in which the flaw propagates, in particular the way in which it aligns itself with respect to loading. Tensile loading usually causes the flaw to propagate unstably, however, in compression the flaw propagates in a manner so as to align itself parallel to the applied stress. Compression can be carried out in two ways, either by uniaxial compression, where the load is applied across a cross-sectional area or by diametral compression where the load is applied across the diameter (Figure 42). The latter, however, can be sensitive to surface defects and machining damage.\(^{90}\)

![Figure 42. Compression testing.](image-url)
2.10.8 Bending strength testing (Flexure strength)

The maximum tensile stress at which failure occurs is known as the bend strength. The two methods of testing that will be discussed henceforth will be three and four point bending tests. The bend testing used to characterise the strength of the ceramic in both cases can accommodate specimens that are fabricated to form a block of material that is uniform in length can have either a circular, square, or rectangular cross-section.

![Diagram of three and four point bend testing](image)

**Figure 43.** Three and four point bend testing.\(^9^0\)

In three point bend testing the specimen is supported at two ends with the load applied at the centre (Figure 43). Four point testing also involves two end supports, however, loading is applied at two positions (Figure 43). For rectangular test specimens a general flexure stress formula can be derived:

\[
S = \frac{Mc}{I}
\]

**Equation 33**

Where
- \( M \) : moment
- \( c \) : distance from neutral axis to surface of maximum tension
- \( I \) : moment of inertia
The moment of inertia for a rectangular specimen is expressed as \( I = \frac{bd^3}{12} \), and \( c \) is given as \( \frac{d}{2} \), where \( b \) and \( d \) are the width and the thickness of the specimen respectively.

Differences in bending strength values between three point bending tests and four point bending tests have been reported and so to understand this the influencing factors must be discussed.\(^{90}\) The measured strength will ultimately vary as a function of size also, this can be explained. The probability of a specimen containing a large strength controlling defect is expected to be higher for larger specimens which exhibit lower bend strength values compared to smaller specimens. Although smaller specimens exhibit higher results the variation in data is much greater.\(^{90,106}\)

The differences in the data values observed for the two tests can be illustrated using simple test distributed curves.\(^{90,106}\) For three point bending the stress increases linearly from the supports to a peak stress corresponding to the surface region on the specimen that is opposite the point at which it is located (Figure 44). Additionally, the stress also decreases linearly from the surface region under stress towards the neutral axis.

![Figure 44. Stress distribution in three point bending.\(^{90}\)](image-url)
The presence of the largest flaw occurring at the line of peak stress is very unlikely, and so the probability of fracture in this exact region is very low. The specimen will most probably fracture at a region of stress that is lower or at a smaller flaw. The fracture stress value for Si$_3$N$_4$, for instance, was measured at 930MN.m$^{-2}$, this corresponds to a flaw that is 10µm in depth at the mid-span. Furthermore, the fracture stress halfway between the mid-span and the support would theoretically measure at 465MN.m$^{-2}$, half the value of the peak stress. This corresponds to a critical flaw size of 41µm. If fracture occurs at the mid-span due to the latter flaw size mentioned, this would give a stress of 326MN.m$^{-2}$. This therefore indicates that this method does not give an accurate enough representation of the strength limit of the material or location of stress and also the flaw size that causes fracture.\textsuperscript{90}

The stress distribution for four point bending differs considerably compared to three point bending. The region of peak stress in this case covers a much larger region of the tensile surface, in fact this is located between the load points (Figure 45). The values that are obtained for this test are usually lower than the strength values expressed in three point bending. The distribution shows that from the load points to the supports the stress decreases linearly from peak stress to zero stress. Similarly, as mentioned for three point bending, the linear decrease in stress from the tensile surface to the neutral axis is also applicable. The higher surface region in which the peak stress covers results in a higher probability of finding the largest flaw in that region, and so the resulting stress occurring due to that flaw is more representative of the strength of the ceramic. As such the scatter of data will be much less than in three point bending, even though the mean value is lower.\textsuperscript{90,106}
Figure 45. Stress distribution in four point bending.\(^{90}\)

2.10.9 Biaxial strength

Biaxial strength can be thought of as an extension of the four point bending test in two dimensions. Instead of measuring the strength of a specimen bar across a certain length one can imagine rotating the bar around the vertical axis of symmetry (360°) in which the characteristic strength of the specimen is more representative of a flat disc. The test specifications require a specimen disc that is supported by a circular ring in which the load is applied on its opposite side by a concentrically positioned ring (Figure 46). This method exhibits lower strength values compared to uniaxial compression because the probability of fracture occurring due to critical flaws that are subjected to peak stresses is higher.\(^{107}\) However, since the peak stress is distributed over a larger region in biaxial flexure and that flaws are randomly orientated and uniformly distributed the variation in data therefore should be less. The effect of edge defects are minimised when using the concentric ring test, this subsequently allows the strength of the material to be determined by pure surface effects.\(^{90,107}\) The biaxial strength can be expressed by the following equation.\(^{99}\)
\[ \sigma_r = \frac{3F(1 + \nu)}{4\pi t^2} \left[ 1 + \ln \frac{r_2}{r_1} + \frac{1 - \nu}{1 + \nu} \left( 1 - \frac{r_1^2}{2r_2^2} \right) \frac{r_2^2}{r^2} \right] \]

Where

- \( F \): load
- \( t \): sample thickness
- \( \nu \): Poisson’s ratio
- \( r_1 \): radius of loading ring
- \( r_2 \): radius of supporting ring
- \( r \): radius of disc specimen

Figure 46. Concentric ring on ring biaxial flexure test.

2.10.10 Weibull statistics

The presence of defects within brittle materials, whether they are microscopic, structural or material, can act as small stress concentrators which can consequently initiate propagation
as a result of loading. Specimens that are used in testing can be manufactured from the same brittle material, however, the distribution of defects between each specimen is random, with respect to size and severity. This presents the material with a large variability in strength. So, for a material to express an accurate enough value that is characteristic of its strength can prove to be difficult. The distribution of crack lengths can also influence the strength of a material. Larger specimens will tend to exhibit lower strength values than smaller ones, since the probability of finding one of the larger flaws in the larger specimen is greater. This suggests that the strength is dependent on the volume of the sample. Hence, a useful method of predicting whether a material can express a given strength is by estimating the survival probability or Weibull probability distribution.\textsuperscript{90,106}

\[
P_s(V_0) = \exp \left[ - \left( \frac{\sigma}{\sigma_0} \right)^m \right]
\]

Equation 34

Where
\[
\begin{align*}
m & \text{ : constants} \\
\sigma_0 & \text{ : Tensile loading} \\
\sigma & \text{ : Tensile loading}
\end{align*}
\]

The equation shows the survival probability of a number of identical samples with a given volume $V_0$. The survival probability is expressed as a function of the specimens that survive loading (tensile stress $\sigma$). For all specimens to survive, $\sigma$ must equal zero, hence $P_s(V_0) = 1$. As $\sigma$ increases the $P_s(V_0)$ value decreases, meaning that the number of samples failing increases progressively. $P_s(V_0)$ approaches zero as the value of $\sigma$ approaches infinity. This implies that the greater the value of $\sigma$ the lower the probability of specimens that survive loading. A value of $\sigma = \sigma_0$ (where $m = 0$) would give a probability of survival of 37%, this is equated as $P_s(V_0) = 1/e$, moreover it can be said that if $\sigma$ approaches $\sigma_0$ at a
rapid rate then the range of loads applied (between $P_s(V_o) = 1 \leftrightarrow P_s(V_o) \equiv 0$) is small, which suggests that the variability in strength data is small. This rate is influenced by the magnitude of the Weibull modulus $m$. The Weibull modulus $m$, can be determined by the slope from the log of the equation:

$$\ln \left[ \ln \left( \frac{1}{P_s(V_o)} \right) \right] = m \ln \left[ \frac{\sigma}{\sigma_o} \right]$$

Equation 35

For a given batch of $N$ samples the $P_s$ values can be calculated:

$$P_{si} = 1 - \frac{(i - 0.3)}{N + 0.4}$$

Equation 36

2.10.11 Fracture toughness

Discussions made on the subject of fracture and strength up to now have only been concerned with critical flaw size. It is also reasonable therefore to approach this with respect to crack surface displacement and the stresses at the crack tip.$^{90,106}$

There are three stress intensity factors associated with stress concentration at a crack tip, these are known as $K_1$, $K_{II}$ and $K_{III}$. The subscripts associated with these factors can be categorised as follows (Figure 47):
$K_1$ (opening mode): Tensile stress is applied perpendicular (normal) to the plane of the crack.

$K_{II}$: This is a shearing mode where the load is applied parallel to the plane of the crack and also along the same direction in which it propagates.

$K_{III}$: This is referred to as a torsional load where again the load is applied in a parallel plane to the crack, however, the direction of propagation is normal to the applied load.

---

Since ceramics are brittle materials and frequently display this behaviour under tension, and that the direction of crack propagation almost always occurs normal to the direction of maximum tension regardless of the direction in which the load is applied, then $K_I$ is usually used in this case to represent the stress intensity factor. Therefore, for ceramics this mode of fracture can be termed as the critical stress intensity factor or fracture toughness $K_{IC}$. 

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Figure 47. Diagrams showing the three modes of fracture. 

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2.10.12 Fracture toughness measurements

A wide range of techniques have been developed to test the fracture toughness of specimens in which the design of the technique is based on the specimen geometry.

1. 

\[ K_I = Y \frac{3PL}{2bW^2} \sqrt{a} \]

\[ Y = 1.96 - 2.75 \left( \frac{a}{W} \right) + 13.66 \left( \frac{a}{W} \right)^2 - 23.98 \left( \frac{a}{W} \right)^3 + 25.22 \left( \frac{a}{W} \right)^4 \]

Figure 48 Single-edge-notched beam test.\(^{90}\)

2. 

\[ K_I = PWm \left[ \frac{3(1 + \nu)}{W_1 \rho} \right]^{\frac{1}{2}} \]

Figure 49 Double torsion (DT) specimen.\(^{90}\)
3.

\[ K_I = \frac{\text{moment}}{\sqrt{It}} \quad I : \text{Inertia of one arm.} \]

Figure 50 Constant-moment specimen.  

4.

\[ K_I = 2P \left( \frac{m}{nt_n} \right) \]

\[ M = \frac{1}{t} + \frac{3a^2}{h^3} \]

Figure 51 Tapered double cantilever beam specimen.  

165
2.10.12.1 **Indentation method**

The method of indentation has become a more applicable way of measuring the fracture toughness of a material.\textsuperscript{108-112} The use of micro-indentors with Vickers and Knoop geometry create an indent impression when loaded onto the surface of the material that are analogous to the characteristic dimensions of the microstructure. However, what can also be obtained is the measure of hardness of the material, which is related to other properties such as compressive strength, wear and erosion.

The hardness values that can be obtained by either Vickers or Knoop hardness and can be expressed by the following equations. Vickers hardness is a measure of the applied load with respect to the contact area:

\[
H_V = \frac{P}{A_c} = \frac{0.2P}{d_1^2 \sin \frac{136^\circ}{2}} \tag{Equation 37}
\]

Where
\begin{align*}
P & : \text{Load} \\
A_c & : \text{Contact area} \\
d_1 & : \text{Average length of diagonals}
\end{align*}

Knoop hardness, however, is calculated by dividing the applied load by the projected area:

\[
H_K = \frac{P}{A_p} = \frac{2P}{d^2 \cot \frac{172.5^\circ}{2} + \tan \frac{130^\circ}{2}} \tag{Equation 38}
\]

Where
\begin{align*}
d & : \text{length of the major diagonal} \\
A_p & : \text{Projected area of indent}
\end{align*}
A simple diagram (Figure 52) can be shown to illustrate the geometry of a Vickers impression which gives the crack dimensions and indentation lengths:

![Diagram of Vickers indentation](image)

**Figure 52.** Vickers indentation.

It has been reported that the crack length dimensions obtained from Vickers hardness can be related to fracture toughness. A value of fracture toughness, therefore, can be obtained using an equation proposed by Laugier:\(^{109}\)

\[
K_{IC} = 0.015 \frac{(a - d)}{d} \left( \frac{E}{H} \right)^{2/3} \frac{F}{a^{3/2}}
\]

**Equation 39**

Where

- \(a\) : Crack length
- \(d\) : Half the indent diagonal
- \(F\) : Load
- \(E\) : Young's modulus – calculated from concentric ring test
- \(H\) : Hardness
3 Analytical methods
3.1 **Infrared spectroscopy**

The infrared region of the electromagnetic spectrum can provide chemists and material scientists with information about the energy of molecular vibrations. This is a useful tool for distinguishing the different functional groups inherent within a material. When ceramics are heated to very high temperatures they usually undergo either a change in structure and/or may undergo a loss of atoms or functional groups. This behaviour can be detected using infrared and is reflected in the changes of vibrational mode, a shift in vibrational mode frequency or intensity and shape of the peak to which a mode is assigned.\(^{113,114}\)

3.1.1 Infrared spectrometer

The application of infrared spectroscopy involves the emission of infrared light from a source throughout the whole frequency range of the instrument.\(^{113,114}\) The infrared light is then split into two equally intense beams, in which one beam is passed through the sample. Absorption of energy of this frequency may occur provided the frequency of vibration of a sample or molecule lies within the range of the instrument, in which case the intensity of the beam passing through the sample is compared against the other beam. A prism or grating within the instrument will spread this information in the form of transmittance peaks across a wavelength range over which this comparison is made. Each peak will correspond to a characteristic absorption at the wavelength or frequency to which it is assigned. Compounds may be examined in vapour phase, as pure liquids, in solution, and in the solid state.
At an atomic level, the process of infrared is considered as the absorption of infrared radiation by a molecule or solid resulting in the promotion of a photon from a lower vibrational energy state to a higher one (Figure 53). The energy of separation of vibrational states must be equivalent to the energy of the photon for this to occur.

**Figure 53. Infrared absorption.**

Raman spectroscopy, however, concerns the use of visible light. In contrast to infrared the energy here is not absorbed. Since the energy of a visible photon is much greater than the fundamental vibrational transitions in molecules and solids, the spectrum that is obtained measures the scattering of photons. This is where the photon loses some of its energy. This loss corresponds to the vibrational transition energy and also correlates to a shift to lower frequency of the visible photon (Figure 54).
3.1.2 Fourier transform infrared (FT-IR)

Fourier transform infrared has become a widely used technique, this is due to its high sensitivity and the digital data handling capability. This technique provides the user with the same information as traditional infrared, however, it utilises the interference effects between two rays from a broad band source, as a result what is detected is a signal that oscillates in time and is referred to as an interferogram. This signal is converted using a mathematical technique so that it can be interpreted as a function of frequency so that it resembles a normal infrared spectrum.
3.1.3 Selection rules

Any molecular vibrations that occur result in an oscillating dipole moment, when this interacts with the oscillating electric vector of the infrared beam infrared light is then absorbed. The requirement for this can be explained by a simple rule; if there is a difference in dipole moments between the two extremes of a vibration then the interaction will occur. Raman, however, involves the interaction of the light with the molecule’s polarizability, this requires different selection rules.\textsuperscript{113}

The difference in selection rules for infrared and Raman are simple to envisage. If the vibration about the centre of symmetry of a molecule is symmetric then the interaction is active in the Raman and inactive in the infrared, if it is anti-symmetric then it is inactive in Raman and active in the infrared (Figure 55).

![Symmetric and antisymmetric modes](image)

Figure 55. Symmetric and antisymmetric modes.\textsuperscript{114}
3.2 X-ray diffraction

3.2.1 Crystal structure

3.2.1.1 Lattice and unit cells

In order to understand the concept of a lattice in crystal structure, one must be able to visualise this crystal as a structure that contains individual entities of a certain pattern that from the building block of a crystal. A crystal is usually constructed from atoms, ions or molecules which are termed asymmetric units. The locations of the asymmetric units that form the pattern can be represented by points that are referred to as the space lattice, which in effect is considered as an abstract scaffolding for the crystal structure.

The basic three dimensional crystal structure consists of an infinite array of points that defines a basic repeating pattern and vice-versa. This scaffold does not necessarily imply that an asymmetric unit must be centred on each lattice point, in essence, the space lattice and the identical asymmetric unit are associated when one uses these terms to define the crystal structure.

The pattern within a crystal structure, can be thought of as an imaginary parallelepiped that contains one unit or unit cell that is translationally repeated (Figure 56). The unit cell is the smallest (fundamental) repeating unit that makes up the entire structure. Primitive unit cells are composed of neighbouring lattice points that are joined by straight lines, in which each unit cell has one lattice point at its origin. Non-Primitive unit cells can also be depicted, however, the lattice points in this case are located at their centres or on pairs of faces.
Figure 56. Translationally repeating unit cell.\textsuperscript{94}

Unit cell parameters are used to define the length of sides and the angles between them, these are given as \(a\), \(b\) and \(c\) (lengths) and \(\alpha\), \(\beta\) and \(\gamma\) (angles) (Figure 57).

Figure 57. Unit cell defined by its parameters.\textsuperscript{94}
A unit cell has an inherent rotational symmetry to which it belongs. The rotational symmetry of unit cells can be otherwise referred to as crystal systems of which there are seven. A cubic lattice, the simplest structure, has unit cells that have a rotational symmetry in which there are four threefold axes in a tetrahedral array (Figure 58).

![Rotational symmetry of a cube](image)

**Figure 58. Rotational symmetry of a cube.**

Primitive and non-primitive crystal lattices in three dimensions are termed Bravais lattices. Primitive unit cells are labelled P, in which the points are located only at the corners of the unit cells. Unit cells that are denoted as I are referred to as body centred unit cells where the lattice points are located at the centre. Those denoted by F are faced centred unit cells in which the lattice points are found at the corners and at the centres of faces.
3.2.1.2 Lattice planes

A crystal structure contains many different sets of planes, so in order to visualise them and understand their significance it would therefore be much easier to imagine the lattice in two dimensions rather than in three dimensions (Figure 59).

![Diagram of lattice planes](image)

Figure 59. Lattice planes.\(^{94}\)

3.2.1.3 Miller indices

If one imagines a rectangular lattice in two dimensions, each plane in the lattice intersects the a and b axis that define a unit cell at a certain point. Each parallel plane can therefore be denoted by distances which describe the smallest intersection distances in that unit cell.
If this lattice is projected in three dimensions or as an orthorhombic lattice, then the
distances would be expressed as (a, b, c), where c is the length parallel to the z axis.
Therefore, for each set of planes that have been mentioned (Figure 59a to d) the distances
would be expressed as follows:

(a) \((1,1,\infty)\) \hspace{1cm} (b) \((1/2,1/3, \infty)\) \hspace{1cm} (c) \((-1,1, \infty)\) \hspace{1cm} (d) \((\infty,1, \infty)\)

For convenience sake, these intersection distances can be taken as reciprocal values and are
termed Miller indices. Negative indices are denoted by a bar over the number. For each set
of planes that have been mentioned the Miller indices would be expressed as follows:

(a) \((1 1 0)\) \hspace{1cm} (b) \((2 3 0)\) \hspace{1cm} (c) \((\bar{1} 1 0)\) \hspace{1cm} (d) \((0 1 0)\)

Miller indices are usually referred to as \((h, k, l)\) planes, the letters used in this instance
correspond to each of the reciprocal values that define a plane. The \(h, k, l\) indices
correspond to the intersections along the \(a, b\) and \(c\) axis respectively.

3.2.1.4 Separation of planes

The separation of planes is denoted by \(d\) spacing. For a cubic lattice this relationship
between \(d\) spacing and Miller indices can be given:

\[
\frac{1}{d_{hkl}^2} = \frac{h^2 + k^2 + l^2}{a^2}, \quad \text{or} \quad d_{hkl} = \frac{a}{\sqrt{h^2 + k^2 + l^2}} \quad \text{Equation 40}
\]
For a general orthorhombic lattice this relationship is given as:

$$
\frac{1}{d^2_{hkl}} = \frac{h^2}{a^2} + \frac{k^2}{b^2} + \frac{l^2}{c^2}
$$

Equation 41

3.2.2 X-Rays

The emission of x-rays arise from the bombardment of a metal with high energy electrons. The radiation produced is electromagnetic with wavelengths of about 100 pm. Bremsstrahlung is a term that refers to the deceleration of electrons as they bombard the metal upon which radiation is generated with a continuous range of wavelengths. The collision of the incoming high electrons with inner-shell electrons from the atoms result in a few sharp high intensity peaks that are superimposed on the continuum, these correspond to the emission of excess energy in the form of an x-ray photon (Figure 60).

Figure 60. X-ray emission.
3.2.3 The Bragg law

The application of x-rays as a means of establishing the crystal properties of a material was not realised until the early part of the 20th century. Max von Laue suggested that when x-rays are passed through a crystal they are diffracted, and that there was a relationship between their wavelengths and the separation of planes.\(^4\)

A crystal can be considered as being made up of stacks of reflecting planes that are separated by d-spacing, with each lattice plane regarded as a mirror in which x-rays can be reflected.

An incoming beam of x-rays may be reflected if the crystal is orientated at a given angle, this is known as constructive interference and appears as an intense spot. The angle at which this interference occurs can be easily calculated and is based on the model of lattice planes (Figure 61). Thus, a plane at a given angle in a crystal is responsible for this constructive interference.

Figure 61. Bragg model of lattice planes.\(^{94}\)
This diagram depicts the path length difference of two rays. The path length difference is related to the glancing angle of the ray and the d-spacing through the following relationship:

\[ AB + BC = 2dsin\theta \]  \hspace{1cm} \text{Equation 42}

The path length difference can be comparable to the wavelength of the incoming ray, however, most rays do not interfere constructively and are out of phase. This infers that the path length difference is not an integral number of wavelengths. During constructive interference the path length difference is an integral number of wavelengths, hence \( AB + BC = n\lambda \). When the glancing angle satisfies the Bragg law then a bright reflection should be observed, therefore:

\[ n\lambda = 2dsin\theta \]  \hspace{1cm} \text{Equation 43}

The separation of planes \( d \) are associated to an integral number of planes at angle \( \theta \), in which the x-rays are reflected and so for reflections corresponding to \( n = 1,2,3,4 \ldots \text{etc} \), the Bragg law can be simplified:

\[ \lambda = 2dsin\theta \]  \hspace{1cm} \text{Equation 44}

Hence, the \( n^{th} \) order of reflection corresponds to the \((nh, nk, nl)\) planes.
3.2.4 The Debye-Scherrer method

This technique, which was developed by Peter Debye and Paul Scherrer, used a powdered sample instead of a single crystal and monochromatic radiation. The orientation of some of the crystallites in the powdered sample should satisfy the Bragg condition for each set of planes \((h \ k \ l)\). For instance, the set of \((111)\) planes, with plane separation \(d_{111}\), belonging to some crystallites of given orientation will give rise to diffracted intensity at glancing angle \(\theta\). Crystallites having the same glancing angle \(\theta\) will be orientated at all possible angles around the incoming beam, giving rise to diffracted beams that lie on a cone around the incident beam of half angle \(2\theta\).

This applies to other crystallites with orientation of different planes that satisfy the Bragg law. Therefore, each set of \((h \ k \ l)\) planes will give rise to a diffraction cone of associated diffracted intensity and half angle.

The Debye-Scherrer method uses a rotating capillary tube to give a random orientation of crystallites, in which the diffraction cones are detected by a photographic film. Modern diffractometers utilise a flat plate for mounting the sample, the intensities of the reflections and the diffraction patterns are collected and interpreted electronically.

The identification of a sample can be made by using powder diffraction, this can be done by comparing the positions of the diffraction lines and their associated intensities with a large data bank. Phase diagrams can also be determined using this technique. A powder may contain a mixture of phases and so the relative amounts of each phase may be
determined also. Identification of different phases are distinguished by the different
diffraction patterns that they exhibit.

3.2.5 Indexing reflections

The unit cell dimensions of a given sample can be easily deduced. If the angle \( \theta \), which is
determined from a given diffraction intensity, and the corresponding separation of planes \( d \)
is known then the unit cell dimensions \( (a, b, c) \) can be calculated from the Bragg equation.

Taking the cubic lattice as a simple case, the Bragg law would be expressed as:

\[
d = \frac{a}{(h^2 + k^2 + l^2)^{1/2}} , \quad \text{for cubic lattice:} \quad a = b = c \quad \text{Equation 45}
\]

\[
2d \sin \theta_{hkl} = \lambda ,
\]

\[
\sin \theta_{hkl} = \frac{\lambda}{2d} ,
\]

Hence,

\[
\sin \theta_{hkl} = (h^2 + k^2 + l^2)^{1/2} \frac{\lambda}{2a} \quad \text{Equation 46}
\]
4 Materials and Methods
4.1 Preparation of phosphate glasses

The glass composite that was selected for incorporating into HA was of a specific composition. The components that made up the different glass composites varied. In essence, the main components that were introduced included calcium oxide (CaO) and phosphorous pentoxide (P$_2$O$_5$), however, the difference in composition from glass to glass was the combination of the remaining components such as Na$_2$O, MgO and CaF$_2$ (Table 12). The starting reagents used to prepare a glass, depending on the composition selected, consisted of the following compounds; NaH$_2$PO$_4$, P$_2$O$_5$, CaCO$_3$, CaHPO$_4$, MgCO$_3$ and CaF$_2$. The starting reagents required to make the selected glass were mixed thoroughly and placed in a platinum crucible, melted between 1000 and 1100°C for one hour, and then poured onto a steel plate and allowed to cool. The resulting glass was then ground to a fine powder using a ball and agate grinder.

<table>
<thead>
<tr>
<th>Glass Code</th>
<th>CaO (mol%)</th>
<th>P$_2$O$_5$ (mol%)</th>
<th>Na$_2$O (mol%)</th>
<th>MgO (mol%)</th>
<th>CaF$_2$ (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNP</td>
<td>32</td>
<td>45</td>
<td>23</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CP15Mg</td>
<td>21.25</td>
<td>63.75</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>CP15F</td>
<td>21.25</td>
<td>63.75</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>CP7.5Mg7.5F</td>
<td>21.25</td>
<td>63.75</td>
<td>0</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>CP20Mg</td>
<td>16.25</td>
<td>63.75</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>CP20F</td>
<td>16.25</td>
<td>63.75</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>CP10Mg10F</td>
<td>16.25</td>
<td>63.75</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 12. Table of glass composites with their designated codes.
4.1.1 Determination of weight of starting reagents for glass preparation

1. CaO: X (mols); Na₂O: Y (mols); P₂O₅: Z (mols)

\[
\begin{align*}
2\text{NaH}_2\text{PO}_4 & \rightarrow \text{Na}_2\text{O} + \text{P}_2\text{O}_5 + \text{H}_2\text{O} \\
\text{RMM} & \quad 239.8716 \quad 61.97 \quad 141.89 \\
\text{Fraction} & \quad 0.258 \quad 0.592 \\
\end{align*}
\]

\[ \frac{Y}{100} \times 239.8716 = \text{weight of NaH}_2\text{PO}_4 \text{ required as starting reagent (A)} \]
\[ A \times 0.592 = \text{weight of P}_2\text{O}_5 \text{ expelled during reaction (B)} \]
\[ \frac{Z}{100} \times 141.89 = \text{weight of P}_2\text{O}_5 \text{ needed in total (C)} \]
\[ C - B = \text{weight of P}_2\text{O}_5 \text{ required as starting reagent (D)} \]

\[
\begin{align*}
\text{CaCO}_3 & \rightarrow \text{CaO} + \text{CO}_2 \\
\text{RMM} & \quad 100.06 \quad 56.07 \\
\end{align*}
\]

\[ \frac{X}{100} \times 100.06 = \text{weight of CaCO}_3 \text{ required as starting reagent (E)} \]
2. CaO: X (mols); MgO: Y (mols); P$_2$O$_5$: Z (mols)

\[
\begin{align*}
2\text{CaHPO}_4 & \rightarrow 2\text{CaO} + \text{P}_2\text{O}_5 + \text{H}_2\text{O} \\
\text{RMM} & = 272.12 \\
\text{Fraction} & = 0.412 \\
\end{align*}
\]

\[
\begin{align*}
\frac{X}{100} \times 272.12 & = \text{weight of CaHPO}_4 \text{ required as starting reagent (A)} \\
A \times 0.522 & = \text{weight of P}_2\text{O}_5 \text{ expelled during reaction (B)} \\
\frac{Z}{100} \times 141.89 & = \text{weight of P}_2\text{O}_5 \text{ needed in total (C)} \\
C - B & = \text{weight of P}_2\text{O}_5 \text{ required as starting reagent (D)} \\
\text{MgCO}_3 & \rightarrow \text{MgO} + \text{CO}_2 \\
\text{RMM} & = 84.31 \\
\end{align*}
\]

\[
\begin{align*}
\frac{Y}{100} \times 84.31 & = \text{weight of MgCO}_3 \text{ required as starting reagent (E)} \\
\end{align*}
\]
3. CaO: X (mols); CaF₂: Y (mols); P₂O₅: Z (mols)

\[
2\text{CaHPO}_4 \rightarrow 2\text{CaO} + \text{P}_2\text{O}_5 + \text{H}_2\text{O}
\]

\[
\begin{array}{c|c|c}
\text{RMM} & 272.12 & 112 & 141.89 \\
\text{Fraction} & 0.412 & 0.522 \\
\end{array}
\]

\[
\frac{X}{100} \times 272.12 = \text{weight of CaHPO₄ required as starting reagent (A)}
\]

\[
A \times 0.522 = \text{weight of P₂O₅ expelled during reaction (B)}
\]

\[
\frac{Z}{100} \times 141.89 = \text{weight of P₂O₅ needed in total (C)}
\]

\[
C - B = \text{weight of P₂O₅ required as starting reagent (D)}
\]

\[\text{CaF}_2\]

\[
\begin{array}{c|c}
\text{RMM} & 78.08 \\
\end{array}
\]

\[
\frac{Y}{100} \times 78.08 = \text{weight of CaF₂ required as starting reagent (E)}
\]
4. CaO: X (mols); MgO: Y (mols); CaF₂: R (mols); P₂O₅: Z (mols)

\[
2\text{CaHPO}_4 \rightarrow 2\text{CaO} + \text{P}_2\text{O}_5 + \text{H}_2\text{O}
\]

\[
\begin{array}{c|c|c|c}
\text{RMM} & 272.12 & 112 & 141.89 \\
\text{Fraction} & 0.412 & 0.522 \\
\end{array}
\]

\[
\frac{X}{100} \times 272.12 = \text{weight of CaHPO}_4 \text{ required as starting reagent (A)}
\]

\[
A \times 0.522 = \text{weight of P}_2\text{O}_5 \text{ expelled during reaction (B)}
\]

\[
\frac{Z}{100} \times 141.89 = \text{weight of P}_2\text{O}_5 \text{ needed in total (C)}
\]

\[
C - B = \text{weight of P}_2\text{O}_5 \text{ required as starting reagent (D)}
\]

\[
\text{MgCO}_3 \rightarrow \text{MgO} + \text{CO}_2
\]

\[
\begin{array}{c|c|c}
\text{RMM} & 84.31 & 40.3 \\
\end{array}
\]

\[
\frac{Y}{100} \times 84.31 = \text{weight of MgCO}_3 \text{ required as starting reagent (E)}
\]

\[
\text{CaF}_2
\]

\[
\begin{array}{c|c}
\text{RMM} & 78.08 \\
\end{array}
\]

\[
\frac{R}{100} \times 78.08 = \text{weight of CaF}_2 \text{ required as starting reagent (F)}
\]
4.2 Preparation of hydroxyapatite (HA) & glass reinforced hydroxyapatite (GR-HA)

4.2.1 Milling of HA

HA (200g) and methanol (300ml) were placed into a porcelain mill pot and the mixture was then wet milled for 24 hours.

4.2.2 Milling of GR-HA

The glass (5g or 10g) was placed into a porcelain mill pot (Pascall Engineering Co. Ltd, UK) and milled dry for 24 hours. HA (supplied by Plasma Biotal Ltd. UK) was then added to the mill pot at either 195 or 190g to glass additions of 2.5% and 5% glass additions, respectively, depending on the weight addition chosen. Methanol GPR grade (BDH Laboratory Supplies, UK) (300ml) was also added and the mixture was then wet milled for a further 24 hours.

4.2.3 Preparation of HA and GR-HA specimen discs

The resulting slip that was obtained from milling was then dried at 70°C and the dry powder was then sieved to give a particle size of less than 75μm using a vibratory sieve shaker (Fritsch, Germany) through Endecotts sieves (supplied by Christisons, Newcastle, UK) with aperture sizes of 1mm, 200μm, 150μm, 100μm and 75μm. Four grams of powder was placed into a custom made steel die and uniaxially pressed at 20 tons using a hydraulic press to give discs of 30mm diameter and thickness of ≈2.5mm. The discs were fired at a heating rate of 4°Cmin⁻¹ to 1200°C, 1250, 1300°C and 1350°C in a furnace (Lenton Thermal Designs Ltd, UK). The specimens were held at the appropriate temperature for 1
hour and then furnace cooled. The specimens obtained were ≈25mm in diameter and ≈2.5mm in thickness.

4.3 Hot pressing

The sieved HA/GR-HA powder (25g) was poured into a graphite sheet lined carbon mould and the powder was compacted, initially, to ≈ 1 ton using a manual hydraulic pump. The mould was constructed to form 40 × 40mm square ceramic blocks. The graphite mould was then placed into a vacuum hot press chamber (JungMin High Vac. Co. Korea). The chamber was then pumped with a mechanical rotary pump to between 200 - 300 mTorr. The rotary vacuum pump was turned off and the diffusion pump was then turned on and the chamber was pumped to obtain a vacuum level of about 30 to 60 mTorr. This vacuum level was often difficult to attain due to degassing and so the chamber was purged several times with Ar gas. Once this vacuum level was reached the chamber was heated at a rate of 10°C min⁻¹ to sintering temperature. During heating and when the temperature reached 600-650°C the vacuum was turned off and Ar gas was allowed to flow through the chamber. From this point the powder was compacted stepwise using a hydraulic oil press until it reached the sintering temperature where full load (3500kg) was applied. The powder was compacted and sintered at this temperature for 1 hour. After heating at the required temperature the hydraulic pump was then turned off, the inlet and outlet gas valves where shut, and the furnace was allowed to cool. The resulting hot pressed blocks that were produced were 40 × 40 × 5mm in dimension.
Ideally, it would have been more appropriate to have hot pressed 25 x 2.5mm discs so as to compare against the pressureless sintered specimens. However, this may not have been possible since the equipment available was designed to produce 40 x 40 x 5mm blocks.

4.3.1 Cutting / Grinding / Polishing / Bevelling

The blocks were cut using a diamond saw (Daisan machine Co, Korea) to 40 x 5 x 4mm bars suitable for four point bending tests. They were then ground to a thickness of 3mm to 40 x 4 x 3mm. The tensile test surface of the specimen blocks were polished to 1μm and the edges of that surface were bevelled to eliminate edge cracks/flaws.

4.4 Shrinkage

The determination of linear shrinkage before and after firing was done by measuring the change in diameter of the specimen disc before (\(l_o\) - initial diameter) and after sintering (l) and dividing by the initial diameter:

\[
\text{Shrinkage} = \frac{l_o - l}{l} \times 100
\]

Equation 47

4.5 Measured density determination

Density measurements were performed on each sintered specimen using the Archimedes principle. The measurements were carried out in distilled water on a Mettler Toledo AG204
balance and density kit. These were then compared with the theoretical densities calculated from the Rietveld analysis to give the porosity.

\[
\rho = \frac{\text{actual weight}}{(\text{actual} - \text{suspended})} \times \rho_w
\]

\text{Equation 48}

Where

\[\rho_w\] : density of water

The density measurements for the hot pressed specimens were carried out slightly differently. This method uses the saturated weight of the sample instead. This measures the amount of water that is saturated by the sample and takes into consideration the porosity of the sample. This can be calculated by the following equation:

\[
\text{Real density} = \frac{\text{dry weight}}{(\text{saturated weight} - \text{suspended weight})} \times \rho_w
\]

\text{Equation 49}

4.6 X-ray diffraction

Tested samples were ground to a fine powder, placed in a specimen holder and then analysed on a Philips PW1070 diffractometer with Ni filtered CuK\(_{\alpha}\) radiation (K\(_{\alpha1}\) = 1.5406 Å, K\(_{\alpha2}\) = 1.5444 Å) at 40kV and 30mA. The data were collected with a scintillation counter between 10<20<100° with a step size of 0.02° and count time of 12s using flat plate geometry. However, the data presented in the results focused on the pattern between 20 and 40°.
4.6.1 Structure refinement

The structure refinement was carried out using General Structure Analysis Software (GSAS, Los Alamos National Laboratory). The glass reinforced HA will always exhibit a diffraction pattern containing the HA phase, however, depending on the composition of the glass incorporated, the pattern may exhibit β-TCP or both β-TCP and α-TCP secondary phases. A standard model for each of the three phases was used for the refinement of the samples and they were determined from the Daresbury Crystal Structure Database. The HA model was based on the single crystal structure determination with P63/m space group and lattice parameters of $a = 9.45\,\text{Å}$ and $c = 6.88\,\text{Å}$. The second phase β-TCP consists of a R3CH space group and lattice parameters of approximately $a = 10.4\,\text{Å}$ and $c = 37.4\,\text{Å}$. The standard model used to refine α-TCP was based on the P21/a space group and unit cell dimensions of $a = 12.887\,\text{Å}$, $b = 27.28\,\text{Å}$, $c = 15.219\,\text{Å}$, and $\beta = 126.2^\circ$.

The peak shapes for the refinement were modelled on the pseudo Voigt distribution. Background parameters, a scale factor, four peak shape variables, an asymmetry factor, cell parameters, and a zero point correction were all refined. Isotropic thermal parameters and atom positions were refined for all HA atoms. For β-TCP and α-TCP all the parameters were refined; however, the thermal parameters and atom positions were fixed.

From the data output from the GSAS list file, a number of other parameters are calculated and these data are presented. The software calculates the phase weight percentage and the theoretical density (assuming no porosity). The calculation for these numbers does not include any error determination.
From the data for the measured density and the theoretical density, the porosity is calculated using:

\[
\frac{\text{recorded density}}{\text{theoretical density}} \times 100(\%) = \text{porosity}
\]

Equation 50

This again does not have an associated error with it.

4.7 Fourier Transform Infrared (FT-IR)

FT-IR analysis was carried out on powdered samples using a Perkin Elmer system 2000 FT-IR. The samples were placed on a diamond ATR (attenuated total reflectance) specimen holder. The source that was passed through the specimen was in the mid infrared range. The incident beams were split using a KBr beamsplitter and the infrared peaks were collected as an absorbance versus wavenumber pattern within a scan range of 400 cm\(^{-1}\) to 4000 cm\(^{-1}\).

4.8 Mechanical testing

The test carried out was the flexural bend strength test method using a concentric ring testing jig with a loading ring of 10 mm and outer supporting ring of 20 mm. Ten specimens were tested for each of the firing temperatures on an Instron machine at a crosshead speed of 5 mm min\(^{-1}\) to failure. The point of failure is indicated by a maximum load (F) value taken from the load-displacement graph. The flexural bend strength in biaxial bending was determined by the following equation:
\[
\sigma_r = \frac{3F(1+v)}{2\pi^2} \left[ \ln \frac{r_2}{r_1} + \frac{(1-\nu)(r_2^2-r_1^2)}{(1+\nu)2r^2} \right]
\]

Equation 51

Where

- \( \sigma_r \): Flexural bend strength
- \( F \): Load
- \( \nu \): Poissons ratio (assumed value: 0.28)
- \( t \): thickness of specimen
- \( r_1 \): radius of loading ring
- \( r_2 \): radius of supporting ring
- \( r \): radius of disc

4.8.1 Four point bend test

The specimen bars that were prepared by hot pressing were mechanically tested using the four point bending test (Tesilon Toyo Baldwin, A&D Co Ltd, Japan) at a crosshead speed of 1mm min\(^{-1}\) to failure. The bars were supported by two support noses that were 30mm apart, with each 15mm away from the midpoint of the bar. The load span consisted of two loading noses that were 10mm apart, with each 5mm away from the midpoint of the bar.

The flexural bend strength was determined by the following equation:

\[
\sigma_{b4} = \frac{3P(L-I)}{2Wt^2}
\]

Equation 52

Where

- \( \sigma_{b4} \): Flexural strength in four point bending
- \( P \): load(N)
- \( L \): outer span
- \( I \): inner span
- \( W \): width of specimen
- \( t \): thickness of specimen
4.8.2 Modulus of elasticity (flexural modulus)

The modulus of elasticity was obtained from the slope of the initial straight line (elastic) portion of the load displacement graph. This is the ratio of stress to strain and can be calculated from the following equation.

\[
E = \frac{0.17L^3m}{bd^3}
\]

Equation 53

Where

- \( E \): modulus of elasticity
- \( L \): support span
- \( m \): slope of the tangent to the initial straight-line portion of the load – deflection curve
- \( b \): diameter of disc tested
- \( d \): thickness of specimen

The modulus of elasticity for the hot pressed specimens was determined using the ultrasound method. This measures the time it takes for an ultrasound to travel the thickness of the sample, in both longitudinal and transverse directions. This value is converted to velocity. Together with the density of the sample and Poisson’s ratio the value for elasticity can be calculated:

\[
V_t = \frac{2d}{T_{l}}, \quad V_t = \frac{2d}{T_{l}}, \quad \text{Poisson’s ratio } v = \frac{1 - 2\left(\frac{V_t}{V_i}\right)^2}{2 - 2\left(\frac{V_t}{V_i}\right)^2}
\]

Elastic Modulus (E) = \[
\frac{V_t^2 \rho(1 + \nu)(1 - 2\nu)}{(1 - \nu)}
\]

Equation 54
Where,

\[ V_l \text{ and } V_t : \text{longitudinal and transverse velocities respectively} \]
\[ T_l \text{ and } T_t : \text{longitudinal and transverse time respectively} \]
\[ d : \text{thickness of the sample} \]
\[ \rho : \text{density of the sample} \]

4.8.3 Hardness

The vickers hardness of fractured specimens were carried out using a Wallace micro-indendation tester with a 3N load. Each indent that was made was instantaneous, therefore the loading time was not considered in this case. The hardness value for a set of specimens fired and tested (biaxial flexure) at a given sintering temperature was obtained from a fractured test piece. The average hardness from 15 indents was taken as the value representing that set of specimens. This value was determined from the following equation:

\[ H_v = \frac{1854.4 \times F}{(2d)^2} \]

Equation 55

Where

\[ H_v : \text{Hardness} \]
\[ F : \text{Load} \]
\[ 2d : \text{is the average mean of the two indent diagonals} \]
4.8.4 Fracture toughness

Indentation method

Fracture toughness was determined using the indentation method proposed by Laugier and is expressed by the following equation:

$$K_{ic} = 0.015 \left( \frac{a - d}{d} \right)^{\frac{1}{2}} \left( \frac{E}{H} \right)^{\frac{2}{3}} \frac{F}{a^2}$$

Where

- $K_{ic}$ : fracture toughness
- $a$ : crack length
- $d$ : half indent diagonal
- $E$ : modulus of elasticity
- $H$ : Vickers hardness
- $F$ : indentation load

The crack lengths and half indent diagonals for a set of specimens fired at a given temperature were represented as average values taken from 15 Vickers indents (see section 4.8.3) carried out on a fractured piece. These values represented all ten specimens belonging to that set fired at that given temperature. The crack lengths though were very hard to measure since their lengths are known to change as a function of time.

The fracture toughness values for the hot pressed specimen bars were determined by the indentation strength method as well as the indentation method. Both indentations were carried out using a Vickers microhardness indenter (Mitutoyo). The dimensions of the cracks that were induced by an indentation load and also the dimensions of the indentations were used to calculate the fracture toughness with respect to indentation alone.
Fracture toughness with respect to indentation strength (I-S) measures the strength of a pre-cracked sample rather than taking into account the crack dimensions. This value was determined using an alternative equation:

\[ K_c = 0.016 \left( \frac{E}{H} \right)^{1/2} \frac{P}{C^2} \] \hspace{1cm} \text{Equation 56}

Where

- \( E \) : elastic modulus
- \( H \) : hardness
- \( P \) : indentation load to induce a crack
- \( C \) : crack length : half the average of the two cracks

\[ K_c = \eta \sqrt{\frac{E}{H}} \left( \frac{1}{\sigma P} \right)^{1/4} \] \hspace{1cm} \text{Equation 57}

\[ \eta = 0.59 \pm 0.12 \hspace{1cm} ; \hspace{1cm} H_v = \frac{1.8544P_H}{(2a)^2} \]

Where

- \( E \) : elastic modulus
- \( H \) : hardness
- \( P \) : indentation load to induce a crack
- \( P_H \) : indentation load
- \( \sigma \) : indentation strength
- \( a \) : half the average of the two indent diagonals

The fracture toughness results that are presented later for the hot pressed specimens were based on the indentation method, not the indentation strength method. This was because the indentation strength for the HA samples were not recorded due to lack of specimens.
4.9 Microstructure

4.9.1 Specimen preparation

Fractured sintered samples (HA and GR-HA composites) were placed into Struers cups, embedded in epoxy resin (Struers, Glasgow, UK) and left to set. The resin blocks containing the embedded specimens were then milled so as to obtain parallel surfaces. The surface containing the fractured specimens was ground and polished to 1μm using a Struers Rotopol 11 polishing machine (see below for protocol).

Grinding

<table>
<thead>
<tr>
<th></th>
<th>Step 1</th>
<th>Step 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>MD Fuga</td>
<td>MD Largo</td>
</tr>
<tr>
<td>Media</td>
<td>SiC</td>
<td>Diamond spray</td>
</tr>
<tr>
<td>Grain size</td>
<td>220 microns</td>
<td>9 microns</td>
</tr>
<tr>
<td>Lubricant</td>
<td>water</td>
<td>Blue (ethanol)</td>
</tr>
<tr>
<td>Pressure</td>
<td>30N</td>
<td>25N</td>
</tr>
<tr>
<td>Speed</td>
<td>300 RPM</td>
<td>150 RPM</td>
</tr>
<tr>
<td>Time</td>
<td>1 minute</td>
<td>5.5 minutes</td>
</tr>
</tbody>
</table>
Polishing

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Base</strong></td>
<td>MD Dac</td>
</tr>
<tr>
<td><strong>Media</strong></td>
<td>Diamond spray</td>
</tr>
<tr>
<td><strong>Grain size</strong></td>
<td>3 microns</td>
</tr>
<tr>
<td><strong>Lubricant</strong></td>
<td>Blue (ethanol)</td>
</tr>
<tr>
<td><strong>Pressure</strong></td>
<td>25N</td>
</tr>
<tr>
<td><strong>Speed</strong></td>
<td>150 RPM</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>4.5 minutes</td>
</tr>
</tbody>
</table>

The blocks were then cut using a diamond saw (Testbourne Ltd, Hampshire, UK) to a suitable thickness for mounting. The surface of the specimens were then etched in 10% HCl (AnalR) (BDH Laboratory supplies, UK) solution for 1.5 minutes. The blocks were then mounted onto stubs using Araldite (Devcon; Northants, UK) with the specimens facing upwards, these were left to dry for 24 hours. Graphite was then applied from the top surface of the mounted blocks to the stub, this allows a better electron conduction. The specimens were then sputter coated with gold using an S150A sputter coater. The surface of the specimens were then observed using a Cambridge Stereoscan S90B (Cambridge Instruments; Crawley, UK).
4.9.2 Image Analysis

The images were recorded on photographic film and developed. Grain size measurements were carried out by placing an acetate sheet on top of the image and drawing around the grain boundaries of each grain in the image. The acetate was then scanned and captured as a black and white image. The grain count and grain size (area) were carried out by image analysis using the Image Pro Plus software (Media cybernetics, USA). Hence, the mean grain size and grain size distribution for 100 grains for specimens sintered at a given temperature were therefore determined.

\[
\text{Grain size} = \left( \frac{\text{Area}}{\pi} \right)^{\frac{1}{2}} \times 2
\]

Equation 58

4.10 Rheology

4.10.1 Precipitation of HA

HA was prepared from solutions of calcium nitrate tetrahydrate (118.06g in 900ml of double distilled water (DDW)) (BDH Laboratory Supplies, UK) and diammonium hydrogen phosphate (39.64g, 1600ml DDW) (BDH Laboratory Supplies, UK), both solutions were adjusted to approximately pH11 with ammonium hydroxide (Sigma-Aldrich, Gillingham, Dorset, UK). The diammonium solution was then added dropwisie to the calcium solution (held at either 60 or 80°C) and then left to mature at that temperature for 24 h. The remaining suspension was then rinsed five times with DDW (5 × 1 L), filtered via a Buchner funnel and then dried in a drying cabinet at 70°C.
The commercial HA was supplied by Plasma Biotal (Plasma Biotal, Tideswell, Derbyshire, UK) and had a batch code P120.

4.10.2 X-ray diffraction (XRD)

XRD of samples was carried out on a Phillips PW1070 powder diffractometer, in a flat plate geometry using Ni filtered CuKα radiation (wavelength 1.5406Å). Samples were ground to a fine powder and mounted in the holder of the diffractometer. Data were collected from 10 to 100° 2θ with a step size of 0.02° and a count time of 12s. Particle size was determined using the following equation.

\[ B = \frac{0.9\lambda}{t \cos\theta} \]

Equation 59

Where B is the broadening of the diffraction measured at its half maximum intensity (radians) and t is the diameter of the crystal particle. Corrections for instrument broadening were made using powdered silicon. Full width at half maximum (FWHM) measurements were made on the 0 0 2 peak for HA and the 1 1 1 peak for silicon.

4.10.3 Transmission electron microscopy (TEM)

Commercial and precipitated HA solutions were prepared by dispersing 1g of HA in 50ml of water. This was placed in an ultrasonic bath for 30 min, to ensure thorough dispersion. A drop of the solution was placed on a Formvar carbon-coated copper 200 grid and left for 1 min. Excess liquid was removed with filter paper and the grid was allowed to dry for about
10 min. The HA particles were then viewed with a JOEL 100CX TEM at an accelerating voltage of 80kV.

4.10.4 Flow curves

Both commercial and precipitated HA powders (20g) were suspended in DDW (30ml) to produce slips containing 40 wt% solids. The rheological properties of these suspensions were then investigated using a Brookfield Rotating Rheometer DVIII+. The HA slips were stirred at a constant rate on a magnetic stirrer for 5 min to break down any aggregates within. The slip was then transferred to the rheometer. The shear rate was started at 0.13s\(^{-1}\) (the lowest rate obtainable with this rheometer) and then varied between 2.5 and 55s\(^{-1}\) at intervals of 2.5s\(^{-1}\). Each shear rate was given a time lag of 1 min to establish an equilibrium before proceeding to the next shear rate step. Measurements of shear rate vs. viscosity were taken to produce flow curves. The standard deviation was calculated from at least two repeat measurements. The method was repeated with deflocculant additions at 0, 0.03, 0.06, 0.13 and 0.19 wt%.

The deflocculant used was Dispex N40, sodium polyacrylate (Allied Colloids, UK).
4.11 In vitro analysis

4.11.1 Cell culture

The human osteosarcoma cell line HOS (TE85) was cultured at 37°C in a humidified atmosphere of 5% CO₂ in air, in flasks containing 10ml of Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% (v/v) fetal calf serum, 2mM L-glutamine, 50 IU/ml of penicillin and 50µg/ml of streptomycin (Gibco; Glasgow, UK). The culture media were changed twice weekly and for subculture the cell monolayers were washed with phosphate-buffered saline (PBS) and incubated with trypsin EDTA for 5 min at 37°C to detach the cells, prior to reculture on the control tissue culture plastic (Marathon Lab, Supplies; London, UK) and on the HA/GR-HA discs.

4.11.2 Cell proliferation

To measure the effects of the HA materials on the growth of the HOS cells in each of 3 separate experiments, 5×10⁴ cells in 0.5ml were plated onto 6 replicate discs (4.9cm²) which had previously been placed into individual wells of a 6-well plate. These were left for 2 hours to allow cell attachment and then 3 ml of culture medium was added to each well, a volume sufficient to cover the surface of the discs. The cultures were incubated at 37°C for a further 2, 5 and 7 days. Cell proliferation was measured at these time periods using the MTT test according to the manufacturer’s instructions (Chemicon; Temecula Ca., USA). 1×10⁵ control cells were plated per well into 6 replicate wells of the (9.6cm²) plastic
culture dishes, giving the same cell density as on the HA/GR-HA discs (approximately 1×10⁵ cells per cm²).

The reagent, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is enzymatically converted by living cells to a dark blue/purple formazan product. The intensity of the colour produced is therefore directly related to the number of viable cells, and thus to their proliferation in vitro. To measure cell growth, each of the 6 replicate HA/GR-HA discs was removed from its well and placed in new 6-well plates with medium prior to assay. This was necessary to avoid including in the assay any residual cells that may have grown on the plastic surface of the wells in which the discs were initially cultured. Then, 100μl of MTT reagent was added to each disc surface and the plates incubated at 37°C for 4 hours. The blue formazan product was solubilised with 500μl of isopropanol-HCl, and 100μl aliquot of each sample removed for assay, which was performed in a 96-well plate. This latter procedure was also performed simultaneously for the control cells grown on plastic.

Absorbance was measured at 570 nm (A₅₇₀) using a TiterTek Multiskan Plus spectrophotometer (Labsystems; Helsinki, Finland) and the average A₅₇₀ of the 6 replicate wells was calculated. The growth of the cells is shown as the average A₅₇₀ of 3 separate experiments ± standard deviation (±SD).
4.11.3 Scanning electron microscopy

For SEM, cells cultured to logarithmic phase on glass coverslips (used as a control), on HA discs and on HA composite discs were fixed in 3% gluteraldehyde in 0.14M sodium cacodylate buffer (pH 7.3) at 4°C overnight. The specimens were then dehydrated in a graded series of alcohols (50%, 70%, 90% and two changes of 100%), washed with hexamethyldisilazane (TAAB Laboratories; Berkshire, UK) for 5 min and placed in a desiccator overnight. After 24 hours, the dehydrated control samples and HA/GR-HA were mounted onto stubs using Araldite (Devcon; Northants., UK) and liquid Dag (Neubauer Chemikalen; Munster, Germany) and left to air dry for 24 hours. Specimens were then sputter coated with Gold/palladium using a Polaron E5100 coating device and observed using a Cambridge Stereoscan S90B (Cambridge Instruments; Crawley, UK).

4.11.4 Flow cytometry (FCM)

FCM measures certain physical and chemical characteristics of cells as they move in a fluid stream past a fixed laser light beam. Light scattered by the cells at small angles (<2°) (forward scatter; FSC) is related to cell size, whereas light scattered at angles of 90° (side scatter; SSC) is related to the granularity and intracellular complexity of the cell. The FCM technique is also able to detect emitted fluorescence light and, in the present study, fluorochrome-conjugated antibodies were used to measure the relative levels of certain antigens considered to play an important part in the integrity and function of bone, as noted
below. It has been shown that the levels of fluorescence obtained in this technique are directly proportional to the actual molecular levels of the antigen present in the cell.

For FCM, cultures of HOS cells were seeded onto the HA/GR-HA discs and into 6-well culture plates at the densities described and incubated for 5 days. After this time, the cells were washed with PBS and harvested by scraping the monolayer with 20 mM EDTA in PBS and no trypsin, to avoid proteolytic cleavage and thus loss of any membrane-associated antigen. The cells were centrifuged and fixed with 1% paraformaldehyde (Merck; Poole, England) in PBS containing 0.1%(w/v) saponin, for 30 min at room temperature, to permeabilise the cells to allow entry of antibodies. After washing twice with 2% FCS in PBS containing 0.1% saponin, the cells were centrifuged and the pellet resuspended in this washing buffer. Replicate aliquots containing approximately $1 \times 10^5$ fixed cells were placed into separate tubes and incubated for 60 min at room temperature with the following primary antibodies: rabbit polyclonal antibodies against human bone sialoprotein (BSP), osteonectin (ON) and osteopontin (OP) (courtesy of Dr. L. Fisher; NIH, Bethesda, MD, USA); mouse monoclonal antibodies (mAbs) against human fibronectin (FN) (DAKO; High Wycombe, England) and type I collagen, (COL I; clone M-38) (Developmental Studies Hybridoma Bank; Iowa City, USA). All primary antibodies were used at a 1:100 dilution in washing buffer. After reaction with the antibodies, the cells were washed twice with washing buffer. Normal, pre-immune rabbit serum and non-specific mouse IgG1 (both DAKO) were used as negative controls, respectively. Fluorescein isothiocyanate (FITC)-conjugated swine anti-rabbit IgG (1:20) and FITC-conjugated rabbit anti-mouse IgG (1:50) (both DAKO) were added for 30 min at room temperature.
The cells were washed and resuspended in 500μl of washing buffer and analysed by FCM using a FACScan flow cytometer (Becton Dickinson, US). The results were analysed using CellQuest software (Becton Dickinson, US) and are represented as arbitrary units of FSC and SSC and as the average fluorescence intensity (AFI) of 10,000 cells (±SD).
5 Results and Discussion
5.1 Comparison of two biaxial flexure strength equations

Investigation into the Biaxial flexure strength values were determined, initially, using the following equation as used by Santos:

\[
\sigma = \frac{3F(1 + \nu)}{4\pi t^2} \left[ 1 + \ln \left( \frac{r_2}{r_1} \right) + \frac{(1 - \nu)(r_2^2 - r_1^2)}{(1 + \nu)r^2} \right]
\]

Biaxial flexure strength data has also been presented for the ring supported / ring loaded system were determined from the ISO 6474 standard formula:

\[
\sigma = \frac{3F(1 + \nu)}{2\pi t^2} \left[ \ln \left( \frac{r_2}{r_1} \right) + \frac{(1 - \nu)(r_2^2 - r_1^2)}{(1 + \nu)2r^2} \right]
\]

Where

- \(\sigma\) : Biaxial bending strength (MN.m\(^{-2}\))
- \(F\) : Load (N)
- \(\nu\) : Poisson’s ratio (0.28)
- \(t\) : Thickness of disc (mm)
- \(r_2\) : Radius of supporting ring (mm)
- \(r_1\) : Radius of loading ring (mm)
- \(r\) : Radius of specimen disc (mm)

The results shown from Figure 62 compare the values determined from the first equation and the ISO standard equation for three different materials, HA, 2.5wt% CNP GR-HA and 5wt% CNP GR-HA. Even though there is considerable overlap in the overall data at any given sintering temperature, the mean values given for the ISO standard formula are lower than those given by the first formula.
As it was felt that the ISO equation was the more complete, this was used for the rest of the testing.

![Graph](image1.png)

**Figure 62.** Comparison of biaxial flexure strengths of two equations.
5.2 Shrinkage

Measurements carried out on the shrinkage (Figure 63) of specimens as a function of sintering temperature indicate densification and in addition show a direct correlation with recorded density measurements and porosity (Figures 71 & 72). The graph shows shrinkage occurring at a lower temperature for commercial HA compared to the GR-HA composites prepared from commercial HA. Furthermore, the data also shows that HA undergoes the greatest shrinkage at the final temperature of 1350°C compared to the GR-HA composites.

![Shrinkage Graph](image)

Figure 63. Shrinkage.
5.3 Crystallographic, Physical and Morphological properties of P120 GR-HA and precipitated GR-HA.

- **GR-HA's containing 2.5wt% CNP glass**

This section highlights the crystallographic, physical and morphological aspects of P120 GR-HA (commercially processed HA) and precipitated GR-HA at 80°C.

Figure 64 and Figure 65 display X-ray diffraction patterns for both P120 GR-HA and precipitated GR-HA at four different sintering temperatures. Both patterns show how P120 GR-HA and precipitated GR-HA decompose to the β-TCP phase at the lower sintering temperatures and then gradually undergo a decomposition from the β-TCP to the α-TCP phase at higher sintering temperatures. The diffraction patterns showed that both the P120 GR-HA and the precipitated GR-HA display similar levels of stability.

The main reflections for HA may be seen at around 32° 2θ and the main reflections for β and α-TCP are located at about 31.1 and 29.9° 2θ respectively. These phases also account for the small decrease in density (Figure 66) at 1350°C, and is most certainly related to the relatively large unit cell of the α-TCP phase. Figure 66 compares the different measured densities for both commercial and precipitated GR-HA and also illustrates the behaviour of the two materials as a function of temperature. P120 GR-HA undergoes a greater increase in densification relative to precipitated GR-HA between 1200 and 1250°C. This observation is significant, since the correlating porosity values at each temperature show a
Development of Glass Reinforced Hydroxyapatite for Hard Tissue Surgery

George Georgiou

decrease from 5.9% porosity at 1200°C to 1.3% at 1250°C for P120 GR-HA, compared to a decrease of 3.2% to 2.5% for precipitated GR-HA.

For the precipitated GR-HA, severe delamination problems were encountered. Examination of the precipitated HA particle morphology revealed highly acicular crystals and these could be oriented during the pressing process, causing the delamination. This greatly diminishes the strength of the material. As a consequence no flexural bend strength measurements could be carried out for precipitated GR-HA.

Figure 64. X-ray diffraction for P120 GR-HA.
Figure 65. X-ray diffraction for precipitated GR-HA.

Figure 66. Density for P120 GR-HA and precipitated GR-HA.

The preferred orientation occurring for the precipitated HA at 80°C can be correlated with the morphology and rheological behaviour discussed later. Although both HA P120 and precipitated HA at 80°C exhibit acicular particulate in the images shown (Figure 91) and
(Figure 93), it was found that the presence of a large particle size distribution, the evidence of some fine particles and the relatively small mean particle size of the precipitated HA compared to HA P120 accounted for the full dispersion of precipitated HA particles at 0 wt% deflocculant addition. This rheological behaviour expressed for precipitated HA can explain the ease in which the crystals orientate themselves during pressing.
5.4 Mechanical properties and characterisation of HA and GR-HA containing 2.5 and 5wt% CNP glass.

CaO: 32mol%; Na₂O: 23mol%; P₂O₅: 45mol%: CNP (ternary glass system)

The biaxial flexure strength values determined for HA P120 and GR-HA composites with different glass additions are shown in Figure 67. Typical values for HA between 1200 and 1350°C were given at between 36.79 and 47.2MN.m⁻². However, for the GR-HA containing 5wt% addition of glass [GR-HA (5CNP)] the values were higher at 1200 and 1250°C compared to HA, these were recorded at 55.65 and 67.39MN.m⁻² respectively. At 1300°C the flexural strength drops to 44.23MN.m⁻², similar to the value recorded for HA at this temperature. At 1350°C there is a slight increase to 48.39MN.m⁻². The GR-HA containing 2.5wt% addition of glass [GR-HA (2.5CNP)] did not exhibit this drop. At 1300 and 1350°C the flexural strength displayed a sharp increase to 104.83 and 129.05MN.m⁻² respectively.

![Figure 67. Biaxial flexure strength of HA and GR-HA with 2.5wt% and 5wt% glass additions.](image-url)
Flexural modulus values were ascertained from the tangent of the straight line portion of the load-deflection curve of the flexural bend strength graph. The values that were calculated showed similarities for all three specimens, except at 1350°C where GR-HA (2.5CNP) displayed the only notable difference in the flexural modulus (Figure 68). This was measured at 64.31GN.m\(^{-2}\). The values for the flexural modulus in general, ranged from 43.49 to 64.31GN.m\(^{-2}\).

![Figure 68. Flexural modulus of HA and GR-HA with 2.5wt% and 5wt% glass additions.](image)

The hardness values determined from Vickers indentation displayed mean values that were generally higher for HA at all four sintering temperatures compared to the GR-HA composites. The mean hardness values for the GR-HA composites were approximately 30% lower at 1200, 1250 and 1350°C and 16% lower at 1300°C. However, the large standard deviation that is usually associated with ceramics with respect to hardness can be seen in this instance. Due to the large variation the difference in the mean values between HA and GR-HA can be considered statistically insignificant (Figure 69).
GR-HA (5CNP) generally showed enhanced fracture toughness values compared to both HA and GR-HA (2.5CNP), typically between 0.5 and 0.6 MNm$^{-3/2}$. GR-HA (2.5CNP) showed a similar value at 1200°C to GR-HA (5CNP) but dropped towards values that were more comparable to those of HA, particularly at 1300 and 1350°C, which gave fracture toughness values of approximately 0.35 and 0.42 MNm$^{-3/2}$ respectively (Figure 70). Although the GR-HA composites in this case do show enhanced fracture toughness compared to HA, in particular GR-HA (5wt%CNP), it must be noted that these values are considerably lower compared to other GR-HA composites and even other ceramics worked on by other authors. The fracture toughness values for cortical bone have been reported at between 2 –12 MNm$^{-3/2}$, and so therefore these GR-HA composites do not possess adequate
fracture properties for implantation. This can be said for most of the GR-HA composites worked on throughout this study.

![Fracture toughness of HA and GR-HA with 2.5wt% and 5wt% glass additions.](image)

**Figure 70.** Fracture toughness of HA and GR-HA with 2.5wt% and 5wt% glass additions.

Actual density measurements that were carried out from the Archimedes principle showed densification occurring between 1200 and 1250°C for both HA and GR-HA composites (Figure 71). The values recorded at 1200°C were below 3g.cm\(^{-3}\) for both GR-HA composites and 3.032g.cm\(^{-3}\) for HA. At 1250°C the density values increase considerably for all specimens with the GR-HA composites undergoing the most densification with values increasing from 2.931 to 3.067g.cm\(^{-3}\) for GR-HA (5CNP) and from 2.957 to 3.098g.cm\(^{-3}\) for GR-HA (2.5CNP). HA, however, shows the highest density overall reaching an optimum value at 1300°C and remaining constant at 1350°C. This same trend was also observed for GR-HA (2.5CNP) with optimum density of 3.108g.cm\(^{-3}\). GR-HA (5CNP), which displayed the lowest density overall, exhibited a drop in density from 1250 to 1300°C and a further drop at 1350°C.
Figure 71. Density values for HA and GR-HA with 2.5wt% and 5wt% glass additions.

The actual density measurements as expected showed an inverse relationship with porosity. In ceramics, it is expected that a decrease in porosity is observed as densification proceeds, this can be seen in Figure 72. The drop in density observed for GR-HA (5CNP) can be correlated to the increase in porosity from 1300 to 1350°C.

Figure 72. Porosity values for HA and GR-HA with 2.5wt% and 5wt% glass additions.
Porosity values were determined from the theoretical densities and phase element fractions which were taken from the Rietveld refinement of the X-ray diffraction patterns (Figures 73-75).

Figure 73. X-Ray diffraction pattern of HA.

Figure 74. X-Ray diffraction pattern of GR-HA containing 2.5wt% CNP.
Figure 75. X-Ray diffraction pattern of GR-HA containing 5wt% CNP.

Figure 73 shows the diffraction patterns for HA at all four sintering temperatures. There are no apparent peaks observed that are associated with secondary phases, in fact at all sintering temperatures the sintered specimens exhibited characteristic HA phase peaks, with the most intense peak at approximately 32° 20. However, for the GR-HA specimens decomposition has occurred and this can be seen in Figure 74 and Figure 75. Characteristic secondary phase peaks, for β-TCP and α-TCP occur at 31 and 30° 20 respectively. For GR-HA (2.5CNP) there is decomposition of HA to β-TCP at all four sintering temperatures, this can be detected by a drop in intensity for the HA peak and the appearance of a β-TCP peak at 30° 20. At 1350°C inversion of some of the β-TCP phase to α-TCP occurs. This is evident by the drop in the intensity of the β-TCP peak and the appearance of the α-TCP peak. The GR-HA (5CNP) composite also exhibited inversion to α-TCP, however, this occurred at a lower sintering temperature of 1300°C.
From the Rietveld refinement the phase element fractions can be refined as a variable and hence measured. The phase element fractions can therefore be represented in a more simplistic way and show a correlation with the changes in intensity of peaks in the diffraction patterns that correspond to each phase, these can be shown in Figures 76 to 78.

Figure 76. Amount of HA in the three ceramics.

Figure 77. Amount of β-TCP phase in the three ceramics.
The Rietveld refinement assumes that there is no porosity in the sample and so the overall theoretical densities that are determined may not necessarily correspond to the actual densities measured. In fact at 1200°C it is very evident that there is a substantial difference between the theoretical densities and the actual measured densities (Figure 79). The porosity therefore can be estimated from the actual measured densities and the overall theoretical densities, which are determined from the theoretical densities and the phase element fractions of each phase in the sample. The overall theoretical densities shown in Figure 79, as with the actual density measurements, showed higher density values for HA compared to the GR-HA composites over all four sintering temperatures with the GR-HA (5CNP) exhibiting the lowest density.
Grain size measurements were also carried out in order to examine the changes in grain size as a function of sintering. The relationship between grain size and sintering temperature is that as temperature increases the mean grain size increases, this occurs for HA and GR-HA (Figure 80). However, it is evident that there is a large variation in the data, in which the variation increases as a function of sintering temperature also.

Figure 79. Overall theoretical densities of HA and GR-HA containing 2.5wt% and 5wt% glass additions.

Figure 80. Grain size measurements of HA and GR-HA containing 2.5wt% and 5wt% glass additions.
Figure 81. Grain size distribution for HA.

Figure 82. Grain size distribution for GR-HA (2.5CNP).

Figure 83. Grain size distribution for GR-HA (5CNP).
Figure 84. HA sintered @1200°C.

Figure 85. HA sintered @1250°C.
Figure 86. HA sintered@1300°C.

Figure 87. HA sintered@1350°C.
The four images (Figures 84-87) show the surfaces of HA sintered from 1200 to 1350°C. The specimens that were analysed were fractured pieces taken from mechanically tested specimens and etched with a 10% concentration citric acid solution. The SEM images showed an increase in the mean grain size as a function of sintering. The scale bar shown at the top of the image, can usually give a good initial indication of the grain size. Data for the mean grain size measurements and grain size distribution previously shown were obtained from these images and support this observation. The grain size distribution graph (Figure 81) for HA exhibits a small grain size relative to specimens fired at higher temperatures which is indicated by the peak of the curve, however, its distribution is very narrow. As processing proceeds to higher sintering temperatures the distribution broadens with the peaks for each sintering temperature becoming lower in intensity and also displaying a greater distribution of larger grains. The specimens sintered at 1350°C showed a bimodal distribution, which indicates the continual growth of larger grains through the consumption of smaller grains. This can be shown by the two peaks at approximately 4 and 6.5μm. This variation in distribution from 1200 to 1350°C is also evident in the GR-HA composites, with the bimodal distribution being more apparent at lower sintering temperatures, particularly at 1300°C (Figures 82 and 83).
The addition of a glass that consists of CaO, NaO and P₂O₅ to form a GR-HA composite demonstrates the effect a glass addition has on the porosity of the material and furthermore the mechanical properties. The incorporation of glass in HA acts as a sintering aid, which enhances densification, and as a result reduces porosity. This occurs through a liquid phase sintering mechanism, which accelerates inter-particulate diffusion and bonding, thus causing elimination of porosity and shrinkage. However, in this system, it is further complicated by the occurrence of decomposition and phase changes that can also have a detrimental or beneficial effect on the mechanical properties.

HA, GR-HA containing 2.5wt% CNP and GR-HA containing 5wt% CNP exhibit a sharp decrease in porosity as temperature increases and these gave values of approximately 0-1% at 1250°C, 1300°C and 1350°C. One would arrive at the assumption that since the porosity value for the specimens mentioned are comparable, then one would therefore expect similar flexural bend strength values. This is not seen however. What we observe is an improvement for the glass reinforced HA relative to HA, and in particular for GR-HA containing 2.5wt% CNP.

The phase element analysis from the Rietveld refinement carried out for these specimens gives an indication of the amount of each of the phases present. For GR-HA (2.5CNP) and GR-HA (5CNP) there is decomposition of HA to β-TCP at all four sintering temperatures, and furthermore, inversion of β-TCP to α-TCP. With GR-HA (5CNP) significantly more β-TCP is found and the β-TCP phase inverts more readily to α-TCP at a lower temperature. This inversion is due to the origin of β-TCP. The decomposition of HA is attributed to the
presence of a reactive glass, which can drive off the hydroxyl groups when entering the HA structure and as a result causes decomposition.\textsuperscript{118-121} The glass may also affect the Ca:P ratio.\textsuperscript{118} However, for GR-HA (5CNP) the inversion of β-TCP to α-TCP, occurring at a lower temperature, is due to β-TCP containing more residual ions, which destabilise β-TCP and facilitates the formation of more α-TCP.

The presence of these secondary phases has a major influence on the mechanical properties of the material. This can be correlated with the values illustrated for flexural bend strength. The decrease in flexural strength for GR-HA (5CNP) at 1300°C and 1350°C correlate with the phase weight fractions, where the α-TCP phase content appears at around 15% and 19% respectively. Alternatively, GR-HA (2.5CNP) displays a gradual increase as the temperature increases. At 1350°C, where the α-TCP is present at 5%, there does not appear to be a reduction in flexural strength as was observed with GR-HA (5CNP). There is also probably a positive influence from the virtual complete absence of porosity.

The presence of β-TCP and α-TCP phases in the HA structure would give a volume increase and thus can give rise to residual stress.\textsuperscript{4} However, the presence of α-TCP at high levels can disrupt the integrity of the GR-HA.\textsuperscript{118} As a result of these findings, one can postulate that a significant amount of α-TCP in the structure can be detrimental to the mechanical properties of the material, but low amounts, as observed in HA (2.5CNP), can be advantageous.\textsuperscript{120}
For this glass system it can be said that both porosity and phase decomposition are influential factors in the outcome of the mechanical properties. However, what the results seem to suggest is an overall improvement in flexural strength for glass reinforced HA which is associated primarily with phase decomposition and subsequent phase inversions.

The flexural modulus and hardness values exhibited for HA, GR-HA(2.5CNP) and GR-HA(5CNP) show no significant differences between these materials and so one cannot provide any factors to explain this.

The fracture toughness values though show overall enhanced values for GR-HA (5CNP) relative to HA and GR-HA (2.5CNP) between 1250 and 1350°C. Since all three materials display similarities in porosity and grain growth factors, such as grain size and distribution, one cannot therefore associate the differences in fracture toughness solely to these factors. The presence of β-TCP in HA as a result of decomposition during the sintering process is believed to cause an increase in the fracture toughness of HA \(^{122}\). GR-HA (2.5CNP) displays lower β-TCP content compared to GR-HA (5CNP), therefore, the lower fracture toughness exhibited for GR-HA (2.5CNP) may probably be attributed more to the presence of surface flaws rather than to differences in β-TCP content. In addition the lower fracture toughness shown for this composite could be explained by the presence of surface flaws. These can manifest through abrasion of the surface during polishing. Grinding of material surfaces prior to polishing can leave particles on the surface of the material. During polishing these particles act as an abrasive which leave scratches on the surface and hence give rise to surface flaws.
5.5 Characterisation of the rheological properties of a range of hydroxyapatite powders.

The use of deflocculant (Dispex N40) during the milling process is known to aid dispersion of particles within the hydroxyapatite slip and can potentially optimise the processing method. In order to determine the quantity of deflocculant required to optimise the slip, the rheological properties must be investigated and understood. Three HA powders were investigated; commercial HA (batch P120 supplied by Plasma Biotal, Tideswell, Derbyshire, UK), HA precipitated @ 80°C, and HA precipitated @ 60°C. The viscosity of the HA slips were measured as a function of shear rate for a given deflocculant addition to give flow curves (Figures 88 and 89). These measurements were undertaken for 5 deflocculant additions; 0, 0.03, 0.06, 0.13 and 0.19wt%.

![Flow curve](image)

Figure 88. Viscosity against shear rate for 3 HA’s with 0wt% deflocculant.
Figure 89. Viscosity against shear rate for the 3 HA’s with 0.13wt% deflocculant.

The two flow curves displaying viscosity versus shear rate at 0 and 0.13wt% deflocculant addition show Newtonian behaviour for HA precipitated at 80°C. At 0wt% deflocculant addition HA P120 and HA precipitated at 60°C exhibit pseudoplastic behaviour, with the viscosity of HA P120 being considerably higher compared to the precipitated HA’s. The flow curves with 0.13wt% deflocculant addition show a significant decrease in the viscosity for HA P120, however, HA P120 starts to deflocculate at this addition and begins to exhibit Newtonian behaviour. HA P120 undergoes full dispersion and exhibits Newtonian characteristics at 0.19wt% deflocculant addition. In this case the 0.19wt% deflocculant addition can be considered as the optimal addition required to cause full dispersion in the HA P120 slip. This can be shown from Table 13 and Figure 90.
Figure 90. Effect of deflocculant addition on viscosity for the three HA’s with a shear rate of 50s⁻¹.

<table>
<thead>
<tr>
<th>wt%</th>
<th>HA P120</th>
<th>HA @60°C</th>
<th>HA @80°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Pseudoplastic</td>
<td>Pseudoplastic</td>
<td>Newtonian</td>
</tr>
<tr>
<td>0.03</td>
<td>Pseudoplastic</td>
<td>Pseudoplastic</td>
<td>Newtonian</td>
</tr>
<tr>
<td>0.06</td>
<td>Pseudoplastic</td>
<td>Newtonian</td>
<td>Newtonian</td>
</tr>
<tr>
<td>0.13</td>
<td>Almost Newtonian</td>
<td>Newtonian</td>
<td>Newtonian</td>
</tr>
<tr>
<td>0.19</td>
<td>Newtonian</td>
<td>Dilitant</td>
<td>Newtonian</td>
</tr>
</tbody>
</table>

Table 13. Rheological properties for three HA’s.
Figure 91. TEM of HA P120.

Figure 92. TEM of HA precipitated @ 60°C.
Figure 93. TEM of HA precipitated @80°C.

The TEM images (Figures 91 to 93) show the morphology of the three HA powders. Particle size measurements taken for the three powders were determined from line broadening effects via x-ray diffraction, this was due to the fact that measurements from the images were made difficult by the high aspect ratio of the particles.

The particle size for HA P120 was found to be much larger (129.99nm) compared to the precipitated HA samples. The effect of precipitation temperature on the precipitate morphology was made evident and is confirmed by the particle size measurements taken for HA precipitated at 60 and 80°C. The HA precipitated at 60°C has a particle size of 61.06nm, whereas HA precipitated at 80°C gives a particle size of 82.24nm.
Although the HA precipitated at 80°C gives a larger mean particle size compared to HA precipitated at 60°C, the presence of fine particles are observed in the TEM indicating a relatively large particle size distribution and therefore would explain the Newtonian behaviour, low viscosity, and the ease with which the slip remains in suspension. The need for a greater amount of Dispex N40 to fully deflocculate the HA P120 system is attributed to the relatively large particle size compared to the precipitated HA’s. Furthermore, all three HA’s exhibit an acicular morphology.
5.6 Mechanical properties and characterisation of GR-HA composite milled at different powder to methanol ratio.

CaO: 21.25mol%; P₂O₅: 63.75mol%; CaF₂: 15mol%; CP15F (ternary glass system)

Glass addition: 2.5wt%

GR-HA(1:2) : 150g:300ml
GR-HA(1:4) : 75g:300ml
GR-HA(2:3) : 200g:300ml

The process of milling so far involved milling 200g of powder with 300ml of methanol. A variety of powder to methanol ratios were selected for the milling process to examine whether they had an effect on the mechanical and crystallographic properties of the material. A parameter that is considered to potentially affect the properties of the material with respect to powder/methanol ratio is the viscosity. The powder/methanol ratio selection was based on the GR-HA (CP15F) composite.

The data gathered showed a correlation between the mean biaxial flexure strength and the powder to methanol ratios. The 75g:300ml powder to methanol composite [GR-HA (1:4)] in this case showed the lowest mean values over the sintering range (Figure 94).

The flexural modulus values also showed a correlation with the powder to methanol ratios. The flexural modulus values found were again assigned to the GR-HA (1:4) composite (Figure 95).
Figure 94. Biaxial flexure strength for GR-HA milled with varying powder to methanol ratios.

Figure 95. Flexural modulus for GR-HA milled with varying powder to methanol ratios.

The values measured for hardness in this case showed no significant difference between the composites, except at 1250 and 1300°C where the mean values for the GR-HA (1:4) composite were recorded at 103.91 and 81.37 H, respectively (Figure 96).
Differences were observed though in the fracture toughness values, particularly between the three materials at 1200°C and also between 1250 and 1350°C for the GR-HA (1:4) composite, which showed enhanced fracture toughness compared to the GR-HA (1:2) and (2:3) composites (Figure 97). Both GR-HA (1:2) and (2:3) composites displayed a drop in value from 1200 to 1250°C followed by a minimal increase between 1250 and 1300°C. In contrast GR-HA (1:4) exhibited an increase in fracture toughness between 1200 and 1250°C followed by a minimal drop between 1250 and 1300°C.
Density and porosity data presented displayed the expected inverse relationship, with GR-HA (1:2) and (2:3) composites showing the expected increase in density between 1200 and 1250°C and is concurrent with the drop in porosity between these temperatures (Figure 98). However, this trend was not followed for the GR-HA (1:4) composite. The increase in density for this composite occurred at 1350°C, but the value expressed at this temperature was considerably lower compared to the other composites.

Figure 97. Fracture toughness.

Figure 98. Density and Porosity.
The X-ray diffraction patterns for all three materials showed decomposition to β-TCP secondary phase along with inversion to α-TCP at 1350°C (Figure 99). It is apparent from the phase element fraction graphs that the GR-HA (1:4) composite is not represented (Figure 100). The x-ray pattern for this composite showed the presence of large amounts of β-TCP at each temperature, this subsequently presented difficulties in the refinement. As a consequence the phase element fractions, theoretical density and also porosity could not be determined.

Figure 99. X-ray diffraction.
Figure 100. Amount of HA, β-TCP and α-TCP phases in composites.
Figure 101. Grain size and grain size distribution.
The grain size and grain size distribution graphs shown in Figure 101 represent the GR-HA composites milled with a 2:3 and 1:2 powder to methanol ratio. These showed enhanced mean grain size and greater distribution of larger grains for the GR-HA (2:3) composite compared to the GR-HA (1:2) composite. The grain size and distribution data for GR-HA (1:4) were not shown. The images taken for this composite did not exhibit the characteristic grain structure as is typically seen in ceramic materials, subsequently this made it difficult to make any kind of measurement (Figures 102 to 105).
Figure 102. GR-HA (1:4) Sintered @ 1200°C.

Figure 103. GR-HA (1:4) Sintered @ 1250°C.
Figure 104. GR-HA (1:4) sintered @ 1300°C.

Figure 105. GR-HA (1:4) sintered @ 1350°C.
The results for GR-HA milled with varying powder to methanol ratios show a correlation between the mean values of flexural strength and flexural modulus and the powder to methanol ratio.

In general an increase in flexural strength and flexural modulus values were observed for an increase in powder to methanol ratio. The purpose of this study was to examine whether or not the mechanical properties vary as a function of viscosity, a property that is known to change with varying powder to methanol ratio. The viscosity of the GR-HA slip is expected to increase as the powder to methanol ratio increases. Therefore, the results suggest that the most viscous suspension, in this case the 2:3 milling ratio, gave the greatest mean values for flexural strength and flexural modulus. So, it is reasonable to suggest that further work could be carried out to examine the effect of increasing the powder to methanol ratio beyond 2:3 on the mechanical properties.

Porosity values, phase element fractions, grain size and grain size distribution curves were not given for GR-HA milled at a 1:4 ratio. Firstly, there were extreme difficulties in the refinement of the HA phase, this was probably due to the fact that β-TCP was the dominant phase with very broad peaks, as can be seen from the x-ray diffraction pattern figure 162. Hence no theoretical values, porosity values and phase element fractions were obtained. Secondly, the SEM images showing the surface of the material at all sintering temperatures did not exhibit any distinct grains or grain boundaries and this therefore made it difficult to distinguish between individual grains and consequently image analysis could not be carried
The low flexural strength and flexural modulus values expressed for GR-HA milled at a 1:4 ratio may be due to the large amount of secondary phases, \(\beta\) and \(\alpha\)-TCP, present in the material. Variations in density between the HA phase and secondary phases may account for the differences in stress at regions of the material where these phases coexist.\(^4\) The lower density secondary phases in this case can be responsible for lowering the flexural strength, flexural modulus and density of the material at these sintering temperatures.

Moreover, the presence of large amounts of secondary phases for this composite can be explained by the effect of viscosity. A low viscosity slip may facilitate the dispersion of the phosphate glass powder during milling thus providing greater diffusion of ions from the glass to the HA lattice during sintering and therefore promote a greater rate of decomposition to secondary phases.

In addition, grinding during milling process can prove to be effective in reducing the particle size for a low viscosity slip, as a result this can affect the diffusion of HA particles by providing sufficient free surface energy during sintering, thereby promoting a greater decomposition to secondary phases. This may also account for the low flexural strength, flexural modulus and density values measured. The effect of grinding on a low viscosity slip may have affected the nature of grain growth and could have consequently reduced surface flaws, such as porosity on the surface. The high fracture toughness values expressed
for GR-HA milled at a 1:4 ratio between 1250 and 1350°C is most probably due to this reduction in surface flaws and so this difference may be attributed to surface effects.
5.7 Mechanical properties and characterisation of HA and a GR-HA composite containing 2.5wt% CP15F glass milled under different conditions.

CaO: 21.25mol%; P₂O₅: 63.75mol%; CaF₂: 15mol%: CP15F (ternary glass system)

The results presented in the following section focuses on how the different milling conditions (i.e. milling with water, methanol, water with a deflocculant addition) affect the properties of one particular material. The following results compare the different milling conditions on the properties of HA.

The biaxial flexure strength for HA milled with methanol expressed a minimum mean value of 36.79 MN.m⁻² at 1350°C and a maximum value of 47.20 MN.m⁻² at 1250°C (Figure 106). Taking into consideration the variation in data with respect to this material it can be said that the values over the sintering range are similar. HA milled with water and deflocculant expressed slightly enhanced mean values overall compared to HA milled with methanol, however there was a considerable overlap in the data between the two materials. The mean values expressed for HA milled with water showed a slight increase from 60.03 to 74.54 MN.m⁻² between 1200 and 1250°C respectively, followed by a drop to 32.83 MN.m⁻² at 1350°C. The highest mean value expressed for the different milling conditions was given for HA milled with water sintered at 1250°C.
Figure 106. Biaxial flexure strength of HA milled under different conditions.

The flexural modulus values determined from the flexural strength experiments did not exhibit any major differences between the three materials (Figure 107). HA milled with water and deflocculant gave enhanced mean values compared to HA milled with methanol and showed a correlation with the flexural strength data (Figure 106). This correlation could also be observed for HA milled with water where there was an initial increase from 1200 to 1250°C followed by a drop to 1350°C.

Figure 107. Flexural modulus of HA milled under different conditions.
Hardness data for HA milled with methanol and HA milled with water did not exhibit any significant changes in hardness from 1200 to 1350°C (Figure 108). Although HA milled with methanol exhibited higher mean values compared to HA milled with water from 1250 to 1350°C, the overlap in data presented difficulty in interpreting any significant differences between the two materials. A significant change could be observed between 1200 and 1250°C for HA milled with water and deflocculant and was indicated by a significant drop in hardness from 252.98 to 142.47 H<sub>v</sub>.

![Figure 108. Hardness of HA milled under different conditions.](image)

Fracture toughness data determined for HA milled under different conditions exhibited significant differences at 1300 and 1350°C (Figure 109). The lowest mean values at these temperatures were given for HA milled with water, which were calculated at 0.22 and 0.19 MN.m<sup>-3/2</sup> respectively. The highest values were given for HA milled with methanol and these were given at 0.35 and 0.42MN.m<sup>-3/2</sup> respectively. The fracture toughness for HA milled with water and deflocculant expressed a value of 0.28MN.m<sup>-3/2</sup> at both 1300 and 1350°C.
Figure 109. Fracture toughness of HA milled under different conditions.

The inverse relationship between density and porosity for the three materials showed a definite correlation in data (Figure 110). This can be seen at 1200°C, where the highest recorded density gave the lowest porosity and vice versa. Large differences in both density and porosity between the three materials are expressed at this temperature, however, at 1250 to 1350°C porosity is diminished with the values for all three materials expressing similarities between them, except at 1350°C. At this temperature HA milled with water and deflocculant showed an increase. The reason for this has been previously discussed.

Figure 110. Density and porosity of HA milled under different conditions.
Grain size measurements for the three materials showed an increase as a function of sintering temperature as expected, with all three exhibiting similar mean values at a given temperature (Figure 111).

![Figure 111. Grain size of HA milled under different conditions.](image)
The effect of milling a ceramic material using different solvents, with the addition of a deflocculant in one case, on the properties was investigated for a GR-HA containing 2.5wt% (CP15F). The biaxial flexure strength values showed enhanced mean values for GR-HA milled with methanol compared to the other milling conditions at all sintering temperatures (Figure 112). All three materials generally showed an increase in flexural strength with increasing sintering temperature.

Figure 112. Biaxial flexure strength of GR-HA milled under different conditions.

An increase in flexural modulus for these materials could also be observed between 1200 and 1350°C, however, this increase was not substantial (Figure 113). The only differences in the mean values between these materials was seen at 1200 and 1250°C, where GR-HA milled with methanol exhibited slightly higher values compared to the other materials. These values at 1200 and 1250°C were given at 61.19 and 69.21 GN.m$^{-2}$ respectively.
Hardness data recorded at 1200, 1250 and 1350°C showed similarities in the mean values between the three materials, however, there was a difference observed at 1300°C for GR-HA milled with water and deflocculant, in which it displayed an enhanced mean value compared to the other materials (Figure 114). The value was recorded at 207.55 Hv.

Figure 113. Flexural modulus of GR-HA milled under different conditions.

Figure 114. Hardness of GR-HA milled under different conditions.
The fracture toughness values calculated exhibited a similar trend for all three materials. An initial drop in fracture toughness could be observed from 1200 to 1250°C, followed by an increase from 1250 to 1350°C (Figure 115). Two differences in values between materials could be seen at 1200 and 1300°C, where GR-HA milled with methanol exhibited slightly higher mean values compared to the other two materials. These were calculated at 0.86 and 0.52 MN.m$^{-3/2}$ at 1200 and 1300°C respectively.

![Graph showing fracture toughness of GR-HA milled under different conditions.](image)

**Figure 115.** Fracture toughness of GR-HA milled under different conditions.

Density measurements recorded for these materials showed similar values between GR-HA milled with water and deflocculant and GR-HA milled with methanol, however, from 1200 to 1300°C HA milled with water gave slightly lower density values (Figure 116). Similarities in porosity could be seen at 1200°C for all three materials. The large drop in porosity observed between 1200 and 1250°C, which is typically seen during densification, is noticeable, however, it seems as though GR-HA milled with methanol exhibited the greatest reduction in porosity in this case. The value given at 1250°C (1.32%) in this
instance was lower than that expressed at 1300°C (1.60%) for the same material. This could be explained by the drop in theoretical density shown at this temperature (Figure 117).

![Figure 116. Density and porosity of GR-HA milled under different conditions.](image)

![Figure 117. Theoretical density of GR-HA milled under different conditions.](image)

X-ray diffraction patterns showed decomposition to secondary phases for all three materials at all sintering temperatures. Decomposition to β-TCP occurred at all sintering temperatures, with inversion to α-TCP taking place at 1350°C (Figures 118 and 119).
Figure 118. Amount HA, β-TCP and α-TCP in GR-HA milled under different conditions.
Figure 119. X-ray diffraction of GR-HA milled under different conditions.
The grain size measurements showed an increase in grain size as a function of sintering temperature for these materials (Figure 120). At 1350°C the grain size of the three materials showed divergence, with GR-HA milled with methanol exhibiting the greatest mean grain size. This could also be shown in the grain size distribution graphs representing these materials (Figure 121). The correlation between the difference in mean grain size between each material at 1350°C and the distribution data is indicated by the shift in the maximum intensity peaks. The images from Figures 122 to 124 represent the surfaces of the three materials sintered at 1350°C.
Figure 121. Grain size distribution of GR-HA milled under different conditions.
Figure 122. GR-HA milled with methanol sintered @ 1350°C.

Figure 123. GR-HA milled with water sintered @ 1350°C.
Figure 124. GR-HA milled with water and deflocculant sintered at 1350°C.
The purpose of using a deflocculant is to enable the full dispersion of particles within the HA slip during milling and therefore potentially optimise the processing method. This is pertinent since it can enhance sintering and provide greater mechanical properties to the material.

It is clear from the data, which represents HA milled under different conditions, that no significant improvement is made on the mechanical properties of HA when milled with water or with water and deflocculant compared to HA milled with methanol. This is evident from the considerable overlap in data.

It must be noted, though, that the fracture toughness data for the aforementioned composites do differ somewhat. Such differences in these values may be attributed to the presence of pores on the surface that manifest through grain boundary migration/ grain growth during sintering, and can have a detrimental effect on the fracture toughness. Evidence of the presence of pores are represented by the porosity data for these materials (Figure 110). These pores can act as surface flaws in conjunction with other flaws that may be created through grinding and polishing, and is a means by which surface abrasion can play a role in significantly altering the fracture toughness.

A correlation is shown between the greater presence of β-TCP and the overall increase in fracture toughness over the sintering range for GR-HA milled with methanol. This suggests that the presence of β-TCP could be responsible for the enhancement in toughness. The mechanism that could be responsible for this would involve an increase of the energy
development of Glass Reinforced Hydroxyapatite for hard tissue surgery

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required to extend a crack and stems from a combination of crack bridging, crack deflection and microcracking in which the latter results from a mismatch in properties between adjacent grains and by phase transformations.\(^8\)

Observation of the data representing GR-HA milled under the same three conditions shows no significant improvement is made on the mechanical properties, in particular flexural strength, of GR-HA milled with water and GR-HA milled with water and deflocculant compared to GR-HA milled with methanol. The greater presence of secondary phases, in particular β-TCP, for GR-HA milled with methanol is probably responsible for providing the greater mean flexural strength values compared to the other milling conditions over the sintering range.\(^4,11\) The presence of secondary phases and the role they play in determining the mechanical strength have been discussed in a previous section (see section 5.4).

The viscosity of the milling solvent is a factor that may affect the grain size. The viscosity of methanol is lower than water, hence a difference in the grain size between composites milled water and those milled with methanol can be expected. A lower viscosity solvent should enhance the milling process, breaking down the particle size further and may also maintain the homogeneity of the slip. This should lower the mean grain size values relative to composites milled with a higher viscosity solvent. However, the results suggest that there is no significant difference in the grain size when comparing each composite at a given temperature.
5.8 Mechanical properties and characterisation of HA and GR-HA composites containing 2.5wt% ternary and quaternary glass systems.

- Composites were milled at 2:3 powder to methanol ratio

The three GR-HA composites that were examined here consists of three different glass systems at 2.5wt% addition, two of which are ternary glass systems and one that is a quaternary glass. All three glass systems consists of 21.25mol\%CaO and 63.75mol\%P_2O_5 but differ in the amount of MgO and CaF_2. One ternary glass consists of 15mol\%MgO and no CaF_2 [GR-HA (CP15Mg)], the other 15mol\%CaF_2 and no MgO [GR-HA (CP15F)]. The quaternary glass consists of 7.5mol\% CaF_2 and MgO [GR-HA (CP7.5Mg7.5F)].

The mean biaxial flexure strength values exhibited for GR-HA composites overall are significantly greater compared to those of HA (Figure 125). The data for GR-HA (CP15F) displayed a gradual increase in mean flexural strength from 1200 to 1350°C. This ranged from 96.43MN.m^{-2} at 1200°C to 142.59MN.m^{-2} at 1350°C. GR-HA (CP15Mg), however, exhibited similar strength to GR-HA (CP15F) at 1200°C but dropped to 70.73 and 73.38MN.m^{-2} at 1250 and 1300°C respectively, this was then followed by an increase to 120.4MN.m^{-2} at 1350°C. GR-HA (CP7.5Mg7.5F) exhibited a lower strength value compared to the other GR-HA composites at 1200°C. With further sintering the strength
increased to approximately 88MN.m\(^2\) at 1250 and 1300°C, and then increased further to 130.23MN.m\(^2\) at 1350°C.

The flexural moduli for the GR-HA composites also exhibited enhanced values compared to HA overall (Figure 126). These range from between 56 to 70GN.m\(^2\). The GR-HA composites in this instance show similar values at all four sintering temperatures.

Figure 125. Biaxial flexure strength of HA and GR-HA composites.

Figure 126. Flexural modulus of HA and GR-HA composites.
The hardness values for the GR-HA composites in this case also showed a similar trend as those discussed with the previous system, where the mean hardness values for HA exhibiting higher values compared to the GR-HA composites (Figure 127). However, again due to the large variation, the data represented showed no significant difference.

The data shown for the GR-HA composites concerning fracture toughness also exhibited enhanced values compared to HA overall (Figure 128). At 1200°C, GR-HA (CP15Mg) and GR-HA (CP15F) expressed higher values compared to GR-HA (CP7.5Mg7.5F), these are given as 0.86 and 0.73 MN.m$^{3/2}$ respectively. This was followed by a drop to approximately 0.46MN.m$^{3/2}$ at 1250°C for both GR-HA (CP15Mg) and GR-HA (CP15F) and then a gradual increase between 1250 and 1350°C, with both composites showing similarity in data.
Figure 128. Fracture toughness of HA and GR-HA composites.

The GR-HA (CP7.5Mg7.5F) composite exhibited the lowest fracture toughness values compared to the other GR-HA composites. From 1200 to 1350°C these values did not show much change.

Actual density measurements obtained for the GR-HA composites as expected show lower values compared to HA, but like the GR-HA (2.5wt%CNP) and GR-HA (5wt%CNP) composites there is improved densification from 1200 to 1250°C (Figure 129).

Figure 129. Density of HA and GR-HA composites.
Porosity is greatly reduced for all three GR-HA composites as a result of sintering, however, at 1350°C GR-HA (CP15F) dropped to zero (Figure 130).

Figure 130. Porosity of HA and GR-HA composites.

Figure 131. X-ray diffraction pattern for GR-HA (CP15Mg).
The X-ray diffraction patterns representing the GR-HA composites showed decomposition to secondary phases for all sintering temperatures, in particular β-TCP (Figures 131 to 133). The β-TCP content that is present in these sintered composites is approximately 20% between 1200 and 1300°C. At 1350°C inversion to α-TCP occurred for all GR-HA composites, with GR-HA (CP15F) expressing the largest content of α-TCP. From Rietveld analysis, the α-TCP content was estimated as 18% for GR-HA (CP15F), 5% for GR-HA (CP7.5Mg7.5F) and 3% for GR-HA (CP15Mg). There is also evidence of inversion from β-TCP to α-TCP rather than continued HA decomposition for the GR-HA composite (CP15F). This is represented by the phase element analysis (Figure 134).
Figure 133. X-ray diffraction pattern for GR-HA (CP7.5Mg7.5F).

Figure 134. Amount of β-TCP and α-TCP in HA & GR-HA composites.
The grain size measurements determined for this set of composites show the trend that is expected when these materials undergo sintering. The results showed an increase in the mean grain size from 1200 to 1350°C, however, a levelling off of the mean grain size for GR-HA (CP15Mg) was observed at 1350°C (Figure 135). The similarity in grain size for GR-HA (CP15Mg) between 1300 and 1350°C can also be seen in the distribution curves (Figure 136).

Figure 135. Mean grain size for HA & GR-HA composites.
Figure 136. Grain size distribution for GR-HA composites.
The image representing the etched surface of GR-HA (CP15F) (Figure 137) sintered at 1300°C is remarkably different to that of HA in that a significant amount of grains do not seem to be present. Etching of GR-HA surfaces results in the removal of any β-TCP present and can account for this surface characteristic.

The porosity of GR-HA (CP15F) sintered at 1300°C shown in Figure 130 is given as 1.56%. The presence of this amount of porosity is evident in the image. Inter granular porosity can clearly be seen at the grain boundaries.
It is apparent that for the same material fired at 1350°C that the mean grain size increases and this can be observed from the image (Figure 138). The distribution curve (Figure 136) and the grain size graph (Figure 135) exhibit a large rise in the mean grain size from 1300 to 1350°C for GR-HA (CP15F) and also a greater distribution of large grains indicating grain growth. In addition, the presence of pores along the grain boundaries and junctions seen at 1300°C has been greatly reduced at 1350°C. This can help explain the difference in porosity (Figure 130) seen between 1300 and 1350°C. The porosity remaining is intragranular.

Figure 138. GR-HA (CP15F) sintered @ 1350°C.
5.9 Mechanical properties and characterisation of HA and GR-HA composites containing 2.5wt% ternary and quaternary glass systems.

CaO: 16.25mol%; P$_2$O$_5$: 63.75mol%; MgO: 20mol%; CP20Mg (ternary glass system)

CaO: 16.25mol%; P$_2$O$_5$: 63.75mol%; CaF$_2$: 20mol%; CP20F (ternary glass system)

CaO: 16.25mol%; P$_2$O$_5$: 63.75mol%; MgO: 10mol%; CaF$_2$: 10mol%; CP10Mg10F (quaternary glass system)

- Composites were milled at 2:3 powder to methanol ratio

The same components were used to formulate the next three glass composites that were to be incorporated into HA. However, the molar composition of MgO and CaF$_2$ were increased to 20mol% in the ternary glasses and 10mol% in the quaternary glass. This was carried out in order to assess whether or not this induced any changes with respect to the mechanical, structural and crystallographic properties of the material. In particular the role of MgO in the stabilisation of the β-TCP phase was of interest.

The Biaxial flexure strength data obtained for this set of GR-HA composites expressed lower values overall compared to the previous set (Figure 139). As a result of increasing the molar composition of MgO and CaF$_2$ there doesn’t appear to be an improvement in the flexural strength. The GR-HA (CP10Mg10F) composite sintered at 1200°C showed the only improved flexural strength. This was recorded at 80.38MN.m$^{-2}$, whereas the GR-HA (CP7.5Mg7.5F) composite gave a flexural strength of 58.95MN.m$^{-2}$ at this temperature (Figure 125). Within this set of GR-HA composites the only apparent difference in strength occurred at 1300°C, with the GR-HA (CP20F) giving a higher value compared to the other GR-HA composites.
The flexural modulus values for this set of GR-HA composites ranged from 54 to 68.9GN.m$^2$, they also showed no significant difference between sintering temperatures (Figure 140). The values recorded for the previous set gave a range of 56.05 to 71.52GN.m$^2$, these GR-HA composites showed no significant change between sintering temperatures also. Hence, the flexural modulus for both sets of GR-HA composites can be considered very similar.

Figure 139. Biaxial flexure strength of HA & GR-HA composites.

Figure 140. Flexural modulus of HA & GR-HA composites.
Similarly, the range of results concerning hardness for both sets of GR-HA composites can be considered similar, in that they both exhibited lower values compared to HA and also expressed a similar range of results from 1200 to 1350°C (Figures 127 and 141).

![Figure 141. Hardness of HA & GR-HA composites.](image)

The fracture toughness values for this set of GR-HA composites showed enhanced values compared to HA also (Figure 142). The GR-HA (CP20Mg) composite in this instance exhibited similar fracture toughness over all four sintering temperatures these range from 0.44 to 0.49MN.m\(^{3/2}\). At 1200°C GR-HA (CP20F) expressed the highest fracture toughness value, which was estimated at 0.55MN.m\(^{3/2}\). The other two composites showed similar values at this temperature, but were lower compared to GR-HA (CP20F), these were estimated at 0.46MN.m\(^{3/2}\). At 1250 and 1350°C there were obvious differences in the data. At 1250 GR-HA (CP10Mg10F) expressed the lowest value which is between 0.36 and 0.41MN.m\(^{3/2}\) lower than the other two composites. The most notable difference can be observed at 1350°C, where GR-HA (CP20F) showed the highest values compared to GR-HA (CP20Mg), these differed by approximately 0.09MN.m\(^{3/2}\). The similarities between the two sets of GR-HA composites could be seen with the GR-HA (CP15F) and GR-HA
(CP20F) composites (Figures 128 and 142). Both composites showed a drop in value between 1200 and 1250°C, followed by a rise at 1350°C. However, the fracture toughness for GR-HA (CP15F) sintered at 1200°C is considerably higher compared to GR-HA (CP20F). This is also evident between the two MgO containing glass GR-HA composites.

![Fracture toughness of HA & GR-HA composites.](image)

Figure 142. Fracture toughness of HA & GR-HA composites.

Actual density measurements (Figure 143) and porosity data (Figure 144) for this set of GR-HA exhibited more or less the same correlation as with the previous GR-HA composites discussed.

![Actual density of HA & GR-HA composites.](image)

Figure 143. Actual density of HA & GR-HA composites.
Figure 144. Porosity of HA & GR-HA composites.

The obvious difference in the porosity shown for this set of GR-HA composites occurred for GR-HA (CP20F) and GR-HA (CP20Mg) (Figure 144). GR-HA (CP15Mg) (Figure 130) displayed a drop in porosity from 1200 to 1250°C and remained constant from 1250 to 1350°C, for GR-HA (CP20Mg), however, the drop in porosity almost approached zero at 1300°C, this was then followed by a rise at 1350°C. GR-HA (CP20F) though showed two points of zero porosity, at 1250 and 1350°C, whereas GR-HA (CP15F) (Figure 130) exhibited zero porosity at 1350°C.
Figure 145. X-ray diffraction patterns for GR-HA composites.
X-ray diffraction carried out on these composites revealed decomposition to $\beta$-TCP at all sintering temperatures and inversion to $\alpha$-TCP at 1350°C, with GR-HA (CP20F) containing the greatest amount of $\alpha$-TCP. The $\alpha$-TCP phase content determined from the Rietveld refinement in this case was measured as 9.6% for GR-HA (CP20F) (Figures 145 and 146).

Figure 146. Amount of $\beta$-TCP and $\alpha$-TCP phases in HA & GR-HA composites.
Grain size measurements for these GR-HA composites, as with previously discussed GR-HA composites, displayed an increase in the mean grain size and variation in data as a function of sintering temperature. For each sintering temperature, the mean grain size for these composites appeared to be similar (Figure 147).

Figure 147. Mean grain size of HA & GR-HA composites.
Figure 148. Grain size distribution of GR-HA composites.
An anomaly was observed when analysing the SEM images (Figures 150 to 153) and the porosity data (Figure 144) for this set of GR-HA composites. The porosity data that was discussed earlier for GR-HA (CP20F) emphasised the drop in porosity to zero at 1250°C and then a rise at 1300°C, followed by a drop to zero again at 1350°C. This, however, is not the case when looking at the images of the surfaces. This anomaly can clearly be observed at 1250°C, where the presence of pores do exist and contradict the calculated porosity seen in Figure 144. This can be explained by the overall theoretical densities determined from the Rietveld refinement (Figure 149). The theoretical density from the refinement assumes no porosity in the structure. At 1250°C this was calculated at a value that was close to the actual density, subsequently giving zero porosity. One must bear in mind that observations made from the SEM images are qualitative and only give a rough indication of the porosity from the material surface, moreover the Rietveld refinements in this instance may have been subject to human error.

Between 1300 and 1350°C the amount of porosity at the surfaces observed from the images appears to correlate with the calculated porosity data.

![Figure 149. Theoretical densities of HA & GR-HA composites.](image-url)
Figure 150. GR-HA (CP20F) sintered @ 1200°C.

Figure 151. GR-HA (CP20F) sintered @ 1250°C.
Figure 152. GR-HA (CP20F) sintered @1300°C.

Figure 153. GR-HA (CP20F) sintered @1350°C.
Discussion for sections 5.8 and 5.9

The relationship between the porosity and the mechanical properties, in particular flexural strength which have been previously discussed with respect to the GR-HA’s containing CNP glasses, can also be observed with GR-HA’s systems with glasses containing MgO and CaF₂.

It has already been mentioned that the addition of glass to HA facilitates densification and reduces porosity in the material. However, as with the GR-HA’s containing CNP glass, the GR-HA’s containing MgO and CaF₂ show comparable porosity with HA. This would again suggest the porosity is not the predominant factor responsible for the enhanced mechanical properties exhibited for this system of GR-HA’s.

The Rietveld refinement carried out on the x-ray diffraction patterns for these specimens gave phase element fractions which indicate decomposition of HA to β-TCP at all four sintering temperatures and further inversion to α-TCP at 1350°C for all GR-HA composites. The pros and cons that these secondary phases present to the mechanical properties and structural nature have already been previously mentioned. However, as a result of these findings, one would arrive at the assumption that phase decomposition and subsequent phase inversions are the predominant factors influencing the mechanical properties of these GR-HA systems.

This system of GR-HA’s show enhanced flexural moduli values compared to HA. This infers that these materials exhibit a greater stiffness compared to HA. The flexural modulus
for the GR-HA’s collectively shown in this case are significantly different to HA, however, this not the case with the GR-HA’s containing CNP glass. The enhanced flexural modulus values for these GR-HA systems can be attributed to the nature of the inter-atomic spacing and bonding, and would suggest that the atomic bonding in the materials are greater. One explanation for this may due to the bonding nature of Mg$^{2+}$ and F$^-$. Mg$^{2+}$ ions that are present in the crystal lattice have a tendency to behave more covalently and have a smaller ionic radius compared to Ca$^{2+}$, F$^-$ ions have a smaller atomic radius than OH$^-$ and are also more electronegative, therefore the introduction of these ions increase bonding and decrease inter-atomic spacing. Another explanation could involve the viscosity of the glass, and may be influenced by the composition. A low viscosity glass at sintering temperature could enhance dissolution of atoms into the liquid phase and therefore enhance particle packing. This can enhance shrinkage and densification and therefore ultimately enhance the flexural modulus of the GR-HA. The type of glass composition introduced and its relation to viscosity may also help to explain why differences are seen in the mean flexural strength values between GR-HA composites. For instance GR-HA (CP15F) shows the highest overall mean values in flexural strength, this may be attributed more to the viscous nature of this particular glass at a given sintering temperature, since the data regarding porosity, phase content and grain size show comparable values between each GR-HA at all four sintering temperatures.

The initial drop in fracture toughness shown for GR-HA (CP15F) and GR-HA (CP15Mg) between 1200 and 1250°C is probably attributed to grain growth and porosity. The presence of pores in a material act as flaws that can initiate cracks during fracture and
which provide regions of stress concentration. Even if there seems to be a reduction in porosity between 1200°C and 1250°C for these materials this does not necessarily imply that there should be a reduction in fracture toughness. The nature of the flaw may change, in particular flaw size, as a function of grain growth, which would suggest that the pores present in these materials may vary in stress concentration between 1200 and 1250°C and would consequently influence the fracture toughness. This could also explain the differences in fracture toughness values generally seen between HA and GR-HA's over the sintering range.
5.10 Mechanical properties and characterisation of HA and GR-HA composites containing 2.5wt% glass with CaF$_2$. 

CaO: 21.25mol%; $P_2O_5$: 63.75mol%; CaF$_2$: 15mol%: CP15F (ternary glass system) 
CaO: 16.25mol%; $P_2O_5$: 63.75mol%; CaF$_2$: 20mol%: CP20F (ternary glass system) 

- Composites were milled at 2:3 powder to methanol ratio 

The following set of results compares the mechanical and crystallographic properties of GR-HA composites with glasses containing CaF$_2$. The GR-HA composites that will be discussed are GR-HA (CP15F) and GR-HA (CP20F). Since only two composites will be compared, this may make it easier to make clear distinctions between the data.

The mean biaxial flexure strength for these GR-HA composites were considerably greater compared to HA, however, the mean values for GR-HA (CP15F) gave slightly enhanced values compared to GR-HA (CP20F) (Figure 154). At 1300 and 1350°C the mean flexural strength for GR-HA (CP20F) approached those of GR-HA (CP15F), however, the mean values show no significant difference indicated by the overlap in error bars. The flexural strength at 1300 and 1350°C for GR-HA (CP20F) are given as 106.75 and 131.99MN.m$^{-2}$ respectively. For GR-HA (CP15F) these are given as 119.46 and 142.59MN.m$^{-2}$ respectively.
Figure 154. Biaxial flexure strength of HA & GR-HA composites.

Flexural modulus values for these GR-HA composites were also enhanced compared to HA (Figure 155). The overall values at 1250 to 1350°C for the GR-HA composites were significantly different compared to HA, except at 1200°C where there is a considerable overlap in data. Between 1200 and 1300°C the mean values for GR-HA (CP15F) were slightly enhanced compared to GR-HA (CP20F). However, at 1350°C the mean values for the two composites were the same. The values at 1350°C for these composites were recorded as 70GN.m² and were significantly greater than HA at this temperature, which was given as 46.81GN.m².
Figure 155. Flexural modulus of HA & GR-HA composites.

The hardness for these composites displayed lower mean values compared to HA, however, a large variation in data was seen. This is shown in Figure 156.

Figure 156. Hardness of HA & GR-HA composites.

The fracture toughness for these GR-HA composites exhibited enhanced values compared to HA over the sintering range (Figure 157). Differences in fracture toughness that were
observed between the two GR-HA composites occurred at 1200 and 1300°C. At 1200°C GR-HA (CP15F) exhibited a value of 0.86 MN.m$^{-3/2}$, whereas GR-HA (CP20F) exhibited a value of 0.55 MN.m$^{-3/2}$. At 1300°C GR-HA (CP15F) exhibited a value of 0.55 MN.m$^{-3/2}$, whereas GR-HA (CP20F) exhibited a value of 0.39 MN.m$^{-3/2}$ and is close to the value of HA at this temperature, which was calculated at 0.35 MN.m$^{-3/2}$. The most noticeable change over the sintering range could be seen for GR-HA (CP15F). This showed a large drop from 0.86 to 0.47 MN.m$^{-3/2}$ between 1200 to 1250°C and then a slight increase from 1250 to 1350°C.

![Fracture toughness of HA & GR-HA composites.](image)

**Figure 157.** Fracture toughness of HA & GR-HA composites.

The density and porosity data (Figure 158) for these two composites showed a similar trend. A large increase in density for both GR-HA composites was observed from 1200 to 1250°C with the values remaining constant from 1250 to 1350°C. The porosity for both GR-HA composites exhibited a similar trend, however the anomaly in porosity noted at 1250°C for GR-HA (CP20F) has been previously discussed.
Figure 158. Density and Porosity of HA & GR-HA composites.

Figure 159. X-ray diffraction patterns of GR-HA composites.
It is clear from the x-ray diffraction patterns (Figure 159) for these composites that at all sintering temperatures there is decomposition of HA to β-TCP and inversion from β to α-TCP at 1350°C, with GR-HA (CP15F) exhibiting the greater content of α-TCP compared to GR-HA (CP20F). This can be reflected by the phase element fractions determined from the Rietveld refinement (Figure 160).

![Graph of Beta TCP weight fraction](image)

![Graph of Alpha TCP weight fraction](image)

**Figure 160.** Amount of β-TCP and α-TCP in HA & GR-HA composites.

Analysis of grain size for these composites showed a distinct difference at 1350°C, where GR-HA (CP15F) displayed an enhanced mean grain size value compared to HA and GR-HA (CP20F) (Figure 161). This observation can be supported by the grain size distribution

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for these composites (Figure 162). This clearly showed a shift in distribution to larger grains for GR-HA (CP15F) compared to GR-HA (CP20F) (Figure 162).

Figure 161. Grain size measurements of HA & GR-HA composites.

Figure 162. Grain size distribution of GR-HA composites.
Figure 163. GR-HA (CP15F) sintered @1200°C.

Figure 164. GR-HA (CP15F) sintered @1250°C.
Figure 165. GR-HA (CP15F) sintered at 1300°C.

Figure 166. GR-HA (CP15F) sintered at 1350°C.
The images shown (Figures 163 to 166) display the surfaces of GR-HA (CP15F) sintered between 1200 and 1350°C. The calculated porosity for this GR-HA showed a large drop in porosity from 1200 to 1250°C, with the value increasing slightly at 1300°C and finally being eliminated at 1350°C. This behaviour can be observed from the images. In this instance there appears to be a correlation between the calculated data and the observation made from the surface images.
Incorporating phosphate based glasses such as CP15F and CP20F into HA during the milling process shows overall improvement in the flexural strength, flexural modulus and fracture toughness of the final material. The factors that may contribute to this, however, have been previously covered and consider effects such as decomposition to secondary phase β-TCP and subsequent inversion to α-TCP, the bonding nature of fluoride ions in the lattice structure and the nature of flaws on the material surface.

The aforementioned mechanical properties all express slightly greater mean values for GR-HA containing CP15F glass compared to GR-HA with CP20F glass. The fact that there is no indication of major differences in the amount of secondary phases present and also in grain size for both composites over the sintering range would suggest that some other predominating factor is responsible for the differences in the mechanical properties.

It has been previously mentioned that the electronegativity and atomic radius of the fluoride ion influence the bonding nature in the HA lattice as a result of A-type substitution with hydroxyl ions, and as a consequence increase bonding and decrease inter-atomic spacing. The results would indicate that the optimisation of the mechanical properties is influenced by the molar addition of CaF₂ in the glass which subsequently determines the extent of fluoride ions that are incorporated in the HA lattice. Therefore a glass that constitutes 15mol% CaF₂ seems to be the optimal addition in this case.
5.11 Mechanical properties and characterisation of HA and GR-HA composites containing 2.5wt% glass with MgO.

CaO: 21.25mol%; P₂O₅: 63.75mol%; MgO: 15mol%: CP15Mg (ternary glass system)
CaO: 16.25mol%; P₂O₅: 63.75mol%; MgO: 20mol%: CP20Mg (ternary glass system)
CaO: 11.25mol%; P₂O₅: 63.75mol%; MgO: 25mol%: CP25Mg (ternary glass system)
CaO: 2.75mol%; P₂O₅: 63.75mol%; MgO: 33.5mol%: CP33.5Mg (ternary glass system)

- Composites were milled at 2:3 powder to methanol ratio

The following set of data compares the properties of GR-HA composites containing glasses with varying composition of MgO.

![Graph showing biaxial flexure strength of GR-HA with varying MgO composition](image)

Figure 167. Biaxial flexure strength of GR-HA with glasses containing varying MgO composition.

All the GR-HA composites exhibited enhanced mean biaxial flexure strength compared to HA (Figure 167). GR-HA (CP15Mg) and GR-HA (CP25Mg) displayed the same trend, where from 1200 to 1300°C there is a drop in flexural strength, followed by a sharp increase at 1350°C. GR-HA (CP33.5Mg) did not show much change from 1200 to 1350°C, the only drop in flexural strength is observed at 1250°C, but this only differs by 6 to 15MN.m². However, the errors bars in the data for this composite between these sintering
temperatures seems to suggest that there is no significance difference in these values. There was also a substantial overlap in data for GR-HA (CP20Mg) from 1200 to 1300°C, however, there was a significant difference between these values and those at 1350°C. From 1200 to 1300°C the mean values ranged from 61 to 71MN.m$^{-2}$, whereas at 1350°C the mean value was given at 107.77MN.m$^{-2}$. This could also be seen for GR-HA (CP15Mg) where the mean value at 1350°C was higher than those between 1200 and 1300°C. In addition, it can be shown that the mean values at 1350°C show a relationship between biaxial flexure strength and composition.

![Figure 168. Flexural modulus of HA & GR-HA composites.](image)

The flexural modulus of these composites displayed overall enhanced mean values compared to HA, however, from the graph there did not seem to be a clear relationship between the flexural modulus values between each composite at a given sintering temperature (Figure 168). This is also true when comparing the values for each composite over the range of sintering temperatures given.
Yet again, as discussed before, the general trend for hardness with respect to these composites is more or less the same in which they express a large variation in data making the analysis of this data rather difficult (Figure 169). It is apparent that there were no significant differences in the overall values even if the mean values express obvious differences.

GR-HA (CP15Mg) exhibited the highest fracture toughness at 1200 and 1350°C compared to the other composites, however, at 1350°C the value was significantly lower than that expressed at 1200°C, these were estimated at 0.73 and 0.62 MN.m$^{3/2}$ respectively (Figure 170). At 1250°C the fracture toughness for GR-HA (CP15Mg) approached a value similar to that of GR-HA (CP20Mg), which was given at approximately 0.48 MN.m$^{3/2}$. Similarities in fracture toughness was also observed at 1300°C, where GR-HA (CP15Mg) exhibited a value of 0.48 MN.m$^{3/2}$ and is close to those shown for GR-HA (CP20Mg) and GR-HA (CP33.5Mg).
Figure 170. Fracture toughness of HA & GR-HA composites.

Actual density measurements for these GR-HA composites indicated densification by a sharp increase in density between 1200 and 1250°C, with GR-HA (CP15Mg) having the highest densification rate (Figure 171). This densification was reflected by the porosity data calculated by a large drop in porosity from 1200 to 1250°C for all composites (Figure 171), with GR-HA (CP20Mg) exhibiting the lowest porosity at 1300°C. However, between 1200 and 1300°C GR-HA (CP15Mg) still showed the highest porosity but dropped to values comparable to the other composites at 1350°C.

Figure 171. Actual density and porosity of HA & GR-HA composites.
Figure 172. X-ray diffraction patterns of GR-HA composites.
X-ray diffraction carried out showed decomposition of HA to β-TCP for all four composites over the given sintering range (Figure 172). Inversion to α-TCP taking place at 1350°C only occurred for GR-HA (CP15Mg) and GR-HA (CP20Mg) and this can clearly be seen from the phase elemental analysis (Figure 173).

Figure 173. Amount of β-TCP and α-TCP phases in HA and GR-HA composites.

Theoretical densities determined from Rietveld analysis showed higher values over all sintering temperatures for HA and between 1200 and 1300°C for GR-HA (CP15Mg) compared to the other composites (Figure 174). The theoretical densities and actual densities for GR-HA (CP15Mg) can help explain the high porosity values that are shown.
This is simple to envisage, the actual density measurements and theoretical density from the refinement for GR-HA (CP15Mg) are further apart compared to the other composites, hence giving the highest porosity between sintering temperatures of 1200 and 1300°C. However, this needs to be supported by images of their surfaces in order to see if there is a correlation between the calculated porosity and the porosity from the images.

Figure 174. Theoretical density of HA & GR-HA composites.

Grain size measurements carried out for these composites showed an increase in the mean values as a function of sintering temperature as expected (Figure 175), except for GR-HA (CP15Mg) which showed a levelling in value at 1350°C.

Figure 175. Grain size measurements for HA & GR-HA composites.
Figure 176. GR-HA (CP15Mg) sintered @1350°C.

Figure 177. GR-HA (CP25Mg) sintered @1350°C.
Figure 178. Grain size distribution of GR-HA composites.

The grain size distribution curves of GR-HA (CP15Mg) and GR-HA (CP25Mg) mirror the grain size data. The levelling in mean grain size of GR-HA (CP15Mg) from 1300 to 1350°C seen in Figure 175 shows a correlation with the distribution of grain size at those temperatures. The distribution of grain size at these sintering temperatures in this case is very similar (Figure 178).
It is also evident from the images (Figures 176 and 177) that there is a difference in grain size when comparing GR-HA (CP15Mg) and GR-HA (CP25Mg) at 1350°C. Even though the magnification between the two images is not the same, it is possible to observe the difference in grain size. From the images it appears that GR-HA (CP15Mg) contains smaller grains than GR-HA (CP25Mg). This can be seen from the grain size (Figure 175) and grain size distribution graphs (Figure 178).

The porosity data discussed earlier showed that GR-HA (CP15Mg) gave the greater porosity over the sintering range. The images from Figures 176 and 177, which show the surfaces of GR-HA (CP15Mg) and GR-HA (CP25Mg) sintered at 1350°C, can be compared. The grain boundaries seen for GR-HA (CP25Mg) appear to be well formed compared to GR-HA (CP15Mg) and seem to contain less porosity, this concurs with the calculated porosity values determined for this sintering temperature.
The flexural bend strength shown for the GR-HA’s with glass containing MgO as expected show enhanced values compared to HA and would, as previously discussed, be due to decomposition of HA to β-TCP and subsequent inversion to α-TCP.

An interesting aspect of these results is the effect of MgO content on the stabilising or in some cases destabilising of the β-TCP phase. It is noticeable that phase inversion to α-TCP does not occur for GR-HA (CP25Mg) and GR-HA (CP33.5Mg). This is due to the stabilisation of β-TCP as result of incorporating MgO in the phosphate glass.\textsuperscript{31,123-125} It has been documented that the incorporation of Mg\textsuperscript{2+} ions into the HA structure can arise from the dissolution of ions from the glass during the sintering process thereby allowing the substitution of ions such as Ca\textsuperscript{2+} for Mg\textsuperscript{2+} in the hydroxyapatite lattice.\textsuperscript{31,123-125} This promotes decomposition of HA to give the resulting β-TCP through the elimination of hydroxyl groups.

The resulting β-TCP that is formed in this instance has probably a very high lattice enthalpy and therefore does not readily invert to α-TCP. This stabilising effect is attributed to the chemical nature of the Mg\textsuperscript{2+} ion that is present in the β-TCP lattice. From this study it can be concluded that high amounts of MgO inhibit β to α-TCP inversion.

By contrast what is observed at 1350°C for GR-HA (CP15Mg) and GR-HA (CP20Mg) is inversion to α-TCP. It seems as though the extent to which Mg ions stabilise the β-TCP is dependent on the amount or molar quantity of MgO introduced into the glass composite. Since there is a lower amount of Mg ions present in this case, there is not a sufficient
enough stabilising affect on the $\beta$-TCP lattice and subsequently makes it easier to overcome the lattice enthalpy at this temperature. This consequently leads to inversion.

Recorded density measurements for almost all GR-HA systems always seem to give lower density values compared to HA even though the porosity values are similar. This effect is due to GR-HA's containing lower density secondary phases.

The high porosity exhibited for GR-HA (CP15Mg) compared to HA and other composites over the sintering range, is ascribed to the difference between the theoretical density values (1200-1300°C) obtained from the Rietveld refinement and the recorded density. The porosity value is obtained by dividing the recorded density measurement by the theoretical density and subtracting that number from 1. Since there is a large difference between the two for this system, the resulting porosity calculated was high. The isotropic thermal parameters, cell parameters and atom positions were refined for all HA atoms. The refinement takes into account the changes in these parameters to determine the theoretical densities and assumes that there is no porosity in the structure. Refinement of the parameters may be limited by the effect of magnesium ions present in the HA lattice, particularly at this molar quantity and might explain why the theoretical density determined in this case is greater than the other GR-HA composites. Furthermore, it can be said that since Mg$^{2+}$ ions have a smaller ionic radii than Ca$^{2+}$ ions then substitution of Ca$^{2+}$ for Mg$^{2+}$ may cause the unit cell to shrink and therefore result in higher theoretical density. However, limited substitution of Mg$^{2+}$ ions may cause detrimental effects. The addition of a
glass with a higher MgO content might bring about the substitution of interstitial ions which can cause an increase in the unit cell and may also cause distortion.

On the other hand the differences seen for the theoretical densities might be related to the viscosity of the glass composites at sintering temperature, which may be influenced by the MgO content and can ultimately affect the extent of substitution of Mg$^{2+}$ ions.\textsuperscript{4}
5.12 Mechanical properties and characterisation of HA and GR-HA composites containing 2.5wt% glass with MgO and CaF₂.

\[ \text{CaO: } 21.25\text{mol}\%; \ P_2\text{O}_5: 63.75\text{mol}\%; \ MgO: 7.5\text{mol}\%; \ CaF}_2: 7.5\text{mol}\%: \text{CP7.5Mg7.5F} \]

(Quaternary glass system)

\[ \text{CaO: } 16.25\text{mol}\%; \ P_2\text{O}_5: 63.75\text{mol}\%; \ MgO: 10\text{mol}\%; \ CaF}_2: 10\text{mol}\%: \text{CP10Mg10F} \]

(Quaternary glass system)

- Composites were milled at 2:3 powder to methanol ratio

The next set of GR-HA composites that were analysed were those reinforced with glasses containing MgO and CaF₂. The improvement in biaxial flexure strength compared to HA for the GR-HA composites that are considered in this case have already been displayed in previous discussions, however, the comparison must be made between the two with respect to the difference in the molar composition of MgO and CaF₂. Both composites generally exhibited an increase in flexural strength as sintering temperature increased (Figure 179).

The flexural strength for both composites between 1250 and 1350°C were similar, except at 1200°C where the values were markedly different. The values given at 1200°C were 80.38MN.m⁻² for GR-HA (CP10Mg10F) and 58.95MN.m⁻² for GR-HA (CP7.5Mg7.5F). Maximum values at 1350°C were given at 123.38MN.m⁻² for GR-HA (CP10Mg10F) and 130.23MN.m⁻² for GR-HA (CP7.5Mg7.5F). However, due to considerable overlap in the overall data at 1350°C, the flexural strength with respect to both composites could be considered similar.
Flexural modulus values for these composites also showed enhanced values compared to HA over the sintering range. Analysis of values for both composites over the sintering range showed no significant difference between them, except at 1200°C where the mean values for both composites displayed a slight difference (Figure 180).
Hardness values for these composites showed more or less the same trend as those composites discussed previously. Mean hardness values for the GR-HA composites were lower compared to HA, however, there was a large variation in data for all specimens (Figure 181).

Enhanced values were observed with respect to fracture toughness for these GR-HA composites compared to HA over the sintering range (Figure 182). The trend for both GR-HA composites were similar in that from 1200 to 1300°C they exhibited a drop in value followed by an increase at 1350°C. Distinct differences in data could be seen at 1350°C between the three materials with GR-HA (CP10Mg10F) exhibiting the highest mean value (0.54 MN.m$^{3/2}$) and was the highest value observed amongst the data.

![Figure 181. Hardness of HA & GR-HA composites.](image-url)
The density and porosity values (Figure 183) for these composites showed the expected inverse relationship and both followed very similar trends.

**Figure 182.** Fracture toughness of HA & GR-HA composites.

**Figure 183.** Density and porosity of HA & GR-HA composites.
Figure 184. X-ray diffraction patterns for GR-HA composites.

The X-ray diffraction patterns for both GR-HA compositions exhibited decomposition to secondary β-TCP phase at all sintering temperatures, with inversion to α-TCP at 1350°C (Figure 184). The phase content for these composites can be best represented by the phase element fraction graphs (Figure 185).
Figure 185. Amount of $\beta$-TCP and $\alpha$-TCP in HA & GR-HA composites.

Analysis of the grain size for these composites showed an increase in the mean grain size as a function of sintering temperature (Figure 186), additionally, the grain size distribution curves exhibited a greater distribution of larger grains as a function of sintering temperature, pointing toward continual grain growth (Figure 187). The mean grain size values at each sintering temperature for both composites were shown to be similar.
Figure 186. Grain size measurements of HA & GR-HA composites.

Figure 187. Grain size distribution of HA & GR-HA composites.
The flexural strength data expressed for both GR-HA’s displays a distinct increase in the mean values at 1350°C. These values are significantly different to those between 1200 and 1300°C. Moreover, there seems to be a correlation between the sharp increase in flexural strength from 1300 to 1350°C and the inversion of β-TCP to α-TCP at 1350°C for both composites. This suggests that the increase in flexural strength at 1350°C seems to be influenced by the presence of α-TCP.

Slight differences in the mean values between these GR-HA’s for flexural strength, flexural modulus and fracture toughness may be attributed to the molar quantity of CaF$_2$ in the glass. As previously discussed only certain molar quantities of CaF$_2$, it seems, can be incorporated in order to optimise the mechanical properties.
5.13 Hot Pressing

The usual method of processing the specimen discs so far has made use of pressureless sintering, where the powder is pressed at 20 tonnes and then fired to the required temperature. Hot pressing involves the application of pressure whilst sintering to produce ceramic blocks. The powder is fired up to a temperature of 600°C before any pressure is applied. Once this temperature is reached pressure is applied stepwise to 20MN.m⁻² (3500Kg) at the required sintering temperature (1100°C). The use of this technique is viewed as a potential means of enhancing the mechanical properties of the material. Three different materials were investigated; HA, GR-HA (CP15F) and GR-HA (CP20F). These were compared against those processed via pressureless sintering.

Analysis made on the flexural strength of these materials showed enhanced values for the hot pressed specimens compared to the specimens processed via the usual route (Figure 188). A value of 78.9 MN.m⁻² was given for hot pressed HA, whereas HA processed via pressureless sintering gave a value of 30.327 MN.m⁻². For both processing routes the flexural strength of the GR-HA composites gave enhanced values compared to HA. However, the values expressed for the hot pressed GR-HA specimens were roughly twice the flexural strength of the pressureless sintered specimens. Hot pressed GR-HA (CP15F) and GR-HA (CP20F) gave values of 91.75 and 88.87 MN.m⁻² respectively. Pressureless sintered GR-HA (CP15F) and GR-HA (CP20F) gave values of 36.58 and 47.35MN.m⁻² respectively.
Figure 188. Flexural strength for hot pressed and pressureless sintered HA and GR-HA composites.

Figure 189. Flexural modulus for hot pressed and pressureless sintered HA and GR-HA composites.
Flexural modulus, hardness, and fracture toughness data for these materials also showed enhanced values for the hot pressed specimens. The flexural modulus values for the hot pressed specimens were approximately four times greater than the values given for the pressureless sintered specimens (Figure 189). The hot pressed specimens give values well above 100GN.m$^{-2}$, whereas the pressureless sintered specimens gave values below 40GN.m$^{-2}$.

The difference in hardness values between the hot pressed and pressureless sintered specimens were quite noticeable (Figure 190). Pressureless sintered and hot pressed HA exhibited values of 106.68$H_v$ and 613.8$H_v$ respectively. The mean fracture toughness values shown for the hot pressed specimens were significantly greater than pressureless sintered HA and GR-HA (CP20F) specimens, however, pressureless sintered GR-HA (CP15F) exhibited a mean value of 0.93 MN.m$^{-3/2}$ which is close to hot pressed HA (0.99 MN.m$^{-3/2}$) (Figure 191).

![Figure 190. Hardness for hot pressed and pressureless sintered HA and GR-HA composites.](image-url)
Density and porosity data determined for these materials show how the hot pressing technique significantly improves densification. This is indicated by the higher density and low porosity values for the hot pressed specimens compared to the pressureless sintered specimens (Figure 192).

**Figure 191.** Fracture toughness for hot pressed and pressureless sintered HA and GR-HA composites.

**Figure 192.** Density and porosity for hot pressed and pressureless sintered HA and GR-HA composites.
Figure 193. X-ray diffraction for hot pressed and pressureless sintered HA and GR-HA composites.
Figure 194. Phase element fractions for hot pressed and pressureless sintered HA and GR-HA composites.

X-ray diffraction analysis of these materials showed no decomposition to secondary phases for HA sintered via both methods, however, decomposition to β-TCP did occur for the GR-HA composites sintered at 1100°C via both methods (Figures 193 and 194).
Figure 195. Grain size and grain size distribution.

Grain size measurements and grain size distribution that were determined for these materials show that hot pressing resulted in larger mean grain size and a broader distribution of grains compared to their respective pressureless sintered specimens (Figure 195). However, the grain sizes of the pressureless sintered GR-HA composites are shown to be comparable with the hot pressed HA specimen. SEM images of the surfaces of these materials can be shown (Figures 196 to 201).
Figure 196. HA Hot Pressed @ 1100°C.

Figure 197. HA pressureless sintered @ 1100°C.
Figure 198. GR-HA (CP15F) Hot pressed @ 1100°C.

Figure 199. GR-HA (CP15F) pressureless sintered @ 1100°C.
Figure 200. GR-HA (CP20F) Hot Pressed @ 1100°C.

Figure 201. GR-HA (CP20F) pressureless sintered @ 1100°C.
The results shown for the hot pressed specimens generally show an improvement in the mechanical properties when compared against the pressure-less sintered materials at 1100°C. This is evident from the flexural strength, flexural modulus, hardness and fracture toughness values obtained from this study.

The combination of pressing and firing at the required sintering temperature serve to improve packing by the rearrangement of particles and enhance particle to particle contact. The resulting sintered materials that undergo this process reach densification at a lower temperature. This effect is reflected by the densification values, which show a higher density for the hot pressed specimens compared to the pressure-less sintered specimens. Furthermore, the relationship between density and porosity is also evident. Generally, one would also expect minimal porosity in hot pressed materials as a result of enhanced densification. This also reflected in the lower porosity exhibited in this instance.

Despite the difference in the rate at which densification occurs, x-ray analysis shows that the β-TCP content is similar for the GR-HA composites processed via both methods. This suggests that the sintering process in this case does not change the effect the glass has on the decomposition of HA.

The higher strength values expressed for the hot pressed specimens is known not only to be due to minimisation of porosity but also to the inhibition of grain growth. However, the mean grain size values for the hot pressed specimens are greater than the pressure-less sintered specimens, and in addition display a broader distribution of grains. Even though
the results do not indicate much inhibition of grain growth, this could suggest that
densification and minimisation of porosity are the predominate factors in influencing the
mechanical properties. In addition, the presence of larger grains in the hot pressed
specimens might account for their translucent appearance.

It must be noted that the four point bend test method was employed to determine the
flexural bend strength of the hot pressed specimens, which were cut, ground, polished and
bevelled to form blocks. This machining method was undertaken to reduce any flaws in the
material. It is therefore important to take into account the elimination of surface flaws, the
dimensions of the blocks and also the parameters of the test rig in addressing whether or not
this test method is more accurate or more representative of the flexural strength of the
material, and whether the factors mentioned in some way influenced the outcome.\textsuperscript{90,106,107}

It is important though to also consider one other detail when comparing the flexural
strength values of the pressureless and hot pressed sintered specimens. The outcome of the
flexural strength measurements is limited by the dimensional parameters of the test
methods.\textsuperscript{90,106,107} Therefore, since the parameters for both test methods are different then
the flexural strength may differ. If a composite was prepared by one method, either by
pressureless sintering or hot pressing, and the test specimens from either preparation were
shaped to comply with either test method, one may assume that the flexural strength values
for that composite would differ between each test method.
5.14 Infrared

The infrared results for sintered HA suggest that there is evidence of hydroxyl ion loss as a function of sintering temperature, which is reflected by a decrease in intensity for the hydroxyl stretching mode at 3568 cm\(^{-1}\) (Figure 202). This is in agreement with what is expected for HA as it undergoes sintering. Decomposition of HA is associated with the formation of an intermediate phase, oxyapatite, through the loss of OH\(^-\) in the matrix when HA is heated in air to above 1200°C.\(^7\)

\[
2\text{OH}^- \rightarrow \text{O} + \text{H}_2\text{O}↑
\]

\[
\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 \rightarrow \text{Ca}_{10}(\text{PO}_4)_6\text{O} • + \text{H}_2\text{O}
\]

Changes in the phosphate \(v_1\) and \(v_3\) modes (~1087, 1016.86 and 962.95 cm\(^{-1}\)) with respect to peak shape and intensity is not so evident which would suggest that there is not much disruption in the HA matrix as a result of sintering (Figure 203).

This, however, is more evident in the GR-HA (CP15F) composite (Figure 204). Even though there are differences in peak broadening and intensity of the phosphate \(v_1\) and \(v_3\) bands between sintering temperatures, there doesn’t seem to be a direct correlation with respect to these changes as a function of increasing sintering temperature.

It has been previously mentioned that changes in peak broadening and intensity are attributed to the presence of \(\beta\)-TCP and \(\alpha\)-TCP secondary phases that responsible for disrupting the HA matrix. Changes in the hydroxyl stretching mode though are not so
significant and so detecting whether there is any loss as a function of sintering is difficult (Figure 205).

![Figure 202. Infrared peak assignments for HA.](image)

![Figure 203. Infrared peak assignments for HA.](image)
Figure 204. Infrared peak assignments for GR-HA (CP15F).

Figure 205. Infrared peak assignments for GR-HA (CP15F).
5.15 Cell Culture

The purpose of this study was to examine the growth and function of an osteoblast-like cell line cultured on HA and two glass reinforced HA composites, compared to the same cells grown on plastic or glass coverslips used as controls. The HOS cell line was used for this study because it has previously been shown to exhibit a number of features similar to those of normal human osteoblasts.  

The physicochemical properties of biomaterials, such as surface chemistry and topography, have profound effects on cell attachment, cell spreading, proliferation, differentiation and function. In addition, many materials, including the HA materials used in this study are known to release inorganic ions into the surrounding fluid, and these substances also markedly influence the biological activity of the target tissues. Thus, the composition of resorbable glass composites, which determines the solubility and biodegradability of these materials, is of crucial importance for their biocompatibility and efficacy in vivo. For example, calcium, which is released at high levels from glass reinforced HA compared with HA alone, is involved in the formation of an apatite mineral phase in hard tissues and Ca\(^{2+}\) ions are known to play an important part in regulating, for example, cell adhesion and activation. Together with surface-associated differences, such as the greater hydrophobicity and higher negative charge of the glass composites, these physico-chemical parameters undoubtedly exert a profound influence on the biological response of host cells. This study was therefore undertaken to delineate the precise effects of HA and glass reinforced HA on the growth and certain key functional activities in vitro.
The results showed that HOS cells were able to adhere, attach, remain viable and then proliferate on both the HA and glass reinforced HA composites (Figure 206). However, the cells grown on GR-HA (2.5CNP) (Figure 206c and g) appeared to have fewer and shorter cytoplasmic extensions on the material surface compared with cells grown on glass (Figure 206a and e), HA (Figure 206b and f) and GR-HA (5CNP) (Figure 206d and h). This does not appear to be due to surface topography only, since such cells have previously been shown to have higher levels of cell attachment to rough sandblast surfaces than to smooth surfaces.\(^{132}\) Thus, despite the roughened surface of the GR-HA (2.5CNP), this composite did not appear to support the cells as well as the HA or the GR-HA (5CNP), possibly because of the particular chemical composition of GR-HA (2.5CNP). Notably, despite the attachment and spreading of the cells on HA and GR-HA composites, none of these materials allowed as high a level of proliferation as on the control plastic surface (Figure 207). Moreover, this effect was prolonged and did not appear to be dependent on the proportion of glass but on the presence of the HA or secondary phases.

Growth on different substrates is also known to be associated with marked changes in cell function.\(^{133}\) In this study, although FCM analysis showed that the HA and the GR-HA composites had little if any effect on the size and intracellular granularity of the HOS cells, all of the materials were found to influence the functional activities of the cells, even after a relatively short period of culture (5 days) (Table 14). In addition, the effects that were observed appear to be antigen specific. Thus, while BSP and ON were down-regulated by growth on HA compared with control cells, OP and also the connective tissue antigens FN and COL I were expressed at similar levels compared with the controls. In contrast, GR-HA (2.5CNP) and GR-HA (5CNP) resulted in elevated expression of BSP and ON compared
with cells grown on HA alone, and protein expression on GR-HA (5CNP) was increased compared with cells grown on GR-HA (2.5CNP), with COL I expression actually exceeding that of the control cells. Although the long-term effects (>5 days) of these materials on bone-antigen expression were not examined, the findings nevertheless support the studies which suggest that certain GR-HA’s, which have clinically beneficial mechanical properties, are also able to elicit favourable responses from osteoblast-like cells.\textsuperscript{134,135,136}

This study also shows that the incorporation of glass into HA, in addition to increasing mechanical strength compared to HA alone,\textsuperscript{66} has differential effects on the functional activity of key connective tissue antigens. In addition, the data indicate that the composite with the higher proportion of glass generally elicits higher levels of bone antigen expression than does the either the lower glass composition or HA. The reason for the apparently improved 'biocompatibility' of the 5\% glass composite is not known, but appears to be more closely associated with chemical composition rather than with surface topography or with porosity, which remain constant as shown by the results of the mechanical properties of these materials show to remain constant. However, the GR-HA (5CNP) material contains high levels of TCP, thus can readily release $\text{Ca}^{2+}$ and $\text{PO}_4^{3-}$ into the surrounding environment \textit{in vitro} and which would undoubtedly have a marked effect on cell activation, cell function, and thus bioactivity of an implant material \textit{in vivo}. 
Figure 206– SEM of human osteoblast-like cells: on control glass surface (a and e); on HA (b and f); on HA2.5 (c and g); and on HA5 (d and h). Under low power (a, b, c & d), note the flattened, well-spread morphology of cells on all surfaces and the cytoplasmic extensions, particularly on cells grown on glass (a) and the HA disc (b). Under high power (e, f, g & h), the underlying surface of each material is clearly visible. Note extensive pitting of the material surface of HA2.5 (f) and HA5 (h).
Figure 207– Effects of HA materials on the growth of HOS cells. Control plastic dishes (black bars), HA alone (white bars), HA2.5 (grey bars) and HA5 (striped bars). The results are shown as the average A570 of 3 replicate cultures ±SD (vertical bars). *Statistically significant difference compared with the control cells (p<0.05; Student’s t-test).

Table 14. Effects of HA materials on antigen expression by HOS cells.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Relative antigen levels(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSP</td>
</tr>
<tr>
<td>HA</td>
<td>58 ± 2.1(^c)</td>
</tr>
<tr>
<td>HA2.5</td>
<td>73 ± 5.0</td>
</tr>
<tr>
<td>HA5</td>
<td>93 ± 3.8</td>
</tr>
</tbody>
</table>

\(^a\)HOS cells cultured for 5 days on control plastic, HA and HA-composite discs, as described in the materials and methods.

\(^b\)Relative antigen levels are expressed as the average percent AFI values obtained for BSP, ON, OP, FN and COL 1 in triplicate experiments, relative to the average level expressed by the control cells grown on plastic surface (defined as 100%).

\(^c\)Statistically significant difference compared with the control cells (p<0.05).
6 Conclusions
Overall it can be shown that for the selected sintering range most GR-HA composites exhibit enhanced flexural strength compared to HA. Through the application of x-ray diffraction information about phase changes can be determined. Decomposition of HA to β-TCP and subsequent inversion to α-TCP at higher sintering temperatures occurred for all GR-HA systems. This may be attributed to the chemical nature of the glass, in particular the chemical interactions that occur between the glass and HA during sintering. The results seem to suggest that the degradation of HA to secondary phases that occur for the GR-HA composites may have a significant effect on the flexural strength.

The primary role of phosphate glasses is to act as a sintering aid so as to enhance densification and reduce porosity. In most cases the GR-HA composites exhibit lower densities over the sintering range compared to HA and also the porosity in many instances were comparable. What is obvious though is that for GR-HA(2.5CNP) and GR-HA(5CNP) changes in density and porosity show a correlation with flexural strength. At 1300°C and 1350°C GR-HA(5CNP) shows a drop in flexural strength, this correlates with the drop in density and increase in α-TCP content and porosity at these temperatures. In this case, it could be argued that phase decomposition, densification and porosity may all influence the flexural strength.

The relationship shown between the flexural strength and phase changes for GR-HA(2.5CNP) and GR-HA(5CNP) seem to suggest that the flexural strength may
have been determined by the extent to which inversion to $\alpha$-TCP takes place and may subsequently be a result of different glass additions. Inversion to $\alpha$-TCP introduces a volume change, in this instance an increase in volume, which as a result creates residual stress within the system. It was shown that for the GR-HA with low glass addition inversion to $\alpha$-TCP occurred at 1350°C and was minimal, however, the GR-HA with high glass addition exhibited a greater presence of $\alpha$-TCP both at 1300°C and 1350°C. This indicates that minimal amounts of $\alpha$-TCP present in the material are advantageous, however, large amounts can be detrimental.

- Data presented for the GR-HA systems with glass compositions containing MgO and CaF$_2$ showed optimal mean flexural strength for GR-HA(CP15F) overall. The mean flexure strength values recorded between 1200 and 1350°C for GR-HA(CP15F) (96.43 to 142.59 MN.m$^{-2}$) were shown to be comparable with the strength of cortical bone (50–150 MN.m$^{-2}$) with the maximum value recorded at 162 MN.m$^{-2}$. The outcome of the flexural strength in this case may be influenced by the molar addition of CaF$_2$ in the glass. In this instance 15 mol% CaF$_2$ seems to be the optimal addition. This effect can be explained by the chemical interactions which take place and involve the substitution of OH$^{-}$ ions with F$^{-}$ ions in the HA lattice. The presence of F$^{-}$ ions leads to greater chemical bonding and a decrease in inter-atomic spacing. It would be reasonable to suggest therefore that GR-HA(CP20F) should show higher flexure strength than GR-HA(CP15F). This was
not shown to be the case and so needs further investigation to explain why excessive amounts of CaF₂ lower the flexural strength.

- The GR-HA composites with a ternary glass containing a low MgO (15mol% and 20mol%) content were shown to have a destabilising effect on the β-TCP phase. However, inversion to α-TCP at 1350°C did not occur for both GR-HA(CP25Mg) and GR-HA(CP33.5Mg). Hence, the extent to which magnesium ions stabilise the β-TCP phase is dependent on the amount of MgO incorporated in the glass.

- The extent of ion substitution in general may also be influenced by the viscosity of the glass at the sintering temperature and is probably related to the glass composition. Viscosity may also have a more direct bearing on the mechanical properties and can be explained by its affect on inter-particulate diffusion. A lower viscosity glass is expected to accelerate inter-particulate diffusion which may lead to enhanced flexural strength and flexural modulus. However, it must be pointed out that such an affect is expected to bring about improved densification and a further reduction in porosity as well as a significant change in grain size. Since the results show no evidence of this, then the former assumption, regarding the extent of ion substitution in relation to viscosity, seems more applicable.

- The presence of β-TCP in the GR-HA composites as results of decomposition during the sintering process is believed to cause an increase in the fracture toughness. Differences in fracture toughness values, therefore, may probably be
attributed more to differences in β-TCP content rather than porosity and/or grain growth factors.

- The fracture toughness values for cortical bone have been reported at between 2 – 12MN.m$^{-3/2}$. It can be said that the fracture toughness values for most GR-HA composites worked on throughout this study were very low compared to cortical bone.

- 0.19wt% of deflocculant was found to be the optimal weight addition to fully deflocculate the HA slip. On examining the affects of deflocculant addition during the milling process in both HA and GR-HA(CP15F), it was shown that there was no significant improvement in the flexural strength or flexural modulus in comparison with the same composites milled with water and methanol. However, the slightly greater fracture toughness values exhibited for the GR-HA composite at 1200 and 1250°C can be explained by the greater presence of β-TCP.

- The mechanical properties of HA and GR-HA composites processed via the hot pressing technique generally showed a great improvement in the mechanical properties. This was mainly owed to the materials undergoing greater densification and a greater reduction in porosity. This method greatly improves packing allowing enhanced particle to particle contact.
However, the implications of using two different test methods (four point bending and concentric ring) and also the machining of the test specimens must be taken into careful consideration. This is to account for differences in strength values expected as result of the test parameters.

- The porosity values determined from the Rietveld refinement and the actual density measurements can be considered reliable and accurate. Each refinement took into account the lattice parameters, thermal parameters, atom positions and phase weight percentage as well as background parameters and peak shape variables. However, the overall theoretical density of the crystal structure was determined without taking porosity into consideration. Therefore, by comparing the actual density against the theoretical density it is possible to obtain a reliable value for the porosity.

- The cell culture study carried out on the HA and GR-HA composites showed that cells were able to attach and proliferate during 7 days in culture. The results also showed marked and highly selective changes in the expression of certain connective tissue proteins. The expression of proteins such as BSP, ON and COL I were shown to be up-regulated on the GR-HA’s.
Future work:

- To investigate the effect of hot pressing over a range of sintering temperatures, i.e. from 1200 to 1350°C on the mechanical properties of GR-HA composites.

- To investigate the fatigue properties of HA and GR-HA composites by cyclic loading and to also carry out Weibull statistics to determine the survival probabilities.

- To investigate the biocompatibility of the GR-HA composites with ternary and quaternary glasses containing MgO and CaF₂ in vitro and in vivo.
Reference List


Development of Glass Reinforced Hydroxyapatite for Hard Tissue Surgery

George Georgiou


117. Santos, J.D., Silva, P.L., Knowles, J.C., Talal, S., Monteiro, F.J. Reinforcement of hydroxyapatite by adding \( P_2O_5-CaO \) glasses with \( Na_2O \), \( K_2O \) and \( MgO \). Journal of Materials Science: Materials in Medicine 7, 187-189 (1996).


121. Lopes, M., Santos, J.D., Monteiro, F.J., Knowles, J.C. Glass-reinforced hydroxyapatite: A comprehensive study of the effect of glass composition on the


Appendix
Papers:


Conference Abstracts:


Characterisation of the rheological properties and zeta potential of a range of hydroxyapatite powders

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Abstract

The effect of precipitation temperature, i.e. particle morphology on the rheological properties of a hydroxyapatite (HA) slip was investigated and compared to a commercial HA (batch P120 supplied by Plasma Biotal, Tideswell, Derbyshire, UK). The commercial HA was highly crystalline and had a particle size much larger than the HA precipitated at 60 and 80°C. With no deflocculant addition, the commercial HA had a viscosity much higher compared to the precipitated HA as expected. The commercial HA and the HA precipitated at 60°C showed similar pseudoplastic behaviour, but the HA precipitated at 80°C showed Newtonian behaviour. This was explained by the HA precipitated at 80°C having mean particle size of 82.24 nm, but a much wider particle size distribution. This is confirmed by the electrophoretic mobility measurements which show that the HA precipitated at 80°C has a much lower zeta potential at a 0 wt% addition of deflocculant. Because of the wider particle size distribution, the need to add deflocculant is much reduced. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Hydroxyapatite; Rheology; Zeta potential

1. Introduction

Hydroxyapatite has been used extensively as a material for implant use and bone regeneration [1-3] due to its bioactivity [2]. It has been used in various forms, such as monolithic block [4], porous foam [5,6] coatings [7,8] and granules [9].

To produce more complex implant designs there are various processing methods and one that is available for producing porous HA implants is reticulated foam technology [10], which makes use of a polyurethane foam, which is coated with an HA slip and then fired to burn off the polyurethane. However, in order to optimise this processing method, an understanding of the rheological properties must be acquired, in order to optimise the slip to coat the foam. The work presented in this paper is an in depth study of the rheology and zeta potential measurements of various HA slips compared to a commercially available HA and a study of the effect of precipitation temperature and hence particle morphology on the rheology and zeta potential of the HA slips.

Work was also carried out to optimise the deflocculation of the slip and this is also presented.

2. Materials and methods

2.1. Precipitation

HA was prepared from solutions of calcium nitrate tetrahydrate (118.06 g in 900 ml of double-distilled water (DDW)) and diammonium hydrogen phosphate (39.64 g, 1600 ml DDW), both solutions were adjusted to approximately pH11 with ammonium hydroxide. The diammonium solution was then added drop wise to the calcium solution (held at either 60 or 80°C) and then left to mature at that temperature for 24 h. The remaining suspension was then rinsed five times with DDW (5 x 1 l), filtered via a Buchner funnel and then dried in a drying cabinet at 70°C.

The commercial HA was supplied by Plasma Biotal (Plasma Biotal, Tideswell, Derbyshire, UK) and had a batch code P120.
2.2. X-ray diffraction (XRD)

XRD of the samples was carried out on a Philips PW1050/1082 powder diffractometer, in flat plate geometry using Ni filtered Cu Kα radiation (wavelength 1.5406 Å). Samples were ground to a fine powder and mounted in the holder of the diffractometer. Data were collected from 10 to 100° 2θ with a step size of 0.02° and a count time of 12 s. Particle size was determined using the following equation [11].

\[ B = \frac{0.9\lambda}{t \cos \theta} \]

where \( B \) is the broadening of the diffraction line measured at half its maximum intensity (radians) and \( t \) is the diameter of the crystal particle. Corrections for instrument broadening were made using powdered silicon. Full width at half maximum (FWHM) measurements were made on the 0 0 2 peak for HA and on the 1 1 1 peak for silicon.

2.3. Transmission electron microscopy (TEM)

An HA solution was prepared by dispersing 1 g of HA in 50 ml of water. This was placed in an ultrasonic bath for 30 min, to ensure thorough dispersion. A drop of the solution was placed on a Formvar carbon-coated copper 200 grid and left for 1 min. Excess liquid was removed with filter paper and the grid was allowed to dry for about 10 min. The HA particles were then viewed with a JEOL 100CX TEM at an accelerating voltage of 80 kV.

2.4. Flow curves

HA (20 g) was suspended in DDW (30 ml) to produce slips containing 40 wt% solids. The rheological properties of these suspensions were then investigated using a Brookfield Rotating Rheometer DVIII+. The HA slips were stirred at a constant rate on a magnetic stirrer for 5 min to break down any aggregates within. The slip was then transferred to the rheometer. The shear rate was started at 0.13 s⁻¹ (the lowest shear rate obtainable with this rheometer) and then varied between 2.5 and 55 s⁻¹ at intervals of 2.5 s⁻¹. Each shear rate was given a time lag of 1 min to establish an equilibrium before proceeding to the next shear rate step. Measurements of shear rate vs. viscosity were taken to produce flow curves. The standard deviation was calculated from at least two repeat measurements. The method was repeated with deflocculant additions at 0, 0.03, 0.06, 0.13 and 0.19 wt%.

2.5. Thixotropic properties

The slips were stirred at a constant rate on a magnetic stirrer for 5 min and then were transferred to the rheometer. Their viscosity and shear stress were measured at a constant shear rate (the variation of velocity with distance) of 50 s⁻¹ for 60 min with data being collected at 1 min intervals, the data for these 60 points were averaged and the standard deviation calculated. The method was repeated for deflocculant additions of 0, 0.03, 0.06, 0.13 and 0.19 wt%.

2.6. Zeta potential

Zeta potential measurements were performed on the HA P120 and the precipitated HA particles using the Rank Brothers electrophoresis apparatus. The HA particles were suspended in a 0.001 mol% potassium chloride (KCl) solution so as to maintain a constant electrical double-layer thickness. 0.05 wt% solutions were used so a good contrast could be achieved when viewing the particles through the microscope. Measurements were taken after each deflocculant addition of 0, 0.06, 0.13 and 0.19 wt% and the mean was calculated from measurements on 20 particles, for each deflocculant addition.

3. Results

3.1. X-ray diffraction

The X-ray diffraction (Fig. 1) shows that the P120 is significantly more crystalline compared to the HA precipitated at both 60 and 80°C. From the particle size measurements, the P120 has a significantly larger particle size with a value of 129.99 nm compared to the HA precipitated at 60 and 80°C, which had a particle size of 61.06 and 82.24 nm, respectively.

3.2. Transmission electron microscopy

Fig. 2a–c shows the morphology of the HA powders after precipitation. Measurement of particle size from the TEM images, is difficult, due to the high aspect ratio of the particles, for all three HA's; however, Fig. 2a is concurrent with the X-ray diffraction measurements, in
Fig. 2. TEM of HA for: (a) P120 (original magnification × 23 000) (b) HA precipitated at 60°C (original magnification × 14 000) and (c) HA precipitated at 80°C (original magnification × 14 000).

3.3. Flow curves

It can be seen from Fig. 3 that at a 0 ml addition the HA precipitated at 80°C acts as a Newtonian system whereas the HA precipitated at 60°C and the HA P120 behave as pseudoplastic systems. Also the viscosity for the P120 is very much higher compared to the precipitated HA's. When a small amount of deflocculant is added, for the P120, the viscosity increases slightly. For the HA precipitated at 80°C, there is no effect on the rheology. The HA precipitated at 60°C shows a change to Newtonian behaviour with a 0.06 wt% addition of deflocculant (Fig. 4). The HA P120 starts to deflocculate at a 0.13 wt% addition (Fig. 5) and is fully dispersed and Newtonian in character at a 0.19 wt% addition (Fig. 6), the HA precipitated at 60°C exhibits dilatant behaviour at a 0.19 wt% addition (Fig. 5). The change in behaviour with deflocculant addition for the three different types of HA is summarised in Table 1.

3.4. Thixotropic properties

Fig. 7 shows the effect of Dispex N40 on the viscosity of the systems when subject to constant shearing at 50 s⁻¹. It can be seen that the HA precipitated at 80°C is at a constant low value of less than 50 MPa throughout all Dispex additions. The HA precipitated at 60°C also stays at constant level of approximately 300 MPa even though its rheological behaviour is changing. The HA P120, however, starts off as a highly viscous system with a viscosity of 1000 MPa and when deflocculated comes down to a viscosity of 70 MPa.

No thixotropic properties were found in any of the systems.

3.5. Zeta potential

The zeta potential of each of the systems at a 0 wt% addition are negative (Fig. 8) with HA precipitated at 80°C being the most negative then the HA precipitated at 60°C and then the HA P120. On addition of Dispex N40 the zeta potential rises. All systems have a maximum around the 0.06 wt% addition.

4. Discussion

The P120 HA is produced via a hydrothermal method and has a significantly larger particle size compared to the precipitated HA samples. For the precipitated HA samples, it is known that temperature significantly affects the precipitate morphology [12] and our findings are concurrent. The HA precipitated at 60°C has a particle size of 61.06 nm and at 80°C, a particle size of 82.24 nm, measured by line broadening effects. This does not explain the deflocculant effects. However, the TEM may
help to explain this. Whilst the mean particle size for the HA precipitated at 80°C is larger, there appears in the TEM to be some fine particulate, which would stay in suspension more easily, hence the findings for the viscosity at 0 ml addition and the effect of deflocculant additions.
Fig. 5. Viscosity against shear rate for the 3 HA’s with 0.13 wt% deflocculant (●—HA P120, ■—HA precipitated at 80°C, ×—HA precipitated at 60°C).

Fig. 6. Viscosity against shear rate for the 3 HA’s with 0.19 wt% deflocculant (●—HA P120, ■—HA precipitated at 80°C, ×—HA precipitated at 60°C).

At a 0 ml addition the HA precipitated at 80°C acts as a Newtonian system indicating that all the particles are already in suspension and are fully deflocculated without the need for any addition of Dispex N40. The HA precipitated at 60°C and the P120 both behave as pseudoplastic systems with no Defloculant added. The HA P120 requires more Dispex N40 to fully deflocculate the system because of its larger particle size. Once the systems have been fully deflocculated a further addition of deflocculant to any of the systems does not have a large effect on the viscosity but their behaviour changes from Newtonian to dilatant.
Table 1
Summary of change in rheological properties with deflocculant addition for the three hydroxyapatites

<table>
<thead>
<tr>
<th>wt%</th>
<th>HA P120</th>
<th>HA @ 60</th>
<th>HA @ 80</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Pseudoplastic</td>
<td>Pseudoplastic</td>
<td>Newtonian</td>
</tr>
<tr>
<td>0.03</td>
<td>Pseudoplastic</td>
<td>Pseudoplastic</td>
<td>Newtonian</td>
</tr>
<tr>
<td>0.06</td>
<td>Pseudoplastic</td>
<td>Newtonian</td>
<td>Newtonian</td>
</tr>
<tr>
<td>0.13</td>
<td>Almost Newtonian</td>
<td>Newtonian</td>
<td>Newtonian</td>
</tr>
<tr>
<td>0.19</td>
<td>Newtonian</td>
<td>Dilatant</td>
<td>Newtonian</td>
</tr>
</tbody>
</table>

Fig. 7. Effect of deflocculant addition on viscosity for the three HA's with a shear rate of 50 s⁻¹ (●—HA P120, ■—HA precipitated at 80°C, x—HA precipitated at 60°C).

Fig. 8. Effect of deflocculant addition on the electrophoretic mobility of the 3 HA's (●—HA P120, ■—HA precipitated at 80°C, x—HA precipitated at 60°C).

No thixotropic properties were noticeable in any of the suspensions, this was because of the low solids content that was used in these experiments.

All the systems begin with a negative zeta potential indicating that they all start with a positive charge. When the Dispex N40 is added to the systems the zeta potential increases slowly until it is positive indicating that the particles have a net negative charge. This is because Dispex N40 dissociates in water to produce a sodium cation and a polymeric anion. Since the HA particles have an overall positive charge, the polymeric anion is attracted to the surface of the particle and interacts. This happens many times over the particle surface. The particle is effectively stabilised by the neutralising effects of the positively charged dispersion determining ion on the surface of the HA particles.

Further addition of this potential determining ion only increases the charge in the liquid phase therefore reducing the potential difference between the surface and the liquid. This reduction in surface potential leads to a reduction in the zeta potential.

5. Conclusions

HA precipitated at 80°C is suspended in solution without the need for any defloculants this is due to the particle size distribution. HA P120 and HA precipitated at 60°C both act as pseudoplastic materials with the HA precipitated at 60°C being the first to defloculate and show Newtonian behaviour with the addition of Dispex N40. This is because the HA precipitated at 60°C has a smaller particle size than the HA P120. If an excess of defloculant was added in any of the systems they would exhibit dilatant behaviour.

Zeta potential measurements show that all the particles start off with a positive charge. When defloculation has occurred, the overall charge becomes negative. This is due to the negative part of the dissociated defloculant attaching itself to the HA particles.

References

Investigation of thermal parameters and crystallisation in a ternary CaO–Na₂O–P₂O₅-based glass system

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Abstract

This paper presents the results of a study of the thermal properties of a range of phosphate-based glasses in the system CaO–Na₂O–P₂O₅. The glasses had a fixed P₂O₅ content of 45 mol% and the CaO:Na₂O ratio was varied. The glasses were produced and ground and their thermal properties measured using a SETARAM differential thermal analyser (DTA). Initial data showed that for the high Na₂O containing glass, NaPO₄ forms and a second unidentified phase that is probably calcium rich. In the middle compositional region, multiple phases precipitated out and were identified as NaPO₄ and Na₄Ca(P₂O₇)₂. For the high CaO content glasses, Na₄Ca(P₂O₇)₂ was the only phase formed. Further studies were carried out to examine whether the phases were bulk or surface nucleating, by grinding the samples to different particle sizes. From this data, it is evident that the NaPO₄ forms via a bulk nucleation mechanism and that Na₄Ca(P₂O₇)₂ forms via surface nucleation. The factors controlling this precipitation process are discussed and ionic radius as a controlling factor is hypothesised. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Phosphate; Glass; Glass transition temperature; Crystallisation

1. Introduction

Phosphate-based glasses offer a unique range of materials, as they are soluble. Furthermore, the solubility can be tailored made to the end application. This high solubility can limit their use. This solubility is linked to the structure of the glass, but little is known, with only recent MAS-NMR studies [1-3] giving an insight into the structure.

Phosphate based glasses also in general, feature low melting points, low glass transition temperatures and low softening temperatures. Despite their solubility, the low processing temperatures have led to their use in applications such as glass to metal seals, low-temperature enamels for metals and they are used for optical elements [4]. For example, a lead indium pyro phosphate glass was used to develop a laser which operates at 933 and 1098 nm [5].

In order to make phosphate glasses more durable only a few workers studied the effect of doping phosphate glasses with various ions. The addition of iron phosphate increases the cross-linkage which makes the glass structure less susceptible to water attack [6]. Xu et al. improved the durability of phosphate-based glasses by using the system SnF₂–SnO–P₂O₅ and adding PbO or B₂O₃ [7].

Work by the authors [8] has shown that a whole range of glasses can be made and their solubility has been measured. However, work has also shown that the useful range of glasses is limited not only by crystallisation effects, but also in vitro studies have shown that too high a solubility is detrimental to cell activity [9]. This work showed that glasses with a fixed P₂O₅ content of 45 mol% gives a good range of glasses, which melt and cast easily and show good biocompatibility in vitro.

The solubility is often seen as a limiting factor, for example, whilst phosphate-based glasses offer unique optical properties, their use as fibre optics is limited because of the solubility. These glasses have also been used for trace element release [10], by doping with various ions. However, these glasses offer great potential for hard tissue surgery. These glasses, because of their chemical affinity with bone may stimulate, via the release of the appropriate ions, new bone formation and this has been
shown by the authors [9]. The controlled release of these ions could be very beneficial for the surrounding tissue, not only in terms of reduced toxicity towards the material, but also, these ions can be used by the surrounding cells to rebuild new hard tissue matrix. Studies with materials chemically similar to bone, such as tricalcium phosphates, have been used as dental implants and were replaced by natural bone [11]. In theory, exactly the same should happen if the glass dissolves in a tissue environment, with the glass being replaced by bone. Using different chemical ratios will give different solubility rates to cover the requirements of hard and soft tissue replacements.

To be able to synthesise glasses which show a controllable breakdown of their glass network, it is important to make stress-free glasses which have been annealed below their crystallisation point. Hence, the only parameter affecting solubility should be the glass composition. A way to obtain data about the glass transition point is by differential thermal analysis.

Uo et al. [10] synthesised a ternary glass system with different P$_2$O$_5$, calcium and sodium oxide content and determined their physical properties via DTA. They found a correlation of glass transition temperature to the composition. The $T_g$ and $T_c$ decrease with increasing P$_2$O$_5$ content. Another study by Shih et al. [4] focused on the effect of CuO in terms of their solubility. For glasses in a compositional range of meta phosphate glasses CuO gave an increase in $T_g$.

In this study glasses have been made out of P, Na and Ca oxides with different chemical compositions. The thermal parameters of the glasses have been measured with differential thermal analysis. Using the data from the DTA, the glasses were cerammed and the phases precipitated, examined by XRD. Work was also carried out to examine the precipitation route for the phases produced on cerammung.

2. Materials and methods

2.1. Glass preparation

Ten glasses were prepared from P$_2$O$_5$, NaH$_2$PO$_4$ and CaCO$_3$ (see Table 1). The precursors were weighted out and then placed in a Pt/10% Rh crucible that was then placed in a furnace at between 1100 and 1200°C for 1 h. Upon removal, the glass was poured into a graphite mould preheated to 350°C. The mould was then placed in a furnace at 350°C and slowly cooled to room temperature to remove any residual stress. Having obtained glass rods, these were cut into discs of 15 mm diameter and 2 mm thickness, using a Testbourne diamond saw.

Three main thermal parameters were measured, the glass transition temperature $T_g$, the crystallisation temperature $T_c$ and the melting temperature $T_m$. This was carried out on a Setaram differential thermal analyser (DTA), using an inert nitrogen atmosphere and a heating rate of 10°C min$^{-1}$ up to a maximum temperature of 1000°C. The data was baseline corrected by carrying out a blank run and subtracting this from the data. For the further investigation into the crystallisation phenomena, the glasses Ca$_8$Na$_4$P$_{45}$ and Ca$_{32}$Na$_{23}$P$_{45}$ were ground using an agate mortar and pestle and then sieved to give different particle sizes. The following particle size ranges were used: <75, 75-100, 100-150, 150-200 and 200-600 μm. DTA data was collected using the same parameters as above.

To investigate the phases that crystallise from the glass, discs of the glass were annealed at 50°C above the measured $T_g$ for 12 h and then data was collected on a Philips PW1780 powder diffractometer in flat plate geometry, using Ni filtered Cu Kα radiation. Data was collected from 10 to 90° 2θ with a step of 0.02° and a count time of 3 s.

3. Results

Fig. 1A shows the trace for a low CaO content glass (Ca$_{12}$Na$_{43}$P$_{45}$). As expected at the lowest contents, the DTA curves show a single crystallisation event at 420°C, and a single melting event at 539°C. There is some evidence for the presence of a secondary phase, indicated by the broad $T_c$ peak. With increasing CaO content, there appears to be more than one crystallisation event, occurring as overlapping peaks (indicated by $T_{c1}$ and $T_{c2}$) as shown in Fig. 1B (Ca$_{20}$Na$_{33}$P$_{45}$) and two melting events (indicated by $T_{m1}$ and $T_{m2}$). If we then look at the high CaO content (Ca$_{32}$Na$_{23}$P$_{45}$), again we get a single crystallisation event, with a very clear $T_g$ and $T_m$ at 507 and 732°C, respectively (Fig. 1C).

![Fig. 1. DTA trace for glass samples Ca$_{12}$Na$_{43}$P$_{45}$, Ca$_{32}$Na$_{23}$P$_{45}$ and Ca$_{32}$Na$_{33}$P$_{45}$](image-url)
Fig. 2 shows the glass transition point plotted against the CaO content. As it can be seen from the graph the glass transition temperature increases almost linearly with the CaO content Table 1.

Fig. 3 shows the crystallisation temperature against CaO content. With increasing CaO content the \( T_c \) increases. Furthermore, two \( T_c \) temperatures may be defined in the mid compositional region as we get two phases from solution. At the high temperatures, again we get a single \( T_c \). The transitions from one phase to the other can clearly be seen.

Fig. 4 plots the CaO content against the melting temperature. This mirrors the data for the \( T_c \), with at low CaO content a single-phase melting occurring, followed by a region with two melting events and finally another region with a single melting event. The variation in \( T_m \) with composition is not linear indicating some slight variation in individual phase composition.

To investigate whether the glasses bulk or surface nucleate, the glasses were ground to different particle sizes and the DTA carried out. From the traces, \( T_c \) was determined and plotted against particle size. Fig. 5 shows the 8 mol% CaO content glass. As can be seen, \( T_c \) varies by only a few degrees with particle size. However, Fig. 6 show \( T_c \) against particle size for the glass with 32 mol% CaO. This glass shows a very marked decrease in \( T_c \) with decreasing particle size.

For the annealed samples, XRD was performed and the data is presented in Fig. 7. Fig. 7A shows the sample with low CaO and high Na₂O content (Ca₁₂Na₄₃P₄₅). This sample is markedly different from the other two traces (Fig. 7B and C). The phase identified for this sample is an NaPO₃ and this was determined by performing a refinement using the LeBail approach and using a space group of C2/m. This phase is monoclinic

---

**Table 1**

<table>
<thead>
<tr>
<th>Code with chemical specification (mol%)</th>
<th>CaO content (mol%)</th>
<th>Na₂O content (mol%)</th>
<th>P₂O₅ content (mol%)</th>
<th>Melting temperature and time (°C/h)</th>
<th>Annealing temperature (°C)</th>
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<td>45</td>
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<td>350</td>
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</tbody>
</table>
4. Discussion

The glass transition temperature varies almost linearly with CaO content. This is expected as CaO is a refractory material and would increase $T_g$ as more is incorporated into the glass. Also $T_e$ is a measure of the bulk, as opposed to parameters for specific phases.

$T_c$ and $T_m$ show some variation and this is related to the phases that crystallise from the glass. As expected, at both low and high CaO content, a single phase crystallises from the glass. However, in the middle region, it becomes more complex, with multiphase formation occurring. From the particle size investigations, if the $T_c$ varies with particle size, this is an indication that nucleation occurs via surface nucleation. If no variation is seen, then it is an indication that bulk nucleation occurs. From this data it is clear that in the glass with $8\text{mol}\%$ CaO the NaPO$_3$ phase nucleates via the bulk and for the glass with $32\text{mol}\%$ CaO, the Na$_4$Ca(PO$_3$)$_6$ nucleates via a surface mechanism.

The complexity may be further explained with the phenomena of phase separation. According to Dietzel [12] for binary glass systems, when a glass cools, three different phenomena can occur, which explain our findings. Dietzel ordered cations according to their field strength. The field strength is defined as $\frac{ze}{r^2}$ where $z$ is the valence, $e$ the elemental charge (Constant and $r$ the radius of ion. Because of the constant factor $e$, to simplify the equation we will erase this constant factor.

For a simple binary silicate glass, Dietzel discussed three simple conditions; if the difference in field strength of two cations $\Delta F$ is above $\Delta F = 0.3Z/a^2$, crystallisation and compound formation occurs, whereas at $\Delta F = 0Z/a^2$ phase separation takes place. Glass formation occurs when $\Delta F > 1.33Z/a^2$. The field strength was altered to $z/a^2$, which means that the field strength is measured in terms of cation distance to anion (oxygen). A similar consideration may be extended to the phosphate glasses in this work. According to Dietzel the element P has a field strength of 2.1, Na of 0.19 and Ca 0.33. In our case, we have a range of glasses with different CaO/Na$_2$O ratio and different phenomena occur. At one end, low CaO high Na$_2$O content no phase separation occurs. In the middle with balanced CaO/Na$_2$O ratio and different phenomena occur. At the other extreme, with high CaO and low Na$_2$O content only one phase is formed. To explain this phenomena of separation in the middle region a binary P$_2$O$_5$–Na$_2$O system should be considered, in which increasing levels of CaO are incorporated. In the case of...
the binary system, one phase will form which means that the relatively low field strength of Na$_2$O is not influencing enough. Hence, the difference of the two field strength's $\Delta F$ will not be 0 (case of phase separation). If one incorporates a third component, such as CaO phase separation can occur but only if CaO is incorporated at a certain level that enough material is present to form a phase. In other words, the more CaO (Ca$^{2+}$ has a higher field strength than Na$^+$) is incorporated the more $\Delta F$ will become zero. If the amount of CaO added is further increased, the sodium content becomes extremely low and is not able to phase separate.

5. Conclusions

For the glass system studied, the glass transition temperature varied almost linearly. As expected, single-phase precipitation occurs at either end of the compositional spectra as seen both by DTA and XRD. These phases have been identified and their thermal parameters characterised. There is also evidence that one phase nucleates via a bulk mechanism whilst the second nucleates via a surface mechanism as evidenced by the particle size effect on the crystallisation temperature.

References

Glass reinforced hydroxyapatite for hard tissue surgery—Part 1: mechanical properties

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Abstract

Commercial hydroxyapatite (HA) was reinforced by adding 2.5 and 5 wt% of a Na2O-CaO-P2O5 glass and then sintered. The resulting composites have chemical compositions that are similar to the inorganic constituent of the mineral part of bone, and are closely related to the trace elements that are present, in this case Na. X-ray diffraction showed no decomposition of HA to secondary phases; however, the glass reinforced-HA composites contained a HA phase and variable amounts of tricalcium phosphate phase, depending on the sintering temperature and the amount of glass added. The HA-composite material exhibited higher flexural strength overall compared to sintered HA. The presence of secondary phases β- and α-tricalcium phosphate in the microstructure of the composites has a major influence on the mechanical properties. Additionally, the presence of porosity also has a bearing on the mechanical properties of the material. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Hydroxyapatite; Decomposition; Tricalcium phosphate; Mechanical properties

1. Introduction

Hydroxyapatite (HA), at present, is of great interest as a material for surgical implants, due to its biocompatibility with living tissue. Connective tissue, which holds together the different structures in the body, is inherent in bone and contains collagen fibres, mineral part and ground substance. The exact composition and relative proportions of collagen depend upon numerous factors, including the location and loading requirements of bone [1-3]. Structural and chemical analyses of the inorganic part of bone have shown that ionic substitutions may occur within the HA lattice [2]. Substitutions include CO32- for OH-, or PO43- and Na+ for Ca2+, and F- for PO43- [2]. Trace ions, particularly Na+, Mg2+, K+ and F- are well known to be prevalent in the inorganic part of bone [1]. In addition, the incorporation of a CaO-P2O5 glass as a sintering aid facilitates the decomposition of HA to β-tricalcium phosphate (TCP). β-TCP can undergo ionic substitutions, which involve the substitution of Ca2+ ions for ions such as Mg2+ in the lattice structure. Due to the smaller ionic radius of Mg2+ relative to Ca2+, the lattice experiences a decrease in the lattice parameters, thus increasing the lattice enthalpy and hence the stability of the β-TCP phase [4]. Thus, it is important to incorporate these trace elements in orthopaedic implants, such as HA, because the biocompatibility of apatites is closely dependent on their composition [5]. The incorporation of a P2O5-CaO glass system with addition of Na, Mg and K oxides allows these substitutions within HA to occur, giving nominal elemental compositions of trace elements in the composites that are analogous to that of the inorganic constituent of the mineral part of bone [6]. Therefore, the glasses within this system are considered to have enormous potential as biomaterials.

Synthetic HA is limited in its use as a biomaterial, primarily due to its low load bearing capacity which can be illustrated by the relatively poor mechanical properties compared with bone [6-11]. However, improvements on its mechanical properties have been undertaken with the inclusion of soluble phosphate based glasses (P2O5-CaO-Na2O) [6-11]. Phosphate glasses, when incorporated into HA, melt at a lower temperature compared to HA and can act to increase density by enhancing the sintering mechanisms which greatly enhances the mechanical properties. Furthermore,
decomposition of HA into secondary phases, \( \alpha \) - and \( \beta \)-TCP, can occur. This is related to the compositional effects, in particular the Ca/P ratio \([7]\). It can also be seen as a function of sintering temperature \([6-11]\). Another physical property observed to change is also a significant decrease in the porosity of the final glass reinforced HA (GR-HA) from 1200°C to 1250°C. This paper will investigate the effect of phases on the mechanical properties along with the porosity and significance of each of these in relation to the mechanical properties.

2. Materials and methods

2.1. Specimen preparation

The glass composite was produced with a composition of 32 mol\% CaO, 23 mol\% Na\(_2\)O and 45 mol\% P\(_2\)O\(_5\). The starting reagents used to prepare the glass consisted of NaHPO\(_4\), P\(_2\)O\(_5\) and CaCO\(_3\). These were mixed thoroughly and placed in a platinum crucible, melted at 1000°C for 1 h, and then poured onto a steel plate and allowed to cool. The resulting glass was then ground to a fine powder using an agate grinder.

The glass (5 or 10 g) powder was placed into a porcelain mill pot and milled dry for 24 h. HA (supplied by Plasma Biotal Ltd. UK) was then added to the mill pot at either 195 or 190 g to give glass additions of 2.5% and 5% glass additions, respectively. Methanol (300 ml) was also added and the mixture was then wet milled for a further 24 h. The resulting slip was then dried at 70°C and the dry powder was then sieved to 75 μm.

Four grams of powder was placed into a steel die and uniaxially pressed at 20 tons using a hydraulic press to give 30 mm discs. The discs were fired at a heating rate of 4°C/min\(^{-1}\) to 1200°C, 1250°C, 1300°C and 1350°C. The specimens were held at the appropriate temperature for 1 h and then furnace cooled.

2.2. Measured density determination

Density measurements were performed on each sintered specimen using the Archimedes principle. The measurements were carried out in distilled water. These were then compared with the theoretical densities calculated from the Rietveld analysis to give the porosity.

2.3. Mechanical testing

The test carried out was the flexural bend strength test method using a concentric ring testing jig with a loading ring of 10 mm and outer supporting ring of 20 mm. Ten specimens were tested for each of the firing temperatures on an Instron machine at a crosshead speed of 5 mm min\(^{-1}\) to failure. The flexural bend strength was determined from the load displacement graph.

2.4. X-ray diffraction

Tested samples were ground to a fine powder, placed in a specimen holder and then analysed on a Philips PW1780 diffractometer with Ni filtered Cu \( K_{\alpha} \) radiation (\( K_{\alpha} = 1.5406 \, \text{Å}, \, K_{\alpha} = 1.5444 \, \text{Å} \)) at 40 kV and 30 mA. The data were collected with a scintillation counter between \( 10 < 2\theta < 90 \) with a step size of 0.02° and a count time of 12 s using flat plate geometry.

2.5. Structure refinement

The structure refinement was carried out using General Structure Analysis Software (GSAS, Los Alamos National Laboratory). A standard model for each of the three phases was used for the refinement of the samples and they were determined from the Daresbury Crystal Structure Database. The HA model was based on the single crystal structure determination, with \( P63/m \) space group and lattice parameters of \( a = 9.45 \, \text{Å} \) and \( c = 6.88 \, \text{Å} \). The second phase \( \beta \)-TCP consists of a \( R3CH \) space group and lattice parameters of approximately \( a = 10.4 \, \text{Å} \) and \( c = 37.4 \, \text{Å} \). The standard model used to refine \( \alpha \)-TCP was based on the \( P21/a \) space group and unit cell dimensions of \( a = 12.887 \, \text{Å}, \, b = 27.28 \, \text{Å}, \, c = 15.219 \, \text{Å}, \) and \( \beta = 126.2^\circ \).

The peak shapes for the refinement were modelled on a pseudo Voigt distribution. Background parameters, a scale factor, four peak shape variables, an asymmetry factor, cell parameters, and a zero point correction were all refined. Isotropic thermal parameters and atom positions were refined for all HA atoms. For \( \beta \)-TCP and \( \alpha \)-TCP all the parameters were refined; however, the thermal parameters and atom positions were fixed.

From the data output from the GSAS list file, a number of other parameters are calculated and these data are presented. The software calculates the phase weight percentage and the theoretical density (assuming no porosity). The calculation for these numbers does not include any error determination.

From the data for the measured density and the theoretical density, the porosity is calculated using

\[
\text{Porosity} = \left( \frac{\text{recorded density}}{\text{theoretical density}} \right) \times 100 \, \%.
\]

This again does not have an associated error with it.

3. Results

Fig. 1 shows the variation of flexural bend strength with change in sintering temperature for all three materials. For HA there is a slight increase in the mean flexural bend strength, which decreases at 1350°C; however, this is not statistically significant.

The GR-HA with 2.5 wt\% glass (HA2.5) addition exhibits a gradual increase in flexural bend strength with
increasing firing temperature. For the GR-HA with 5 wt% glass (HA5), the flexural bend strength increases to approximately 80 MPa and then decreases again at 1300°C and 1350°C to around 50 MPa. Using X-ray diffraction and Rietveld analysis, quantitative phase analysis was performed. The HA showed no sign of decomposition at any firing temperature. For HA2.5 (Fig. 2) the HA levels stay relatively constant at about 85% for all firing temperatures. At 1350°C, it is clear that the β-TCP inverts to α-TCP.

Fig. 3 shows the phase element fractions for HA5. Between 1200°C and 1250°C, there is no α-TCP and the β-TCP levels stay relatively constant at around 35%. However at 1300°C, there is further decomposition of HA, and the β-TCP inverts to α-TCP and this continues at 1350°C.

The presence or absence of porosity in ceramics significantly affects the mechanical properties and so the densities were measured and porosity calculated. For the measured densities (Fig. 4), HA shows a rapid increase in density between 1200°C and 1250°C and remains relatively constant at about 3.13 g cm$^{-3}$ with further increase in firing temperature. HA2.5 exhibits a similar trend to HA, but the density values are a little lower. For HA5 the density rapidly increases between 1200°C and 1250°C and then decreases again with increasing firing temperature. This will be explained.

From the Rietveld analysis, a theoretical density may be calculated from the atom positions, taking into account the phases. The theoretical density assumes no porosity. These data are shown in Fig. 5. For HA, the values are slightly below the maximum theoretical density of 3.16 g cm$^{-3}$ and remain constant for all firing temperatures. These values differ from the theoretical density as the data has been refined for each particular sample and does not assume optimal atom positions and

<table>
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<tr>
<th>Temperature</th>
<th>XRD Data</th>
<th>HA (%)</th>
<th>B-TCP (%)</th>
<th>A-TCP (%)</th>
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<tr>
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<th>HA (%)</th>
<th>B-TCP (%)</th>
<th>A-TCP (%)</th>
<th>Theoretical Density</th>
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<td>1200°C</td>
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Fig. 2. Effect of firing temperature on phase weight percentage for HA2.5.

Fig. 3. Effect of firing temperature on phase weight percentage for HA5.
all OH sites are fully hydroxylated. For HA2.5 there is a reduction in theoretical density with firing temperature, but it is not so marked except at 1350°C. Also the overall values are lower than the values for HA. HA5 shows a significant decrease in density with increasing firing temperature. Also all the theoretical values are significantly lower than the values for both HA and HA2.5.

The difference between the values for the measured and theoretical densities gives the porosity and this is shown in Fig. 6. For all materials, there is a sharp decrease in the amount of porosity between 1200°C and 1250°C. For HA and HA5 the porosity then remains fairly constant. At 1300°C all the samples show similar levels of porosity. However, HA2.5 continues to show a reduction in porosity levels, so at 1350°C the level of porosity approaches zero.

4. Discussion

As previously mentioned, the presence or absence of porosity in ceramics significantly affects the mechanical properties. The incorporation of glass in HA acts as a sintering aid, which enhances densification, and as a result reduces porosity. This occurs through a liquid phase sintering mechanism, which accelerates inter-particle diffusion and bonding, thus causing elimination of porosity and shrinkage. However, in this system, it is further complicated by the occurrence of decomposition and phase changes that can also have a detrimental or beneficial effect on the mechanical properties.

HA, HA2.5 and HA5 exhibited a sharp decrease in porosity as temperature increases and these gave values of approximately 0–1% at 1250°C, 1300°C and 1350°C. One would arrive at the assumption that since the porosity values for the specimens mentioned are comparable, then one would therefore expect similar flexural bend strength values. This is not seen however. What we observe is an improvement for glass reinforced HA relative to HA, and in particular for HA2.5.

The phase element analysis from the Rietveld refinement carried out for these specimens gives an indication of the amount of each of the phases present. For HA2.5 and HA5 there is decomposition of HA to β-TCP at all four given temperatures, and furthermore, inversion of β-TCP to α-TCP. With HA5 significantly more β-TCP is found and the β-TCP phase inverts more readily to α-TCP at a lower temperature. This inversion is due to the origin of β-TCP [7]. The decomposition of HA is attributed to the presence of a reactive glass, which can drive off the hydroxyl groups when entering the HA structure and as a result causes decomposition. The glass may also affect the Ca : P ratio [7]. However, for HA5 the inversion of β-TCP to α-TCP, occurring at a lower temperature, is due to β-TCP containing more residual ions, which destabilise β-TCP and facilitates the formation of more α-TCP [7].

The presence of these secondary phases has a major influence on the mechanical properties of the material. This can be correlated with values illustrated for flexural bend strength. The decrease in flexural strength for HA5 at 1300°C and 1350°C correlate with the phase weight fractions, where the α-TCP phase content appears at around 15% and 19%, respectively. Alternatively, HA2.5
displays a gradual increase as temperature increases. At 1350°C, where α-TCP is present at 5%, there does not appear to be a reduction in flexural strength as was observed with HAS. There is also probably a positive influence from the virtual complete absence of porosity.

The presence of β-TCP and α-TCP phases in the HA structure would give a volume increase and thus can give rise to residual stress [6-8,10]. However, the presence of α-TCP at high levels can disrupt the integrity of the GR-HA [6-8,10]. As a result of these findings, one can postulate that a significant amount of α-TCP in the structure can be detrimental to the mechanical properties of the material, but low amounts, as observed in HA2.5, can be advantageous.

Finally, both porosity and phase decomposition are influential factors in the outcome of the mechanical properties. However, what the results of this study seem to suggest is an overall improvement in flexural strength for glass reinforced HA which is associated primarily with phase decomposition and subsequent phase inversions.

5. Conclusions

HA undergoes significant elimination of porosity during sintering, particularly between 1200°C and 1250°C. However, the mechanical properties are still relatively poor. The inclusion of a glass as a sintering aid significantly improves the flexural bending strength. The majority of the improvement comes from the decomposition of HA to β-TCP with an associated volume change, helping to improve the mechanical properties. At higher firing temperatures, the β-TCP inverts to the high temperature α-TCP form. A relatively small amount of α-TCP can be sustained in the composites without detriment. However, large percentages of glass can promote formation of significant amounts of α-TCP with a resultant decrease in flexural bend strength. The glass can also help to eliminate porosity from the structure and this may have contributed to the improvement in mechanical properties in HA2.5.

Acknowledgements

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References

Glass reinforced hydroxyapatite for hard tissue surgery—Part II: in vitro evaluation of bone cell growth and function

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Abstract

Hydroxyapatite (HA)-based materials are considered to be potentially useful as bone implant materials, particularly those reinforced with glass to improve mechanical strength. However, the precise effects of glass-reinforced HA on the growth and functions of bone cells are still unclear. The present study has therefore examined the response of human osteoblast-like cells to HA and HA reinforced with two different proportions of glass, namely 2.5% and 5%.

All materials enabled the cells to attach and proliferate during 7 days in culture and, although the growth was less than on control plastic surfaces, there was no deleterious effect of the 5% glass composite compared with HA alone. Flow cytometry analysis showed that there was no effect on cell size and granularity, but there were marked and highly selective changes in the expression of certain connective tissue proteins. Thus, while bone sialoprotein and osteonectin were down-regulated on HA alone, the expression of these antigens was relatively enhanced on the composite materials, and collagen type I was also up-regulated on the glass-reinforced HA. Thus, modulation of the glass composition of HA materials could be used to produce not only improved mechanical strength, but also enhanced biocompatibility. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Glass-reinforced HA; Bone cells; Growth; Flow cytometry

1. Introduction

As a result of the very limited supply of autograft material and the potential health risks associated with the use of allografts, hydroxyapatite (HA) has over the last two decades become increasingly important as a bone implant material and has been widely used in both orthopaedic [1-5] and dental applications [6-8]. A comparison of HA with natural bone shows a biologically favourable similarity with respect to overall structure, and pore sizes in particular. However, the use of a synthetic HA material has been limited to areas of low mechanical load [9] because such HA structures are typically brittle and exhibit little deformation to failure [10].

Synthetic HA is relatively easily prepared with a high degree of purity and exhibits similar crystallographic properties to the natural HA found in bone and teeth [11]. The latter are highly imperfect apatites, with substantial CO₃⁻ substitution for PO₄³⁻ and low levels of ions such as Na⁺, K⁺ and Mg²⁺ substituting for Ca²⁺ [12,13]. In contrast, synthetic HA, which tends to lose associated CO₃⁻ ions unless sintered under controlled conditions, can be produced to also contain a number of different elements in the HA lattice to improve its mechanical strength and biocompatibility [14-16]. For example, recent studies have shown that HA reinforced with PO₄³⁻-based soluble glasses within the P₂O₅-CaO-Na₂O system have enhanced mechanical properties and are thus promising biomaterials for hard connective tissues [17-20]. Moreover, such glass-reinforced HA composite surfaces have been shown to support the attachment and proliferation of human bone cells both in vitro and in vivo [21-23]. Furthermore, in the preceding paper we have demonstrated that the addition of 2.5% and 5% soluble glass generates a final HA product with material phases that improve the mechanical characteristics. However, the relationship between the physical and mechanical properties and the
biological activity of these new composites is not yet known. The present study has therefore examined the effects of P2O5-CaO-Na2O glass-reinforced HA composites on the growth and expression of key components of the extracellular matrix by human bone-derived cells.

2. Materials and methods

2.1. HA disc preparation

HA discs were prepared as described in the preceding paper and reinforced with 2.5% and 5% (w/v) of the ternary glass system 45 mol% P2O5-32 mol% CaO-23 mol% Na2O (HA2.5 and HA5, respectively). The HA, HA2.5 and HA5 discs are referred to as HA materials, while HA2.5 and HA5 (those containing glass) are referred to as HA composites.

2.2. Cell culture

The human osteosarcoma cell line HOS (TE85) was cultured at 37°C in a humidified atmosphere of 5% CO2 in air, in flasks containing 10 ml of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal calf serum, 2 mM L-glutamine, 50 IU/ml of penicillin and 50 μg/ml of streptomycin (Gibco; Glasgow, UK). The culture media were changed twice weekly and for subculture the cell monolayers were washed with phosphate-buffered saline (PBS) and incubated with trypsin-EDTA for 5 min at 37°C to detach the cells, prior to reculture on the control tissue culture plastic (Marathon Lab. Supplies; London, UK) and on the HA discs.

2.3. Cell proliferation

To measure the effects of the HA materials on the growth of the HOS cells in each of 3 separate experiments, 5 x 10^4 cells in 0.5 ml were plated onto 6 replicate discs (4.9 cm²) which had previously been placed into individual wells of a 6-well plate. These were left for 2 h to allow cell attachment and then 3 ml of culture medium was added to each well, a volume sufficient to cover the surface of the discs. The cultures were incubated at 37°C for a further 2, 5 and 7 days. Cell proliferation was measured at these time periods using the MTT test according to the manufacturer's instructions (Chemicon; Temecula Ca., USA). 1 x 10^5 control cells were plated per well into 6 replicate wells of the (9.6 cm²) plastic culture dishes, giving the same cell density as on the HA discs (approximately 1 x 10^5 cells per cm²).

The reagent, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) is enzymatically converted by living cells to a dark blue/purple formazan product. The intensity of the colour produced is therefore directly related to the number of viable cells, and thus to their proliferation in vitro. To measure cell growth, each of 6 replicate HA discs was removed from its well and placed in new 6-well plates with medium prior to assay. This was necessary to avoid including in the assay any residual cells that may have grown on the plastic surface of the wells in which the discs were initially cultured. Then, 100 μl of MTT reagent was added to each disc surface and the plates incubated at 37°C for 4 h. The blue formazan product was solubilised with 500 μl of isopropanol-HCl, and a 100 μl aliquot of each sample removed for assay, which was performed in a 96-well plate. This latter procedure was also performed simultaneously for the control cells grown on plastic.

Absorbance was measured at 570 nm (A570) using a Titrtek Multiskan Plus spectrophotometer (Labsystems; Helsinki, Finland) and the average A570 of the 6 replicate wells was calculated. The growth of the cells is shown as the average A570 of 3 separate experiments ± standard deviation (± SD).

2.4. Scanning electron microscopy (SEM)

For SEM, cells cultured to logarithmic phase on glass coverslips (used as a control), on HA discs and on HA composite discs were fixed in 3% glutaraldehyde in 0.14 M sodium cacodylate buffer (pH 7.3) at 4°C overnight. The specimens were then dehydrated in a graded series of alcohols (50%, 70%, 90% and two changes of 100%), washed with hexamethyldisilazane (TAAB Laboratories; Berkshire, UK) for 5 min and placed in a desiccator overnight. After 24 h, the dehydrated control samples and HA discs were mounted onto stubs using Araldite (Devcon; Northants., UK) and Liquid Dag (Neubauer Chemikalen; Munster, Germany) and left to air dry for 24 h. Specimens were then sputter-coated with gold/palladium using a Polaron E5100 coating device and observed using a Cambridge Stereoscan S90B (Cambridge Instruments; Crawley, UK).

2.5. Flow cytometry (FCM)

FCM measures certain physical and chemical characteristics of cells as they move in a fluid stream past a fixed laser light beam. Light scattered by the cells at small angles (<2°) (forward scatter; FSC) is related to cell size, whereas light scattered at angles of 90° (side scatter; SSC) is related to the granularity and intracellular complexity of the cell. The FCM technique is also able to detect emitted fluorescence light and, in the present study, fluorochrome-conjugated antibodies were used to measure the relative levels of certain antigens considered to play an important part in the integrity and function of bone, as noted below. We have previously shown that the levels of fluorescence obtained in this technique are directly proportional to the actual molecular levels of the antigen present in the cell [24].
For FCM, cultures of HOS cells were seeded onto the HA discs and into 6-well culture plates at the densities described and incubated for 5 days. After this time, the cells were washed with PBS and harvested by scraping the monolayer with 20 mM EDTA in PBS and no trypsin, to avoid proteolytic cleavage and thus loss of any membrane-associated antigen. The cells were centrifuged and fixed with 1% paraformaldehyde (Merck; Poole, England) in PBS containing 0.1% (w/v) saponin, for 30 min at room temperature, to permeabilise the cells to allow entry of antibodies. After washing twice with 2% FCS in PBS containing 0.1% saponin, the cells were centrifuged and the pellet resuspended in this washing buffer. Replicate aliquots containing approximately 1 x 10^3 cells were placed into separate tubes and incubated for 60 min at room temperature with the following primary antibodies: rabbit polyclonal antibodies against human bone sialoprotein (BSP), osteonectin (ON) and osteopontin (OP) (courtesy of Dr. L. Fisher; NIH, Bethesda, MD, USA); mouse monoclonal antibodies (mAbs) against human fibronectin (FN) (DAKO; High Wycombe, England) and type I collagen, (COL I; clone M-38) (Developmental Studies Hybridoma Bank; Iowa City, USA). All primary antibodies were used at a 1:100 dilution in washing buffer. After reaction with the antibodies, the cells were washed twice with washing buffer. Normal, pre-immune rabbit serum and non-specific mouse IgGl (both DAKO) were added for 30 min at room temperature. The cells were washed and resuspended in 500μl of washing buffer and analysed by FCM using a FACScan flow cytometer (Becton Dickinson). The results were analysed using CellQuest software (Becton Dickinson) and are presented as arbitrary units of FSC and SSC and as the average fluorescence intensity (AFI) of 10,000 cells (±SD).

3. Results

3.1. SEM

After 5 days of incubation, the HOS cells were observed to attach and spread on all substrates when viewed at 300 x magnification (Fig. 1a-d). On the control surface, as shown in Fig. 1a, the cells were observed to be flattened, indicating good attachment, and were well spread across the surface. At this low magnification, these cells in particular were observed to have numerous cytoplasmic processes, which could also be seen in the HA cells (Fig. 1b) but were not readily apparent on the HA2.5 and HA5 composites (Fig. 1c and d, respectively). In addition, multiple cytoplasmic extensions were present between neighbouring cells on all surfaces, as were rounded cell bodies indicating that some cells were undergoing cell division at the time of fixation. Furthermore, the underlying surfaces of the HA materials were observed to become increasingly rough and pitted as the proportion of glass increased, as was evident in the cultures grown on HA2.5 (Fig. 1c) and particularly HA5 (Fig. 1d).

At 2000 x magnification, cells grown on the control surface had a highly fibrillar cell membrane, while the underlying glass surface appeared very smooth (Fig. 1e). Cells cultured on HA alone also showed many long cytoplasmic extensions, suggesting their close attachment to the underlying surface (Fig. 1f). In contrast, cells grown on HA2.5 (Fig. 1g) had far fewer and much shorter processes, with many cells less flattened and less-well spread across the pitted surface of this composite. At this high magnification, surface pitting was particularly apparent in the HA5 composite, although cells grown on this material were nevertheless very well-spread, having prominent fibrillar extensions from the cell membrane and a network of processes clearly associated with the cell body (Fig. 1h).

3.2. Effects of HA materials on cell proliferation

Few, if any residual cells were found to be present on the surface of the culture wells from which the HA and HA composite discs were removed for subsequent incubation in new dishes. In addition, no cells or noticeable debris were observed in the medium of the culture wells after the discs had been incubated for 2, 5 and 7 days. The results of the MTT assay for cell growth showed that the HOS cells incubated on all substrates proliferated over a period of 7 days (Fig. 2). However, the increase in the number of cells on the HA and HA composite disc was notably lower than that of the control cells, and this difference became progressively more pronounced as the incubation period increased. Thus, after 2 and 5 days of incubation on the HA2.5 and HA5 composites, the inhibition of cell growth was 40% and 50%, respectively, and these differences were statistically significant (p < 0.05). By day 7, growth on the HA was also significantly reduced (by approximately 50%)
Fig. 2. Effects of HA materials on the growth of HOS cells. The MTT assay was carried out after 2, 5 and 7 days of incubation in control plastic dishes (black bars), HA alone (white bars), HA2.5 (grey bars) and HA5 (striped bars). The results are shown as the average A570 of 3 replicate cultures ± SD (vertical bars). *Statistically significant difference compared with the control cells (p < 0.05; Student's t-test).

compared with the control culture (p < 0.05), as shown in Fig. 2.

3.3. Effects of HA materials on cell size and granularity

FCM analysis of the size and granularity of HOS cells grown on the control and material surfaces is shown in the dot plots of a representative experiment in Fig. 3. The profiles of cells grown on all three HA materials did not appear to differ substantially from the control culture, although there was an indication that an increasing proportion of the cells became more granular with increasing glass composition. However, a summary of three separate experiments in Table 1 shows that the overall average size and average granularity of the cells grown on all HA materials were very similar to each other and not significantly different from the control cells grown on plastic.

3.4. Effects of HA materials on antigen expression

FCM was used to measure the effects of the HA materials on the relative levels of bone-associated antigens, as described in the materials and methods. In the experiment shown in Fig. 4, ON was arbitrarily selected as a representative antigen and the fluorescence profiles measured and compared for cell cultures grown on plastic, HA and the composite discs. In this particular experiment, whereas the relative level of ON in the control cells was an AFI value of 24, the same cells incubated on HA discs had markedly reduced ON expression (AFI of 14). However, cells grown on HA2.5 had a higher level of ON (AFI of 17), and the HA5-grown cells expressed a relative level of ON (AFI of 20) which was more similar to the control cells.

Table 1

<table>
<thead>
<tr>
<th>Materials</th>
<th>Size (%)</th>
<th>Granularity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>112 ± 2.3</td>
<td>112 ± 3.5</td>
</tr>
<tr>
<td>HA2.5</td>
<td>107 ± 3.1</td>
<td>109 ± 3.2</td>
</tr>
<tr>
<td>HA5</td>
<td>109 ± 2.6</td>
<td>108 ± 3.6</td>
</tr>
</tbody>
</table>

*HOS cells cultured for 5 days on control plastic, HA and HA-glass discs were analysed by FCM for size and granularity.

The data from three replicate experiments is summarised in Table 2 and shows that, after 5 days in culture, the bone-associated antigens BSP, ON and OP were
The down-regulation of OP in cells grown on HA was also lower (18% less than control), although this difference was not statistically significant (Table 2). Notably, HOS cells grown on the HA2.5 and HA5 composites showed progressively elevated levels of BSP and ON, compared with cells incubated on HA alone, while OP was also increased when the cells were grown on HA5. In contrast, the expression of FN, a major antigen of soft and hard connective tissue, was unaffected (except by HA2.5), and COL I expression was possibly even up-regulated by incubation on discs with increasing glass composition.

### 4. Discussion

The present study has examined the growth and function of an osteoblast-like cell line cultured on HA and two HA-glass reinforced composites, compared to the same cells grown on plastic or glass as controls. The HOS cell line was used for this study because it has previously been shown to exhibit a number of features similar to those of normal human osteoblasts [25].

The physicochemical properties of biomaterials, such as surface chemistry and topography, have profound effects on cell attachment, cell spreading, proliferation, differentiation and function [26,27]. In addition, many materials, including the HA materials used in this study are known to release inorganic ions into the surrounding fluid [11,28,29], and these substances also markedly influence the biological activity of the target tissues. Thus, the composition of resorbable glass composites, which determines the solubility and biodegradability of these materials, is of crucial importance for their biocompatibility and efficacy in vivo. For example, calcium, which is released at high levels from HA-glass composites compared with HA alone, is involved in the formation of an apatite mineral phase in hard tissues and is known to play an important part in regulating cell adhesion and activation [30]. Together with surface-associated differences, such as the greater hydrophobicity and higher negative charge of the glass composites, these physico-chemical parameters undoubtedly exert a profound influence on the biological response of host cells. The present study was therefore undertaken to delineate the precise effects of HA and HA glass-composites on the growth and certain key functional activities in vitro.

The results showed that HOS cells were able to adhere, remain attached and viable and then proliferate directly on both the HA and HA composites. However, the cells grown on HA2.5 appeared to have fewer and shorter cytoplasmic extensions onto the material surface compared with cells grown on plastic, HA and HA5. This does not appear to be due to surface topography only,
surfaces than to smooth surfaces [31]. Thus, despite the roughened surface of the HA2.5, this composite did not appear to support the cells as well as the HA or the HA5, possibly because of the particular chemical composition of HA2.5. Notably, despite the attachment and spreading of the cells on HA and HA composites, none of these materials allowed as high a level of proliferation as on the control plastic surface. Moreover, this effect was prolonged and did not appear to be dependent on the proportion of glass but on the presence of the HA itself.

Growth on different substrates is also known to be associated with marked changes in cell function [32]. In the present study, although FCM analysis showed that the HA and the HA composites had little if any effect on the size and intracellular granularity of the HOS cells, all of the materials were found to influence the functional activities of the cells, even after a relatively short period of culture (5 days). In addition, the effects we observed appear to be antigen-specific. Thus, while BSP and ON were down-regulated by growth on HA compared with control cells, OP and also the connective tissue antigens FN and COL I were expressed at similar levels as the controls. In contrast, HA2.5 and HA5 resulted in elevated expression of BSP and ON compared with cells grown on HA alone, and protein expression on HA5 was increased compared with cells grown on HA2.5, with COL I expression actually exceeding that of the control cells. Although we have not examined the longer-term effects of these materials on bone-antigen expression, our findings nevertheless support other studies which suggest that certain bioactive glasses, which have clinically beneficial mechanical properties, are also able to elicit favourable responses from osteoblast-like cells [22,23,33].

The present study also shows that the incorporation of glass into HA, in addition to increasing mechanical strength compared with HA alone, has differential effects on the functional activity of key connective tissue antigens. In addition, our data indicate that the composite with the higher proportion of glass generally elicits higher levels of bone antigen expression than does either the lower glass composition or HA composite. The reason for the apparently improved ‘biocompatibility’ of the 5% glass composite is not known, but appears to be more closely associated with chemical composition rather than with surface topography or with porosity, which the preceding paper has shown to remain constant. However, the HA5 material contains high levels of Ca$^{2+}$ and PO$_4^{3-}$ that are likely to be released into the surrounding environment in vitro and which would undoubtedly have a marked effect on cell activation, cell function, and thus the bioactivity of an implant material in vivo.

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References


DEVELOPMENT OF A GLASS REINFORCED HYDROXYAPATITE

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Introduction. A glass-reinforced hydroxyapatite (GR-HA) has been developed as a new high strength bioactive implants material. The work to date has utilised commercially available hydroxyapatite. The aim of this study was to investigate the use of aqueously precipitated HA and to investigate its effect on sintering and phase stability in comparison to the GR-HA using commercially available HA.

Materials and Methods. HA was precipitated at 80°C using the following reaction:

\[ 10\text{Ca}(\text{NO}_3)_2.4\text{H}_2\text{O} + 6(\text{NH}_4)_2\text{HPO}_4 + 2\text{NH}_3\text{OH} \rightarrow \text{Ca}_{10}(\text{PO}_4)_{6}\cdot(\text{OH})_2 + 14\text{NH}_4\text{NO}_3 + 6\text{HNO}_3 + 40\text{H}_2\text{O} \]

Following washing, drying and sieving, a glass of composition 45 mol.% P2O5, 32mol.% CaO and 23 mol.% Na2O was incorporated into both the precipitated HA and the commercial HA (P120, Plasma Biotal, UK) at 2.5wt.% addition using a process documented elsewhere [1]. Pressed discs were fired at 1200, 1250, 1300 & 1350°C. X-ray diffraction was performed along with density measurements.

Results. Figure 1 shows the effect of the different HA’s on densification with temperature. As can be seen, the precipitated HA undergoes densification at a much lower temperature compared to the commercial HA.

![Figure 1. Density against firing temperature for the two composites.](image)

Figure 2. X-ray diffraction trace for GR-HA using precipitated HA.

X-ray diffraction showed that both the P120 and precipitated HA GR-HA’s showed similar levels of stability. Figure 2 shows an expanded view of the precipitated HA composite fired at 1250°C. The main reflections for HA may be seen at around 32° 20 and the main reflections for β and α-TCP are located at about 31.1 and 29.9° 20. Both the P120 and the precipitated HA composites when fired at 1300°C showed small amounts of decomposition to β and also α-TCP. These phases also account for the small decrease in density at the higher firing temperatures.

Conclusions. The mean particle size of the precipitated HA is smaller than the P120. When the glass is added (which acts as a sintering aid) the higher surface energy of the precipitated HA drives the sintering process at lower temperatures as can be seen at 1200°C. This could lead to lower temperature processing, to obtain the same mechanical properties.

HA shows great potential for dental implant use. However, it is limited in its application by its poor mechanical properties. The authors have developed a way to significantly improve the mechanical properties. This work compares the mechanical properties of commercial HA (HA P120, Plasma Biotal, UK) reinforced with a variety of calcium phosphate based glasses as a function of sintering temperature. These glasses act as a sintering aid and are generally used to improve the mechanical properties of the material.

**Materials and Methods**

Two glasses were produced with the following composition:

<table>
<thead>
<tr>
<th>Glass Code</th>
<th>CaF₂ (mol%)</th>
<th>MgO (mol%)</th>
<th>P₂O₅ (mol%)</th>
<th>CaO (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPMg</td>
<td>0</td>
<td>15</td>
<td>21.25</td>
<td>63.75</td>
</tr>
<tr>
<td>CPF</td>
<td>15</td>
<td>0</td>
<td>21.25</td>
<td>63.75</td>
</tr>
</tbody>
</table>

Two composites were produced by first milling 5g of the glass for 24 hours and then adding 195g of the commercial HA and 300ml of methanol and milling for a further 24 hours, to give a 2.5wt% glass addition. Following drying, the mixture was then sieved to 75um. The resulting powder was pressed at 20 tonnes to form discs of 25mm diameter and these were fired using a heating rate of 4°C/min. to 1200, 1250, 1300 or 1350°C. The discs were held at this temperature and then furnace cooled. Density measurements for a set of ten specimens at each sintering temperature were taken using the Archimedes principle in distilled water.

**Results**

Figure 1 illustrates the change in FBS as a function of sintering temperature for HA with and without glass additions. As expected, the GR-HA displays improved mechanical properties compared to HA without glass, with the glass type CPF showing the highest FBS. The main factors that could account for this behaviour are porosity and secondary phases.

**Conclusions**

The glasses are known to have an effect on grain growth and can either promote or retard grain growth. This may be a further effect to investigate and correlate with the mechanical properties.

**Reference**


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