STUDIES OF THE QUALITY OF THE INTRAOSSEOUS DENTAL IMPLANT BED AND OF THERMAL EFFECTS IN IMPLANT PATHOLOGY.

KEVAN WONG

THIS THESIS HAS BEEN SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF SCIENCE UNIVERSITY OF LONDON.

NOVEMBER 1999

DEPARTMENT OF ANATOMY AND DEVELOPMENTAL BIOLOGY UNIVERSITY COLLEGE LONDON
‘Research is to see what everyone has seen, and to think what nobody else has thought’ (Albert Szent-Györgyi)
ABSTRACT

Dental implants give problems if there is deficient or poor quality host bone, particularly in the maxillary sinus region, and operations to augment the bone volume into which an implant is to be placed may be undertaken as a preliminary step in which particulate irradiated mineralised cancellous allograft can be employed.

It was hypothesised, and demonstrated, that practical information might be obtained through analysis of trephine bone cores removed in creating the implant bed. Therefore, such cores were embedded and examined, mainly using quantitative backscattered electron imaging to study the quantity and the quality of bone. New bone formed as woven or lamellar bone on the allograft, which retained many of its original topographical and morphological characteristics. The bone volume fraction was found to be significantly greater within 5 mm of the original sinus floor. Biopsy core specimens from native sites in both maxilla and mandible were treated similarly. The highest mineralisation densities were found in the mandible, and the lowest in the posterior maxilla beneath the sinus floor. The results led to a proposal for a future bone quality scale to include mineralisation density, volume fraction and connectivity.

Another aspect of success concerns vascularity of the implant/graft bed. To this end, the possible clinical use of Laser Doppler Flowmetry to confirm positive blood flow in grafts, sinus membrane, and oral tissues was assessed and proven.

Heat conduction via dental implants may impair bone healing and survival: here, a theoretical study was undertaken, and this predicted food/drink heat to be an element in implant pathology. In addition, the possible influence of temperature on osteoclastic function in vitro was examined using a volumetric resorption pit assay: measured volumes and depths of resorption lacunae were increased at 41° and 43° C compared with the standard 37°C temperature used in previous studies.
List of abbreviations

AU arbitrary units
bis-GMA bisphenol A-glycidyl methacrylate
bFGF basic fibroblast growth factor
BSE backscattered electron
CT computerised tomography
DFDB demineralised freeze dried bone
D1 bone quality according to Misch (1990b) like oak
D2 bone quality according to Misch (1990b) like white pine
D3 bone quality according to Misch (1990b) like balsa wood
D4 bone quality according to Misch (1990b) like polystyrene
EC endochondral bone
IM intramembranous bone
IMCA Irradiated mineralised cancellous allograft
LDF laser Doppler Flowmetry
LM light microscopy
MMA methacrylate monomer
PMMA polymethylacrylate
PDGF platelet derived growth factor
qBSE quantitative backscattered electron
quality 1 according to Lekholm and Zarb (1985) almost completely compact bone
quality 2 according to Lekholm and Zarb (1985) thick layer of compact bone surrounding a core of dense trabecular bone
quality 3 according to Lekholm and Zarb (1985) thin layer of cortical bone surrounding a core of dense trabecular bone
quality 4 according to Lekholm and Zarb (1985) thin layer of cortical bone surrounding a core of low density trabecular bone
RAP regional acceleration phenomenon
rhBMP recombinant bone morphogenetic protein
SCC96 Association of Osseointegration Sinus Concensus Conference (1996)
SEM scanning electron microscopy
TGF-β Transforming growth factor β
µCT computerised micro tomography
ACKNOWLEDGEMENTS

The inspiration for this thesis began with the many unknowns encountered in the practice of implant dentistry. It was to my good fortune that both the facilities and scientific teachers of such imminent standing as Professors Alan Boyde and Sheila Jones were available for guidance. Their accumulated knowledge gave me a profound sense of humility and inspiration by their diverse and complimentary approaches to the scientific inquiry. To Professor Jones, the timely advice, guidance, and encouragement in all aspects of my work is gratefully acknowledged. To the others who have helped me from the Hard Tissue Unit at UCL: Dr Colin Gray for his patience and answers to my many queries of both science and nature; Mo Arora, who continually supplied technical assistance and direction; Roy Radcliffe for his help on the many machines I have used; Dr Peter Howell for his endless patience in giving computer and statistical support, and for the heat conduction graphs in Chapter 5; Dr Virginia Kingsmill for offering lots of useful, ‘student’ advice and camaraderie; To the Graduate School, UCL, for equipment funding; To Moor Instruments Ltd (UK) for the generous loan of the Laser Doppler Instrumentation, and the exceptional help in its implementation from Dr Rodney Gush and Mr Peter Jady.

I would like to thank my patients for giving consent to be included in this study, and to my practice colleague Kevin Churchyard for his encouragement. Thanks are due to my staff at Treetops Dental Practice; Rachael Gaymer, Sandra Mollicone, Kirsty Johnstone, Gwyneth Brabyn, and Anji Thomson who were always there to lend support and ‘hold the fort’, whilst I was away following my interests at UCL. A special tribute is made to Mrs Philomena Hume, deceased, my close friend, and practice manager, who helped to make it possible, but sadly did not live to see the end of this journey.

Of friends, I would like to thank: Mr Hank Wassenaar, and Dr Robert Williamson for their time in numerous discussions on engineering and thermodynamics.
Of family I wish to thank my son Nigel Wong for his computer help, and to my wife, Jan, for her proof reading, waitress services, and whose constant encouragement and support, relieved me of family concerns during the course of this work. Most importantly I thank all my children, as they have been neglected for some time.

Finally, to Professor Alan Boyde, my supervisor whose dedication to science was difficult to emulate. I thank him for his patience, and generosity of time in support of my investigations. For his guidance, close and personal support I owe him a debt of gratitude.
TABLE OF CONTENTS

TITLE PAGE.................................................................................................................1
ABSTRACT......................................................................................................................3
LIST OF ABBREVIATIONS............................................................................................4
ACKNOWLEDGEMENTS.................................................................................................5
TABLE OF CONTENTS.................................................................................................7
LIST OF FIGURES..........................................................................................................8
LIST OF TABLES.............................................................................................................10
CHAPTER 1  Introduction...............................................................................................12

CHAPTER 2  Evaluation of bone quality and quantity in trephine cores ... 31
from sinus graft sites.

CHAPTER 3  Evaluation of bone mineralisation density distribution .......... 80
and percentage bone volumes of native implant sites.

CHAPTER 4  Laser Doppler Flowmetry for clinical detection of blood.....121
flow as a measure of vitality of sinus bone grafts.

CHAPTER 5  Thermodynamics of temperature in dental implants,...........138
a hypothetical model.

CHAPTER 6  Osteoclast resorption assay with temperature....................146

CHAPTER 7  Conclusion...............................................................................................154

BIBLIOGRAPHY............................................................................................................156
APPENDIX 1  Documentation on IMCA supplier.....................................................187
LIST OF FIGURES

Figure 1-1  Trephine drills and retrieved bone cores ................................. 37
Figure 2-1  Packaged IMCA graft and vials of autogenous serum ............... 39
Figure 2-2  Graphs of qBSE results of bilateral sinus IMCA grafts........... 46
Figure 2-3A Graphs of qBSE results IMCA grafts by sex ............................... 47
Figure 2-3B Graphs of qBSE results IMCA grafts by site ............................... 47
Figure 2-4  BSE-SEM micrographs of unused IMCA .................................... 53
Figure 2-5  BSE-SEM micrographs of unused IMCA II ............................... 54
Figure 2-6  BSE-SEM micrograph of micro cracks and woven bone .......... 55
Figure 2-7  BSE-SEM micrograph of sinus graft IMCA ............................... 56
Figure 2-8  BSE-SEM micrograph of fracture at cement lines .................. 57
Figure 2-9  LM micrograph of osteocytes and canaliculi in woven bone...... 58
Figure 2-10 3D-LM photomicrograph from stereo pairs of sinus graft ....... 59
Figure 2-11 BSE-SEM micrograph incorporated HA granules I .................. 60
Figure 2-12 BSE-SEM micrograph of HA-bone interface ......................... 61
Figure 2-13 BSE-SEM micrograph of hypermineralisation ....................... 62
Figure 2-14 BSE-SEM montage of sinus graft bone core ........................... 64
Figure 2-15 BSE-SEM micrograph of mandibular graft IMCA .................... 65
Figure 2-16 BSE-SEM micrograph of osteoporotic trabeculae .................. 67
Figure 3-1  Mineralisation frequency distributions of bins by class.......... 83
Figure 3-2  Mineralisation frequency distributions of bins by class.......... 84
Figure 3-3  Mineralisation frequency distributions of bins by class.......... 85
Figure 3-4  Mean mineralisation distributions of bins 1 to 16 by Class ... 86
Figure 3-5  Box plot of mean mineralisation in bins by class .................. 86
Figure 3-6  Box plot of mean grey scale by sex .................................... 86
Figure 3-7  Box plot of percentage bone volume by sex ......................... 89
Figure 3-8  Box plot of percentage bone volume by class ....................... 89
Figure 3-9  BSE-SEM micrograph of normal lamellar bone structure ....... 92
Figure 3-10 BSE-SEM micrograph of lateral wall of maxillary sinus ......... 93
Figure 3-11 BSE-SEM montages of anterior maxillary cores ............... 95
Figure 3-12 BSE-SEM micrographs of sub-sinus maxillary cores .......... 96
Figure 3-13  BSE-SEM montage of posterior maxillary core............ 97
Figure 3-14  BSE-SEM montages of anterior mandibular cores.........100
Figure 3-15  BSE-SEM of areas of variations in mineralisation .........101
Figure 3-16  BSE-SEM micrograph of extrinsic (Sharpey fibres) fibres...102
Figure 3-17  BSE-SEM micrograph of woven bone formation ............103
Figure 3-18  BSE-SEM micrographs of healing extraction sites ..........106
Figure 3-19  BSE-SEM montage of buccal plate exhibiting RAP.........107
phenomenon.
Figure 3-20  3-D graph to derive area representing Bone Quality........109
Figure 3-21  Graph of quality by bin number..................................113
Figure 3-22  Graph of quality by percentage bone volume...............114
Figure 3-23  Graph of bone quality Scale......................................116
Figure 4-1    DRT 4 and Doppler probes .....................................125
Figure 4-2    Diagram of points of measurement in bone .................127
Figure 4-3    DRTWIN Doppler graphs of blood flow ......................130
Figure 5-1    Graphs of heat conduction down an implant at ..........142
different temperatures.
TABLES

Table 2-1 Material included in Chapters 2 and 3 of the thesis ............ 36
Table 2-2 Classification of anatomical sites of trephine cores............. 38
Table 2-3 Results of mineral density distribution using paired t-test .... 44 for bilateral sinus grafts.
Table 2-4 Results of mineral density distribution using two sample ..... 44 t-test for pooled sinus data.
Table 2-5 Results of two sample t-test for qBSE between unused..... 45 IMCA, sinus and mandibular graft.
Table 2-6 Results of Mann Whitney U-test for percentage ............... 48 bone volume.
Table 3-1 Data for qBSE mean grey scales by class ....................... 82
Table 3-2 Results of two sample t-test for differences in mineral .... 87 density distributions between classes.
Table 3-3 Data for percentage bone volumes per class................... 88
Table 3-4 Results of two sample t-test for differences in percentage ... 90 bone volume between classes.
Table 3-5 Mean grey scales for healing extraction sites ............... 104
Table 3-6 Bone quality table for selected images......................... 117
Table 4-1 LDF data for native implant alveolus implant sites ......... 129
Table 4-2 LDF data for sinus graft implant sites ......................... 133
Table 4-3 LDF data for saline and serum grafts ....................... 133
Table 4-4 LDF pooled data for sinus grafts ............................ 133
Table 4-5 Results of two sample t-test between h1, h2, h3 in ........ 134 sinus graft.
Table 4-6 LDF data for labial mucosa over teeth and............... 134 osseointegrated implants.
Table 4-7 LDF data for sinus membrane .......... 135
Table 5-1 Physical properties of dental materials ......................... 141
Table 6-1 Mann Whitney U-test results of osteoclast assay ........... 152 at 35°C.
Table 6-2 Mann Whitney U-test results of osteoclast assay .......... 152 at 39°C.
Table 6-3  Mann Whitney U-test results of osteoclast assay.............. 152 at 41°C.

Table 6-4  Mann Whitney U-test results of osteoclast assay.............. 152 at 43°C.
Chapter 1

Introduction

An historical review

Endosseous dental implant techniques can provide a cosmetic and functional solution to the problems of removable full or partial dentures (Zarb 1982). Although the need for a preventive approach to the problems of gross jaw atrophy following tooth loss has been recognised (McCord et al 1992), edentulousness partial or total is a present and continuing problem for many people. Dentures are inefficient, often lack retention, are unstable in lateral and protrusive excursions, and inconvenient. Implants offer support, stability and retention for fixed bridgework or removable dentures. Patients who seek dental implant treatment span the spectrum of needs that can be solely related to oral function, facial aesthetics, dental cosmetics, confidence in personal and professional situations and any combination of these factors. Successful implant treatment is dependant on physical and physiological factors. In the absence of psycho-social deficiencies, predictable success is possible when bone health, bone volume, and bone quality factors are balanced by an appropriate restorative regime.

Modern Dental Implants and Techniques

There are in excess of 50 different dental implant designs commercially available today. Current designs have evolved from concepts that were created by the early pioneers of implant treatment. The early implants forms took many shapes and sizes and were manufactured from various materials, but most commonly of metal. Venable et al (1937) described the effects of metals on bone that lead to Strock (1939) to be the first to place a vitallium (cast cobalt-chromium-molybdenum alloy) implant in 1938 at Harvard. Strock recorded clinical evidence of an endosseous spike implant that remained healthy and unchanged after 38 years in function. In 1937 a patent taken out by Adams (1938) had characteristics similar to current implant concepts. It described a submergible cylindrical screw implant with a rounded bottom, smooth gingival collar, and a healing cap or screw. A ball head was used to retain an overdenture and was fastened to the implant by a threaded attachment. Formiggini (1955) developed a spiral implant of stainless steel wire or tantalum. The two ends of the wire were soldered together to form a post. Scialom (1962) invented the tripodial implant consisting of three diverging tantalum pins inserted into bone and then cemented together at the intersections to form support for a crown. Tramonte (1965) developed a bone spiral design, which he called a drive screw and is
still marketed today by Oraltronics (Bremen GmbH). Similarly Lew (1970) designed a vitallium bone screw with a square post for insertion and for attachments that has been available until recently (from Howmedica). Muratori (1964), Pasqualini (1963) and Linkow (1964) produced screw threaded implants with hollow cores. Linkow (1968) developed the innovative endosseous blade implant to overcome the problems of narrow alveolar ridges. Roberts and Roberts (1970) invented the ramus frame implant which incorporates a full arch bar and has a blade implant at its midline which inserts into the mandibular symphysis with the distal ends inserting into the ascending ramus. This implant design is still in use today. Small et al (1970) reported on the successful use of the mandibular staple implant, Bosker et al (1991) recorded a success rate of about 96% over 13 years in a multi-centre study with a similar transmandibular implant. These implants required an extra-oral approach and are specialised for cases of gross mandibular atrophy by utilising the mandibular symphyseal region.

The subperiosteal implant was first introduced by Dahl (1943) and is still utilised (though rarely) in special clinical cases today with very much the same design as the original. This design relied on a close fitting framework lying on the cortical surface of the jaw bone beneath the periosteum. The requirements of extensive surgery to obtain an impression of the jaw bone for fabrication of the implant followed by the same procedure to fit the implant discouraged both patients and clinicians. Further, violation of muscle attachments and intrusion into vital regions by overextended periosteal frameworks were often causes of failure. Major problems were encountered with instability when the implant did not fit accurately to the underlying bone surface morphology leading to micro-motion and instability in function. The failure of subperiosteal implants have been associated with gross bone destruction and infection in the supporting tissues.

Most of these early designs and protocols provided an implant that was primarily supported by a fibrous capsule (osseous union was also evident but not recognised as desirable) which enveloped the implant from the supporting bone. This feature was accepted by clinicians at that time to be almost an ideal solution as the fibrous capsule was seen to mimic the periodontal ligament of the natural dentition (Babbush 1972). Later studies showed this capsule to have fibrous connective tissue fibres aligned parallel to and without adhesions to the implant surface. This did not replicate the periodontal ligament which has dynamic oblique and transverse fibres intimately inserted into cementum and bone (Lavelle 1980). Many treatments were successful but results were
inconsistent, owing to imprecise fit, variable protocols and the lack of knowledge of bone function and implant materials. Features that distinguish today’s successful implants from the earlier devices are the use of biocompatible materials, topographical surfaces that promote bone formation, bioactive coatings, pure titanium and its alloys, and a surgical protocol that is precise and atraumatic, with delayed loading of the implant. These features and techniques predictably produce an ankylosis instead of a fibrous encapsulation of the implant to the bone bed. The earlier implants were commonly loaded with prosthetic superstructures within 1 to 6 weeks post implantation. Current dental implants that show high rates of success are predominately root form and are based on the principles of osseointegration.

Osseointegration

Branemark and co-workers (Branemark et al 1969; Branemark et al 1977; Adell et al 1981) provided the scientific validation for a significant change in surgical and prosthetic protocol with longitudinal studies of 10 and 15 years on the clinical use of endosseous dental implants. They reported success rates of 80 - 100% in edentulous jaws. The term “osseointegration” was coined (Branemark et al 1977) to describe the observed ankylosis as an intimate apposition of vital bone tissue to the surface of pure titanium screw implants. These principles required a bicortical fixation of the implant, care and precision in the surgical preparation of the implant recipient site with minimal trauma to the bone, followed by placement of a tight fitting screw shaped titanium implant, which was left buried beneath the periosteum for undisturbed bone healing to take place for many months. The last element relating to the loading protocol has been recently challenged by the study of Schnitman et al (1997) which indicated that high success rates can still be achieved for up to 10 years with an immediate loading schedule using the osseointegration technique. The success also depended on the meticulous precision, manufacture, sterilisation and surface passivation of the implants before implantation, an issue that was not previously addressed. However, the dental implant protocol “ad modum Branemark” depended on a bicortical fixation of both the coronal and apical regions of the implant and a substantial vital bone bed capable of providing at least a 1-2 mm encasement of alveolus around the implant. Although modifications to the protocol have been successfully advanced, the osseointegration concept has now been universally accepted as the technique of choice for tooth replacement in the edentulous jaw. The success of osseointegration was primarily dependant on providing dental implants with a sound foundation of investing quality bone. Thus the technique
demanded a greater understanding of maxillary bone and its function. Two bone factors have been universally reported to be important in implant success. These were bone quality and bone volume and are central to the investigations in this thesis.

The factor of bone quality

The two systems popularly cited in describing bone quality in dental implantology concern only the macro-anatomy of cortical and cancellous envelopes. The first classification presented by Lekholm and Zarb (1985) was based on a radiographic assessment of jawbone quality, which they defined as below.

Quality 1. Almost the entire jaw is composed of homogenous compact bone.
Quality 2. A thick layer of compact bone surrounds a core of dense trabecular bone.
Quality 3. A thin layer of cortical bone surrounds a core of dense trabecular bone of favourable strength.
Quality 4. A thin layer of cortical bone surrounds a core of low density trabecular bone.

Lekholm and Zarb stated that their scale was drawn directly from experience, and not from any quantitative investigation. They acknowledged the one weakness of the classification, namely that any radiographic assessment would be influenced by variations in density and thickness of the predominating compact cortical structures. Examination of the available radiographic techniques and their salient features provides some insight on the value of this classification.

Periapical radiographs have a limited field of view and provide apparently detailed information on bone structure, but give no reliable information on bone density. The edentulous jaw poses a very difficult clinical problem with the long cone parallelling technique due to the shallow depths of the lingual, palatal, and buccal vestibule. For these reasons these films are of limited diagnostic value for bone density or quantity. The panoramic tomograph is a series of consecutive single exposures of each vertical strip of film of 1 to 2 cm in thickness. A narrow slit collimated X-ray beam less than 1mm is passed through the jaws while film and source are rotating around a centre positioned within the patient. The film cassette movement is synchronised with that of the X-ray beam. This provides a predetermined image layer (the jaws) to be focused. The depth of the good image layer is 1 cm in anterior and 2 cm in posterior regions (Molander 1996). The resolution of the image of a panoramic radiograph is unsuitable for evaluating bone quality (Kraut 1993) although it does indicate height and extension of the sinus floor. Its
diagnostic value is even more compromised by the inconsistency of patient positioning at each sitting, and superimpositions of skull and cervical structures (Lam et al 1995). Images of the maxillary sinus give a two dimensional view of sinus structures that may be 10 to 15 mm in cross-sectional thickness which gives no indication of bone quality. Despite its shortcomings, its value is its general availability, low radiation risk (the average dosage being equivalent to that of 4 bitewing intra oral films) ease of use and the immediate overall 2D visual assessment of the jaw structures for implant placement.

In dual energy x-ray absorptiometry (DEXA) the x-ray photons with two distinct energies are attenuated as the beam passes through the tissues. Emergence profiles of the attenuated beam are produced by computerised analysis for both hard and soft tissues. Bone mineral density is automatically calculated from these profiles. The limitation of DEXA (or single photon absorptiometry, SPA) is that the measurement reflects the projected bone mineral in grams per square centimetre, with no reference to the volume of the bone sampled.

Computerised transverse axial tomography (CAT) or Computed Tomography (CT) was described by Hounsfield (1973) to collect data by multidirectional X-ray scanning with the tissues being well contrasted between air and soft tissues and bone structure. It provides definition of bone trabeculae and exposes any pathology that extends beyond the normal bony anatomy of the sinus. Axial scans can be taken parallel to the orbital-meatal baseline (which is at 10 degrees to the Frankfort plane) while coronal scans are taken in the submentovertical position with the neck extended and give optimal detail of the lateral nasal wall and frontal recesses (McGowan et al 1993b). The summation of views and detail sectioning allows for 2 mm slices of the jaws to be examined in terms of bone height, volume and indications of trabecular structure. The main disadvantages are cost, restricted availability and increased exposure to radiation. Resolution is poor for bone density owing to the large volume sampled.

New Interactive Computerised Axial Tomography Software for personal computers can reformat CAT data into more useful images for dental implant diagnosis and treatment planning. These programs produce three dimensional images, with panoramic and cross-sectional views of the jaws. The clinician is able to model a particular patients reconstruction requirements within the transformed CT images, hence the term, "interactive". Bone density calculations have been incorporated and are able to give
bone density data within a defined region, measured as numbers of Hounsfield units. Quantitative computed tomography can then determine in three dimensions the real volumetric density as grams per cubic centimetre. However the bone mineral density measured is affected by the amount of bone tissue mass, its degree of mineralisation and it is severely disturbed by air pockets (e.g. the sinus) in the sample.

These diagnostic tests have a common major fault in that they sample an area or a volume of bone that encompasses both cortical and cancellous envelopes, and can only offer a quality factor, represented by a grey scale that is dependant on the mineral content within that volume sample. Resolution was in the order of gms/cm^3. Thus a system of classification for quality based on radiographic 2D/3D imaging has inherent problems equal to those criticisms outlined above.

The second system of classification of bone quality into divisions was proposed by Misch (1989), Misch (1990b) and Misch (1993b). These were related directly to tactile feedback when drilling bone and to the heterogeneity of alveolar bone. The divisions were defined as:

D1. Dense compact bone, almost like oak, or maple-like, composed of almost all dense compact bone.

D2. Dense to thick porous compact to coarse trabecular bone, similar to spruce or white pine when drilling.

D3. Porous compact and fine trabecular bone, similar to drilling balsa wood.

D4. Fine trabecular bone, of light density with little or no cortical crestal bone, like drilling Styrofoam.

Misch formulated appropriate treatment protocols in both surgical site preparation, loading schedules and implant geometry’s that accounted for the density variations and character of each division of bone. He also designed special implants (Misch et al 1998) for each division of bone quality. Despite the positive contribution of this classification to the understanding of bone quality, a recent study by Trisi and Rao (1999) reveals the inadequate nature of the system. They utilised histomorphometric analysis to analyse bone cores taken at implant placement. These authors scored total bone trabeculae within total bone, and related these results to the clinical evaluation of bone density at the time of harvest using the Misch divisions. The study confirmed a good correlation between histomorphometry and clinical density for D1 and D4, with no significant differences between D2 and D3. These results reveal the arbitrary nature of such
divisions which did not adequately describe the difference in bone structure between the extremes of D1 and D4.

The present clinical classifications do not reflect the true diversity in bone quality. Bone quality is dependant on both its macro and microanatomy. The macro- divisions of cortical and cancellous bone become more diverse when viewed on the micron scale to reflect bone volume fraction, degrees of mineralisation, cell volume, connectivity, fabric, and structural anisotropy. All of these factors contribute to bone quality. The complexity of the task in any evaluation of bone quality is shown by the innovative and diverse methods employed by bone scientists to quantify these characteristics and their attempts to describe bone quality, by 2-D histomorphometry and 3-D methodology.

**Histomorphometry**

The two dimensional structure of bone has been extensively investigated as a means of understanding the structural biomechanics of this material. Several stereological methods for measurement of trabecular bone have been proposed to gain a greater collective understanding of its quality and behaviour under load. These include trabecular bone volume, marrow space star volume, and trabecular number and intertrabecular distance (Baddeley et al 1986; Gundersen and Jensen 1985; Vesterby et al 1989).

The marrow space star volume provides a stereological estimate of the mean size of an object from a random point when seen unobscured from a random point inside the object along straight lines in all possible directions. When applied as marrow space star volume it becomes an indirect measure of connectivity within the trabecular structures. Large estimations mean more connected holes and vice versa.

Hahn et al (1992) introduced the trabecular bone pattern factor (TBPf) as a measure of trabecular connectivity. This factor relies on the assumption that the connectivity of cancellous structures viewed in two dimensions can be described by the ratio of concave to convex surfaces. Measurements of bone area and perimeter are made, followed by dilatation of the bone area or trabeculae by adding a pixel to each surface. The area and perimeter will either increase or decrease depending on whether the area is dominated by convex or concave surfaces. Higher negative values relate to greater connectivity. Odgaard (1998) demonstrated that this concept was badly flawed.
The secant method utilises an array of parallel matrix of test lines of equal dimensions to scan an image of the bone section from which an average number of intersections of bone crossing the test lines are calculated for the different line orientations. The area fractions and numbers of intersections provides the data for estimation of bone volume fraction, trabecular number, thickness and separation. The above techniques offer some descriptive insights into cancellous bone structure but have shortcomings of which the most serious is that none of the above methods show any relationship to the 3-D structure of bone. These are all derived from histomorphometric analysis of 2-D structural images. True connectivity and fabric of bone structure cannot be evaluated from 2-D images.

3-Dimensional Analysis

The three dimensional (3-D) bone architecture is shown to be highly complex and no single means of evaluation of its quality of structure is yet available. Historically serial sectioning techniques to produce 3-D reconstructions were manual, tedious, time consuming, and prone to errors as pointed out by Odgaard (1998). Feldkamp et al (1989) were among several groups who introduced a new in vitro method to study the 3-D structure of bone using high resolution computed X-ray tomography (CT) which has become known as μCT. More recent versions can reconstruct a 3-D array directly as the clinical CT which produces a series of 2-D slices from which the 3-D reconstruction is formed. The 3-D reconstruction enabled the quantification of bone structure using the Euler number concept. Odgaard and Gundersen (1993) improved the concept and addressed the problems of edges and segmentation. The X-ray point source transmits polychromatic X-rays from a cone beam design. This limits the resolution due to image distortions as well as introducing beam hardening artefacts. The introduction of the X-ray tomographic microscope (XTM) using synchrotron radiation appeared to offer superior 3-D imaging by the higher spatial resolution. It is claimed that structures as small as 1μm may be analysed in 3-D (Bonse et al 1989).

High resolution magnetic resonance (MR) imaging has been applied to 3-D reconstructions of bone. Quantitation of Euler number, trabecular number and volume fraction, have been achieved (Majumdar et al 1995) however, the resolution is worse than μCT with resolution between planes at about 300μm.

From the above, bone quality derives from a composite structure with contributions from each of its components, and that no single scale is yet available to express bone quality.
The factor of bone volume

The loss of jaw bone volume and ridge height is intimately related to tooth loss and is a common feature found in the edentulous jaw. A continuous resorptive process begins following tooth loss that generally results in an atrophied ridge deficient in both volume and height (Atwood 1971; Atwood 1973; Atwood 1979). This three-dimensional change in morphology by resorption was described by Cawood and Howell (1991). The maxilla becomes narrower at the expense of buccal alveolar volume, whilst the opposing edentulous mandible becomes broader at the expense of the lingual alveolar volume and height. Carlsson (1998) reviewed the literature from 1952 to 1996 on factors responsible for alveolar bone loss subsequent to tooth loss. He concluded that no single factor was responsible except that alveolar bone loss was an inevitable consequence of tooth loss. Although Branemark's clinical trials proved the concept, his early studies were located in a single anatomical location, the anterior symphysis of the lower edentulous mandible. This region has the greatest volume of bone in the edentulous jaws. A recent study (Kingsmill and Boyde 1998b) showed this region to maintain sufficient bone height and volume in the edentulous state to satisfy the Branemark criteria for success. Furthermore, this study revealed the mandibular symphysis to progressively increase in bone density with age, and this may be the reason for its resilience in bone volume maintenance following tooth loss. Von Wongem et al (1990) observed increases in mineral density in this area and attributed this to the presence of implants in function.

The edentulous posterior maxilla

In complete contrast, alveolar bone loss in other jaw regions appears fated following loss of the natural dentition, with alveolar loss in the posterior maxilla increasing with age. This region, the posterior edentulous maxilla, presents as a special case where the anatomical factors increase the rate of jaw bone loss. The special anatomical factors of this region are, a predisposition to large fatty marrow spaces with loose connective tissue, that is quality 4 bone according to Lekholm and Zarb (1985), decreased trabeculation (Razavi et al 1995), and the proximity of the maxillary sinus cavity that lies directly superior to the posterior maxillary alveolus and the peculiar characteristic of its enlargement by pneumatisation that continues with age and increases in intra-sinal pressures (McGowan 1993a). This loss of bone can lead to an egg-shell thin dental alveolus which forms both the floor and lateral walls of the maxillary sinus. Although
the author has identified special cases that can avoid this problem (Wong 1996) this region is both difficult and challenging to reconstruct and has a high failure rate. Schnitman et al (1988) reviewed survival rates of 10 variations of implant type that included blades, Branemark, single crystal sapphire, Tubingen, and other dental implant types. A life table showed a significantly reduced success rate of 78% after 24 months in the posterior maxilla compared to 100% in the anterior mandible. Jaffin and Berman (1991) reported a failure rate of 35%. Adell et al (1981) and Adell et al (1990) in a multi-centre study, reported 83% success. Their conclusion was that the reduced success rates are intimately associated with deficiency of bone height and poor bone quality. The sinus graft technique was developed to resolve this problem.

The Sinus Graft

Reconstruction of the edentulous posterior maxilla has been achieved by performing ridge augmentation procedures: onlay rib Terry et al (1974); mandibular and iliac crest onlay grafts Nystrom et al (1996), and Le Fort I osteotomies with interpositional bone grafting Sailer (1989). Some of these techniques have been observed to be unpredictable for the long term retention of the graft. Disadvantages of these techniques are the extensive surgical procedures involved, surgical trauma, hospitalisation, and extended convalescent periods, with donor and recipient site complications. These include adverse healing, bleeding, infection and postoperative trauma associated with major surgery. A new technique to overcome this extreme bone deficiency was first reported by Boyne and Kruger (1962) who found that new bone formed on the antral floor following elevation of the sinus membrane in Rhesus monkeys and dogs. Tatum claims to have been the first to use (in 1977) a modified Caldwell-Luc crestal approach to elevate the sinus membrane to increase the vertical height of the alveolus to accommodate dental implants. Boyne and James (1980) grafted the sinus floor with autogenous marrow and cancellous bone from the iliac crest, and successfully reconstructed 11 cases.

The creation of this type of reconstruction is interesting in that it does create a volume of “new bone” with greater dimensions than is usually endowed by nature and in doing so restricts the maxillary sinus volume for respiratory function. Timmenga et al (1997) followed 45 patients who had undergone sinus augmentation procedures in 85 sinus floors for periods between 12 and 60 months after surgery for evidence of radiographic and clinical pathological changes. They considered many factors, including pre-existing sinus pathologies such as septal deviations and nasal polyps, surgical complications such
as perforations of the sinus membrane during the procedure, and patient allergies. Comparing diagnostic, and clinical data from panoramic pre- and post-operative radiographs with naso-endoscopic examinations showed no clinical problems resulted from the sinus augmentation procedure, even in those patients where perforations of the membrane occurred during surgery. Timmenga et al concluded that sinus augmentation did not increase or produce any signs of sinus pathology. In contrast, a recent report by Zimbler et al (1998) offers a cautionary view and cites four cases of complications resulting from the procedure.

Guided Bone Regeneration
Bone grafting for correction of local defects in jaw ridge height and deficient volume prior to dental implant placement, has become predictable by the development of guided tissue regeneration techniques. Historically, successful bone grafting has been difficult to achieve owing to resorption and dissemination of both block and particulate grafts by the epithelial invasion of the grafts by migrating fibroblasts. Boyne (1964) introduced the barrier concept utilising cellulose acetate filters to restrict fibroblast invasion of the bone surface. He called this an osteophylic response. Recent interest and successful use of this technique with a special emphasis on dental implant reconstructions has been published by various authors using both non-resorbable (Becker et al 1994; Buser et al 1990; Cochran and Douglas 1993; Dahlin et al 1991; Linde et al 1993) and resorbable barrier materials (Colangelo et al 1993; Parodi et al 1996; Pineda et al 1996).

The use of barriers has made bone grafting of particulate materials a predictable and successful technique. Resorbable barriers have reduced the second surgery required to remove the membrane, but Simion et al (1996) observed that the quantity of new bone formed beneath resorbable materials was less than that produced beneath a non-resorbable membrane. The evolution of “guided bone regeneration” by barrier techniques has made bone grafting a predictable procedure in general dental practice. In completion of the sinus graft, closure of the access window was often a problem, with invagination of the periosteal connective tissue through the access window invading the graft itself (Avera et al 1997). This phenomenon was common to the difficulties encountered with particulate bone grafting. Thus guided tissue regeneration has also enabled the final surgical procedure of access closure in the sinus graft.
These bone corrective techniques have provided solutions to the problems of bone loss, with special relevance to the posterior maxilla. Although the sinus graft is predictably used to increase bone volume, the selection of materials has continued to be problematic (Jensen et al 1998).

**Sinus Grafting Materials**

The sinus graft surgical technique is now a well understood and successful procedure with little or no systemic side effects and it has been well documented (Block et al 1998; Lazzara 1996; Smiler 1997; Tatum et al 1993). This has not been true in the selection of the grafting materials. Bone grafts can be autografts derived from the patient’s own bone reserves, allografts derived from a donor of the same species, xenografts derived from another species, or non-biological alloplasts. Grafts can be a composite of two or more of these materials. A recent development is the use of recombinant bone morphogenetic proteins (rhBMPs) which directly stimulate osteogenesis at the recipient site, but are still undergoing animal and clinical evaluation (Boyne, 1996; Nevins et al., 1996; Riley et al., 1996).

Autografts of cortical bone, cancellous bone, and bone marrow from patient donor sites have been considered the gold standard for bone grafting in orthopaedics and maxillofacial surgery. This is a result of the inherent resident osteogenic precursor stem cell population (Friedenstein et al 1968; Salama et al 1973; Burwell 1985) and live differentiated endothelial and osteogenic cells in the tissue. Osteocytes have been reported to produce a bone resorption inhibiting factor (Maejima-Ikeda et al 1997) and reported to survive within the bone matrix after grafting for between 4 hours to over 2 weeks (Berggren et al 1982), but the role of the osteocyte in graft development is unknown. Generally the cells of the autograft are thought to promote re-vascularisation of the graft and may endow early resistance to infection and possible failure (Sadove et al 1990; Marx 1992; Block and Kent 1997; Block et al 1998). Tatum (1977) harvested autogenous bone from the iliac crest for placement into the sinus cavity and reported on 15 cases in 1977. The first publication of sinus grafting with autogenous bone and marrow from the iliac crest on 14 cases was by Boyne and James (1980). Wood and Moore (1988) harvested autogenous bone intra-orally, and used chin bone in a 59 year old women in the right sinus only. Chanavaz (1990) documented 11 years of bone grafting experience associated with dental implants. Of the 241 sinus grafts performed only 12 were with autogenous bone alone. Preliminary reports were published by Jensen et al (1990) on 5 cases and Jensen and Sindet-Pedersen (1991) on 26 cases, using cortico-
cancellous block grafts from the mandibular symphysis for nasal and or sinus grafts in 17 partially dentate patients, and in 9 fully edentulous patients. Jensen et al (1994) published the results of autogenous grafting on 98 patients utilising autogenous bone from the ilium, the lateral aspect of the ipsilateral sinus, and anterior symphysis of the mandible. Isaksson et al (1993) reported on 12 patients with combined nasal and sinus grafting together with Le Fort I maxillary repositioning. Krekmanov (1995) used the same technique in 35 patients, but combined this with Le Fort 1 down tilting of the maxilla. Chanavaz (1996) published data on 15 years of sinus grafting and concluded that “autogenous bone and its combinations with calcium- and phosphorus-containing biomaterials remains undoubtedly the best all-purpose biomaterials” for sinus grafting. Lundgren et al (1997) reported on 20 patients using a trephine harvesting technique from the iliac crest. The Consensus statement I (Jensen et al 1998) from the Sinus Consensus Conference in 1996 (SCC96) on autografts was “Autogenous bone is appropriate for sinus grafting”. Tatum et al (1993) after 18 years experience, stated that, “the finest material we have used is autogenous bone from the ilium”.

There is unanimous agreement on the safety and compatibility in the usage of autogenous bone. Autogenous bone harvest requires a second surgical donor site. In a bilateral sinus graft operation, each sinus can require in excess of 15 cc of donor bone. The iliac crest can provide this volume, but hospitalisation is a necessity, with accompanying costs, post-surgical pain and temporary disablement from normal walking (Hall and Smith, 1981). Complications can include nerve injury, haematoma formation, blood loss, long term pain and functional deficits (Marx and Morales, 1988; Stoll and Schilli, 1981). However, Kalk et al (1996) has recorded a low morbidity at the 1-4 year postoperative period. Because of these greater surgical risks, increased costs for hospitalisation and the morbidity associated with autogenous bone harvesting from the iliac crest, it is widely recognised that there is a need for successful sinus graft substitutes that will eliminate hospitalisation and reduce both morbidity and costs.

Intra-oral sites have been used as a donor site but may not provide sufficient bone volume for extensive sinus augmentations. Sindet-Pedersen and Enemark (1990) reported on alveolar cleft grafting in young patients in a comparative study. Twenty patients received intramembranous mandibular autografts and 20 patients received iliac crest autografts, with equal success. Hirsch and Ericsson (1991) reported on the usage of a mandibular cortical graft for the sinus, but the patient numbers and means of assessment were not described. Autografts from intra-oral sources have been used singularly and in
combination with xenografts, allografts and alloplasts in order to add cellular material and volume to the capacity required to fill the sinus. The harvesting of intra-oral autogenous bone reduces the morbidity of the iliac crest harvest, but has risk factors of its own such as regional temporary or permanent paraesthesia, loss of vitality of the associated natural teeth, gingival recession and scarring of the mucosal tissues. Even with moderate resorption of the jaws, intra-oral sites are depleted and become poor sites for bone harvest in fully edentulous patients.

Rib bone (Terry et al 1974) and tibia bone (Catone et al 1992) as donor sites have been utilised but have the disadvantages of an autograft harvest technique. There is a universal consensus that, although autogenous bone is the gold standard, there is a need for the development of a sinus graft material that can equal or better the autogenous graft, without the restrictions imposed by autogenous harvesting.

In 1996, The Academy of Osseointegration held a Sinus Consensus Conference SCC96 in recognition of the disparity and dissemination of information regarding the sinus graft technique (see Jensen et al 1996). Retrospective data collected from 38 surgeons for 1007 sinus graft cases and approximately 2997 dental implants placed over a 10 year period were reported and their results correlated to provide a consensual view for the sinus graft treatment protocol. This combined database showed a diversity of use in all materials, but the scarcity of scientific analytical data did not allow for any definitive conclusions to be made in respect to graft materials. The singular use of all types and combinations of different grafts produced a 90% success rate in 3 to 5 year periods. Unfortunately, this analysis did not rate failures of grafts alone. Success was rated by implant survival. The majority opinion was that allografts, alloplasts, and xenografts alone, or in combination with each other were effective as a graft material in selected clinical situations and that the limited published data did not allow for any statement to be made about their use in severely atrophic situations. The minority opinion was that published data were too limited to make statements about the use of graft materials for sinus grafting and general use. The general statement concluded, “The consensus of the group is that the combination of autogenous bone and either allograft, alloplast, or xenograft can be effective as a sinus grafting material (unanimous)”. It was suggested that studies were needed on an allograft used alone as a sinus material. There was insufficient data available on the singular use of a graft material (apart from autogenous bone) to allow for any conclusions to be made on its use in the sinus graft.
Irradiated mineralised cancellous allograft (IMCA)

Irradiated mineralised cancellous allograft is an alternative material to the autograft for the sinus procedure. It is a particulate graft form derived from cadaver vertebrae and supplied by Rocky Mountain Tissue Bank (Colorado USA) since 1981. They state that, "There has never been a reported case of infectious disease transmission from an allograft which was processed using the procedure followed by the Rocky Mountain Tissue Bank". This material is designated as a mineralised allograft only because no demineralising processing occurs in its production. Jensen and Greer (1990) reported haematoxylin and eosin histology and 4 years of clinical experience utilising radiated mineralised cancellous allograft for 15 grafts in a group of 10 patients. Forty one implants were placed at the time of sinus graft, of which 9 failed. In this study the alveolar bed was classified for implant placement by the method of Jensen (1989), namely Class B (7 to 9 mm), Class C (3 to 6 mm), and Class D (< 3 mm). 6/24 implants failed in Class C and 3 of 3 failed in Class D. A total of 21% of implants failed in this type of graft. Tatum et al (1993) have been using this material for sinus grafting since 1974 and reported that this allograft "produced the most rapid, consistent and reasonable replacement ratio of new bone". No statistical data were published or described, even though the conclusion was drawn from core biopsy and clinical observations of success. Chanavaz (1996) reported on 15 years of sinus graft materials to produce a Burt Contingency Chart. His results show that irradiated mineralised allograft was clinically next best to autograft in success rates, but no histology was evaluated. Allograft usage alone was analysed by the SCC96. A total of 239 implants were collated of which only 44 were in mineralised allograft, with a cumulative success rate of 85% at 5 years for implants placed in pure allografts. The criteria for sinus graft success was implant survival, which did not include sinus graft failures prior to implant placement. Unfortunately the SCC96 was unable to analyse the failure rate of sinus grafts alone. The latest report is from Jensen and Sennerby (1998) who retrieved one micro-implant with a cylinder of the investing intact bone at 6 and 14 months from each of 6 allograft sites from the lateral wall of the sinus. They reported measurements of bone to implant contact and minor bone formation using microradiography and light microscopy. Although many reports have been published on the use of IMCA in sinus grafts, very little scientific data has been published on the use of this allograft alone.

Another aspect of bone grafting was the paramount need to promote the early consolidation and vitality of the graft material to combat infection and dissemination of
the graft (Berggren et al 1982), being especially important in the use of non-vital material. This can be achieved by any means that may enhance the early neo-vascularisation of the graft which endows it with osteogenic capability and resistance to infection. Examination of the methods in graft preparation provide an opportunity to exploit the potential to enhance graft maturation.

**Graft preparation**

Reports that investigate the use of bone allograft, xenograft, and alloplasts in the dental implant literature do not show a unified approach to the preparation of non-autogenous particulate grafts. Hydration of the graft with physiological saline has been a regularly reported procedure. Tidwell et al (1992) reported on composite autogenous and non-resorbable hydroxyapatite grafts on 48 patients, storing the graft materials in physiological saline. Avera et al (1997) mixed graft in saline (pH, 5.5; isotonic) prior to sinus grafting in an evaluation of resorbable and non-resorbable membranes. Tatum et al (1993) mixed graft with blood and antibiotic in the use of many different materials in the sinus. Wagner (1991) described microfibrillar collagen and resorbable hydroxyapatite mixed with freshly drawn venous blood for grafting. Vlassis et al (1993) described a 1:1 mix of resorbable hydroxyapatite and demineralised allogenic bone matrix in a flowable gel for the sinus graft: no mention was made of the liquid medium. Hurzeler et al (1996) reported on the use of multiple graft types and combinations without any description as to the means of graft preparation of either with blood or saline or other medium. Smiler et al (1992), in a multi-centre study of case reports, described the admixture of graft materials with reconstituted patient’s blood from participating centres. However, in reports on personal cases, they recognised the toxicity of blood by-products in catabolism and the acidic nature of anaesthetic solutions, as did Misch (1993a). They both mixed graft with sterile saline, or D5W (5% dextrose in water, pH, 4.0) as recommended in the study by Marx et al (1979). Marx et al (1998) used platelet enriched plasma to enhance the quality and quantity of new bone formation when used with autogenous grafts.

There is no consensus as to the preparation of graft material prior to placement in the recipient site to promote osteogenesis. An essential growth factor isolated in serum is Platelet Derived Growth Factor, (PDGF) which is released from platelets on blood clotting. Platelet activating factor is also released to promote further platelet activation. Platelet derived growth factors are potent bone cell mitogens that can initiate osteoblastic
proliferation and differentiation, regulate osteoclastic resorption, and indirectly induce vascular-endothelial cell proliferation and angiogenesis. Angiogenesis is a precondition for bone formation, but osteoblasts can also produce PDGF, which attenuates the effects of PDGF. Many years prior to the above, Trueta (1969) considered a syncytium of cells extending from the endothelial cell to the osteocyte from which vascular stimulating factors might be released when they died. These factors could then become chemo attractants for new vasculature into the fracture callus. This concept described the formation of bone radiating from a vascular pattern. This relationship between blood vessels and bone formation has some supporting evidence from Hulth et al (1990) who localised laminin basement membrane proteins in callus cartilage. Contrary to this evidence, Oni (1993) investigated 3-10 day old human callus using lectin binding to target vascular structures and red blood cells: they found no attachments to bone trabeculae, chondrocytes or any other bone cells, even though they were within close proximity to the blood vessels. Paralkar et al (1990) demonstrated the binding of osteogenin to a heparin binding BMP, and of TGF-β (Paralkar et al 1991) to collagen type IV, a constituent of the basement membrane of endothelium. Basic fibroblast growth factor (bFGF), an angiogenic endothelial cell mitogen, has been shown to lie on the surface of vascular endothelium (Folkman et al 1988). Andrew et al (1995) observed PDGF A and B chains to be expressed by endothelial, mesenchymal, bone and chondrocytic cells in healing human fractures. Horner et al (1996) observed PDGF-A mRNA and its protein to be widespread in newly forming heterotopic bone and osteophytic bone.

Angiogenesis is a requirement for bone formation (Bruder et al 1994), and the selective use of platelet and serum growth factors for their properties as described above may influence the rate and magnitude of the osteogenetic response in bone grafts. Marx et al (1998) have reported significant increases in bone quality and quantity when grafts were prepared with platelet enriched plasma. Autogenous serum is known to contain such factors, but the effects of serum in bone graft preparation has not been reported.

Vascularisation of the sinus graft as discussed above is a vital element in its successful maturation to become a suitable bone bed to support dental implants. However, the large volume and geometric form of the sinus cavity creates a situation where the greatest distance between a point in the graft to the closest native bone surface of the sinus can be over 10mm (Anagnostopoulou 1991). One common observation (but often anecdotal) is
that the most superior aspect of sinus grafts have been revealed to be inferior in quality and quantity of new bone than regions closer to the floor of the sinus (Jensen et al 1998). One possible explanation for this observation may be related to a reduced or absence of vitality. No diagnostic means has been presented to investigate this possibility. The contribution that the elevated sinus membrane makes to the development of the graft beneath is unknown.

The quality of bone is known to be affected by high temperatures (Lundskog 1972; Eriksson and Albrektsson 1983), but the effects of high but normal oral temperatures on implants in function has not been investigated. Similarly the bone-implant interface is known to be in a state of virtual remodelling (Garetto et al 1995). Osteoclastic resorption is a key function in the remodelling cycle, but no data is available that relate the effects of oral temperatures on this behaviour.

The foregoing has provided the background and principal requirements of a bone bed for endosseous implants and explains how some deficiencies in bone can currently be resolved and how bone healing may be enhanced. Classifications of bone quality are shown to be inadequate and do not accurately relate to actual bone microstructure. The central theme within this thesis was to address and investigate the concept of bone quality and quantity of the dental implant bone bed in native beds and grafted sites, and to also consider how the quality of the bone-implant interface might be affected in function by oral temperature on implants.

Beginning in Chapter 2, bone quality is discussed in terms of mineralisation density and introduces scanning electron microscopy in the backscattered electron imaging mode (BSE-SEM) as a suitable technique to investigate bone quality and quantity. It was hypothesised in the first instance that essential information would be obtained by examination of bone core specimens removed by trephine drills as part of routine site preparations for implant placement. The sinus graft was chosen as the specific site for study utilising a single allograft. Incorporated within this chapter was also an investigation based on the hypothesis that the use of autogenous serum would enhance angiogenesis and hence graft maturation.

Chapter 3 develops the concept of a new bone quality scale and continues with the same investigative techniques as in Chapter 2, in the examination of bone core specimens
derived from all other native jaw sites. The data provided baseline values in bone quality and quantity for comparative analysis.

Chapter 4, continued the investigation of bone quality within a clinical perspective. The quality of the bone formed directly below the sinus membrane was hypothesised to be influenced by its vitality and that of the membrane. This was explored using Laser Doppler Flowmetry to directly confirm and to measure the blood supply of the sinus membrane, graft and adjacent structures.

Chapter 5, presents a theoretical discussion using thermodynamic principles to assess the effect that oral temperatures might have on the quality of the bone-implant bed.

Chapter 6, continues the investigation of bone quality in tissue culture, by considering the effect of temperature on osteoclasia, on the hypothesis that this phenotype may have preferential temperatures for increased functional efficacy apart from the known preference of osteoclasts for acidic microenvironments. Results are discussed in relation to the increased rates of remodelling at the bone-implant interface.

Chapter 7, provides the collective conclusions found in this thesis.

The Aims of the Thesis are:
1. To characterise and evaluate the quality and quantity of sinuses grafted with irradiated mineralised cancellous allograft at 6 months, by examination of trephine core specimens using light and electron microscopy.
2. To determine if autogenous serum was beneficial when used in particulate allograft preparation.
3. To examine the morphology, bone quality and quantity in the edentulous jaw from sites prepared for dental implants as in 1.
4. To develop a quantitative scale to describe bone quality in the implant bed.
5. To evaluate and utilise Laser Doppler Flowmetry as a clinical means to detect vitality or blood flow in sinus membrane, grafts and adjacent structures.
6. To determine the possible effects of oral temperature on dental implants using a hypothetical model.
7. To observe the effect of temperature variation on osteoclasts in vitro.
Chapter 2

Evaluation of bone quality and quantity in trephine cores from sinus graft sites.

Introduction

As previously discussed the classifications adopted by Lekholm and Zarb (1985) and Misch (1990b) recognised both the structural and physico-chemical diversity of bone structure. Both groups stated that it was only at the point of drilling the bone for implant placement that the quality could be assessed. Adell et al (1981), Jaffin and Berman (1991) and Schnitman et al (1988) showed that the success rate of osseointegrated implants were related to regional differences in bone quality. Success was found to be 25% to 50% less in the posterior maxilla and 10% less in the anterior maxilla compared to the anterior mandible.

Physico-chemical diversity is one of the defining characteristics of bone, and to which it owes many of its mechanical properties. In remodelling of mature or lamellar bone, osteoblasts synthesise and lay down osteoid on bone surfaces (that have an ordered structure of alternating layers or lamellae 2-3μm thick), generally following resorption, and unlike enamel which is mineralised immediately on deposition, undergoes a lag phase prior to mineralisation of 5 to 10 days (Frost 1960). A rapid collagen directed primary mineralisation phase follows, and begins to slow after 80 days. Mineralisation proceeds to completion after a further 30 days (Parfitt 1992) but packets of bone can continue to become highly mineralised over many years, (Boyd et al 1995; Grynpas 1993).

The formation of new or woven bone as in modelling and neo-bone formation takes a different route in mineralisation. Woven bone is immature and forms from a matrix vesicle mineralising process (Anderson 1969; Anderson 1984; Bonucci
that forms mini-calcospherites in the osteoid. The collagen fibres vary widely in diameter, from <0.1 to > 3 micron and are randomly orientated.

Mineralisation of bone is a dehydration phenomenon whereby the water filled intra- and inter-matrix spaces between and within collagen fibrils of the osteoid are replaced with a carbonated apatite mineral phase (Dahllite), similar to, but distinct from hydroxyapatite, owing to the inclusion of carbonate, Mg, and other ions (Driessens and Verbeeck 1990). Immature woven bone is also morphologically distinct from mature bone by its high cellularity.

The relationship between bone mineral content (ash content) and static strength was studied by (Currey 1970). He observed a rapid rise in static strength over a small range of increasing ash content (mineral of 63-71% by mass), with a sharp cut-off at the higher values. This phenomenon is explained by the apatite being unable to bear load until it begins to saturate the collagen matrix at the optimal density. In a later study using human femora, Currey (1979) provided evidence for the loss of bending strength and ability to absorb impact forces above this level. The decrease of elasticity and plastic deformation with increased mineral content allowed crack propagation to occur through the mineral phase, with a resultant loss of mechanical properties.

Others have related bone strength to structure (Compston 1994), and its orientation (Riggs et al 1993a; Riggs et al 1993b). Bone structure is diverse in its mineralisation density, and architectural anisotropy with its variations reflecting age (Reid and Boyd 1987), health and history of the individual (Compston 1994; Schnitzler 1993). Although bone mineralisation density is a major, but not the sole factor that determines bone strength (Boyd and Jones 1998), it is mineral content that defines the physical entity of bone from osteoid that is devoid of mineral to the hypermineralised aged bone.

Quantification of the mineralisation density of bone and its morphology contains information on the individual's, development, turnover, and remodelling at any determined time (Kneissel et al 1994). Many methods have been applied to measure bone mineral content. Density gradient fractionation separates bone
powdered specimens by specific gravity (Richelle 1967). The lowest density represents the least mineralised fraction, which is also the most recently formed bone. Unfortunately, this is a destructive method of analysis and does not allow for further analysis of the specimen. However, the main criticism is the loss of positional, spatial and temporal information that is locked into the bone structure in the mineral phase.

Microradiography was developed by Amprino and Engstrom (1952). In this technique, thin parallel sections of approximately 100μm are examined with X-rays to produce grey scale images correlated to the mineralisation density. Both mineralisation density and the thickness of the section determine the net X-ray absorption. The preparation of thin perfectly parallel sections of 100μm is technically demanding and not always possible to achieve. Furthermore, the volume of tissue sampled at 100μm in thickness does not allow for high resolution. The technique is also considered to be excessively time consuming (Grynpas 1993). Single (SPA) and dual (DPA) photon absorptiometry and dual energy X-ray absorption (DEXA) are techniques that measure the overall quantity of bone in a given volume but are unable to offer information on the distribution of the mineral phase.

Backscattered electron (BSE) imaging in scanning electron microscopy was introduced by Boyde and Jones (1983b) to quantitate the distribution of mineral densities whilst still preserving the tissue morphology. This technique is outstanding in that it samples only a thin layer in the surface of a specimen, which makes it ideal and versatile for examining bulk samples of bone (Boyde et al 1993).

*Scanning Electron Microscopy (SEM)—Quantitative Backscattered Electron (qBSE) Microscopy*

Scanning electron microscopy (SEM) uses an electron-optical lens system to focus a beam of electrons capable of probing the surface of a specimen in a scanning array. The interaction of the specimen and electrons gives rise to a number of signals that can be detected to produce an image.
Secondary electrons are low energy (i.e. <50 eV). Backscattered electrons have higher energies but always less than that of the electrons in the probe beam. These signals are emitted from a volume of the sample much less than that penetrated by the incident electron beam, and dependent on probe energy and probe diameter, the specimen morphology and composition. A secondary electron image provides a detailed three dimensional structure of specimen surface topography and is invaluable in the investigation of osteo-architecture (Boyde and Jones 1996).

Backscattered electrons (e.g. >10keV), originate from within the target specimen surface and can provide images that rely on the composition and crystal structure of the sampled specimen. The fraction of the electrons backscattered increases with the atomic number Z (Z contrast) of the atoms hit by the incident beam. Bone has an organic matrix and mineral elements. In the latter, concentration of Ca is greatest and this element also has the highest atomic number (Z=20), so that it dominates the intensity of the compositional component of backscattered signals.

When BSE is applied to specimen surfaces that are perfectly flat or with minimum topography, the total summed signal reflects the mean atomic number of the elementary volumes sampled in the surface layer. It has been estimated by Howell and Boyde (1994) that within normal conditions when the accelerating voltage is 20 kV, and bone of 40% volume mineral, then the backscattered electrons will be returned from a volume of less than 1 μm deep and 2 μm in radius. The resultant volume resolution would then be of the order of 1μm³. In bone, these flat surfaces can only be produced from a resin embedded sample, which can then be milled or polished. The embedding medium used in this study was polymethylmethacrylate (PMMA).

The image contains grey levels adjusted to cover the full range of mean atomic number variations of the substrate (Boyde and Jones 1983a; Boyde and Jones 1983b). Bloebaum et al (1997) “validated” this technique by observing bones from ten species for mineral content with Fourier-transformed infrared spectroscopy, X-ray diffraction, energy dispersive X-ray spectrometry, ash
measurements, and BSE imaging. They found that BSE image intensity (grey level) had a very strong positive correlation to mineral (ash) content. Compositional and crystallographic variations among bones did not affect backscattered electron grey levels.

Boyde et al (1995) used novel dimethacrylate esters derived from the reaction of halogenated phthalic acids and glycidyl methacrylate synthesised by Davy (1994). These materials exhibited physical and mechanical properties similar to bis GMA and are X-ray opaque: some have values similar to dental enamel. The first material, C_{22}H_{25}O_{10}Br with a defined BSE peak at a level above PMMA and below that of normal bone. The second is C_{22}H_{25}O_{10}I has a peak above bone and between dentine and enamel. These standards enable the calibration and standardisation of qBSE analysis of mineralised tissue samples taking care of any variations of SEM operations such as thermal instability by regular sampling of the standards during every analytical run (Boyde et al 1998a; Boyde et al 1995; Kingsmill and Boyde 1998b). Metals such as aluminium are inherently soft and at room temperatures form an oxide layer. Both these factors make it difficult task to achieve a perfectly flat stable surface that does not act as an insulator. Most important of all is that they are not crystalline and therefore do not show channelling contrast. The use of these halogen standards with qBSE is a principal method employed in this thesis to determine mineral density distribution and morphology in bone.

**Materials and Methods**

*Origin of the samples*

Specimens studied in this thesis are bone fragments and trephine alveolar core biopsies derived from patients who have attended for dental implant treatment at the author’s practice between 1996 and 1998. Ethics committee approval was obtained from the joint UCL/UCLH committee on the ethics of human research.

The material studied was surgical scrap - trephine bone cores - from a total of 35 patients, 19 male and 16 female (14 patients, 24 sinus grafts, from which 20 sinus
grafts were sampled for analysis. A total of 252 implants were placed of which 75 were into sinus graft regions. Ages ranged from 34 to 84 years. These are shown in Table 2-1. From a total of 235 samples embedded, 211 were mounted and analysed for qBSE and BSE for morphological analysis. The 24 samples lost to the study did not offer sufficient bone surface for observation or were unsuitable for examination by BSE-SEM. A total of 532 quantitative analytical images were recorded, with a further 422 BSE images recorded for detailed morphology. The data from each field imaged were then collated to the specimen SEM number and grouped according to Table 2-2.

**Sinus Surgery**

Where a sinus graft procedure was employed, the first stage for the followed that outlined by Tatum et al (1993) and Smiler et al (1992). Briefly, the procedure requires a surgical access window into the lateral wall of the maxillary sinus, through which the sinus membrane is carefully dissected from the bony floor and walls of the sinus, with eventual elevation of the complete membrane to create an intact “pseudo-sinus”. A prepared bone graft, is then placed into this pseudo-sinus which supports the elevated sinus membrane. Implants were placed approximately 6 months later, when a trephine drill was used initially to prepare the site (Figure 1-1).

### Table 2-1. Material included in Chapters 2 and 3 of the thesis.

<table>
<thead>
<tr>
<th>numbers</th>
<th>male</th>
<th>female</th>
<th>age range</th>
<th>No Implants</th>
<th>No Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=</td>
<td>19</td>
<td>16</td>
<td>32-84</td>
<td>252</td>
<td>35</td>
</tr>
<tr>
<td>bilateral sinus grafts</td>
<td>6</td>
<td>4</td>
<td>43-67</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>unilateral sinus graft</td>
<td>3</td>
<td>1</td>
<td>49-66</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>sinus graft implants</td>
<td>41</td>
<td>34</td>
<td></td>
<td>75</td>
<td>14</td>
</tr>
<tr>
<td>maxillary (not sinus)implants</td>
<td>62</td>
<td>24</td>
<td></td>
<td>86</td>
<td>24</td>
</tr>
<tr>
<td>mandibular implants</td>
<td>37</td>
<td>54</td>
<td></td>
<td>91</td>
<td>18</td>
</tr>
<tr>
<td>mean age years</td>
<td>57</td>
<td>54</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median age</td>
<td>53</td>
<td>55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age range</td>
<td>46-84</td>
<td>32-70</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 1-1. Clinical retrieval of bone cores.

Figure 1-la

The standard trephine drill used for sinus implants of 5 mm diameter.

Figure 1-lb

A bone core after removal from the drill approximately 12 mm long.

Figure 1-1c

A smaller trephine drill used for the 3.75 mm diameter implants. The bone as seen on the land was collected for analysis as bone shavings.

Figure 1-1d

The cutting teeth clogged with bone that was collected for examination as bone chips.

Figure 1-1e

An example of the loss of continuity that often occurred when trying to remove the bone core from the bone site.

Figure 1-1f

Bone core that was retrieved from the left anterior maxillary alveolus in the region of the lateral incisor.
**Graft Preparation**

The graft material IMCA was removed from its sterile packaging and placed into a surgical dish ready to be placed into the pseudo-sinus. The graft was packaged moist with saline from the supplier (Figure 2-1) or after for soaking in autogenous serum prior to placement. All grafts were mixed with approximately 250mg of clindamycin antibiotic powder. Sufficient graft was packed, but not compacted, to fill the pseudo-sinus to a height such as to accommodate at least 13-15mm long implants.

<table>
<thead>
<tr>
<th>Table 2-2. Anatomical sites of trephine core specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. native anterior maxilla (canine to canine)</td>
</tr>
<tr>
<td>2. native posterior maxilla (premolar region)</td>
</tr>
<tr>
<td>3. native anterior mandible (canine to canine)</td>
</tr>
<tr>
<td>4. native posterior mandible (premolars to molars)</td>
</tr>
<tr>
<td>5. sinus graft with saline</td>
</tr>
<tr>
<td>6. sub-sinus native alveolus</td>
</tr>
<tr>
<td>7. sinus wall</td>
</tr>
<tr>
<td>8. sinus graft with serum</td>
</tr>
<tr>
<td>9. bone shavings</td>
</tr>
<tr>
<td>10. graft only, posterior mandible</td>
</tr>
<tr>
<td>11. extraction sites</td>
</tr>
<tr>
<td>12. irradiated mineralised cancellous allograft (IMCA)</td>
</tr>
</tbody>
</table>

**Fixation and Embedding**

Trephine core specimens in surgery were fixed in 70% ethanol for fixation and stored for at least two days before further processing via three or more dehydration steps in absolute alcohol over a period of three days; followed by two changes of xylene each day for two days; then immersion in freshly distilled methacrylate monomer (MMA) for 24 hrs with two changes. Samples were then transferred into glass pots previously prepared with a base of set PMMA, and immersed in MMA with 1 gm per litre azo- iso-butyronitrile (AIBN) activator, and incubated at 40°C until polymerisation was complete. Once set, the glass pots were fractured to release the embedded sample.
The allograft when removed from its container is moist and of regular size with a springy resilience.

IMCA is supplied sterile and sealed in small plastic screw topped vials enclosed within a larger outer hard plastic screw topped container.

Off the clot serum. Autogenous serum was produced from peripheral blood taken from patients prior to surgery and transferred into microfuge vials to stand for at least one hour.

The vials were microfuged to separate the serum from the cells. The light yellow fluid as shown is the serum.
A band saw was used to section the cylindrical biopsy block to expose the bone core longitudinally. The final block was shaped such that the base was parallel to the exposed cut face of the sample to eliminate tilt of the sample surface and to reduce the need to refocus during microscopy over successive fields of view. This cut face of exposed sample was then trimmed and polished with successively finer grades of wet carborundum paper. After each grade of polish the embedded blocks were sonicated to remove any retained debris and particles from the previous stage. The final preparation of the samples was completed by hand polishing with successive grades of diamond strips and polishing paste down to 1 μm particle size.

**Mounting**

In SEM the incident electron beam can cause a build up of electrical potential on a non-conductive sample. This potential can discharge to produce artefacts of lines and bright regions in the of non-conducting specimens image called “charging”. To prevent this, the samples were coated with a conductive layer of evaporated carbon: this has the lowest atomic number of materials used for coating thus minimising any signal from the coating itself (Boyde and Jones 1996). Prior to carbon evaporation, the polished samples were mounted on 80 mm square aluminium alloy rafts using double sided adhesive carbon tape. They were painted around the periphery of the surface with carbon paint which was continued down the (corners) sides to join with the base carbon tape.

**SEM - qBSE**

Specimens were analysed using an automated digital scanning electron microscope (Zeiss DSM 962), controlled by an IBAS external computer (Kontron Electronik, Munich, Germany, and using an annular solid state BSE detector KE Electronics, Toft, UK; Boyde et al 1995). Standards were two monosubstituted halogenated dimethacrylates, C\textsubscript{22}H\textsubscript{25}O\textsubscript{10}Br “monobrom” and C\textsubscript{22}H\textsubscript{25}O\textsubscript{10}I “monoiiod”. The BSE signal levels from these materials encompassed the range of levels found in normal bone. Fields with both standards were imaged at the beginning and end of each run, and after every ten images during the run.
Samples were not of identical size, but fields were selected to cover the whole surface of each sample. Care was taken not to overlap adjacent fields and to be able to relate fields as a montage. The working distance was adjusted to focus at 17 mm for each field, viewing at the higher magnification of 2000x. Images of 512*512 pixels at a nominal magnification of 33x gave a field size of 2.70 x 2.70 mm square. Prior to each run the SEM was allowed to stabilise and filament saturation achieved. This was to minimise any change in filament current during the run. The accelerating voltage was 20 kV and the emission current 70 μA. The filament current at saturation was usually between 3.2 and 3.60 A. The SEM was then switched to automatic slow scanning mode to image the previously selected fields, covering all the samples mounted on each raft.

**Image Analysis**

The images were edited prior to image analysis to exclude artefacts and sample debris. The histograms of the edited images were stretched by linear interpolation to cover 256 grey levels in the range of density standards. Any instrumental drift was corrected by stretching each image with reference to the standards.

**Data Analysis**

The histogram bins were divided into 16 (for analysis) and 8 (for image display) equal bins, where zero represented black and 255 represents white, the highest order of mineralisation density. The zero level lies at the mean for the monobromo standard, whilst 255 lies at the mean for the monoiodo standard.

**BSE for Morphology**

When the analytical runs were completed, the specimens were then reviewed using BSE-SEM to record relevant morphological detail over a range of magnifications, usually with a denser image pixel array of 1024 * 1024 with a field width of 5.40 mm. These were recorded directly using the Zeiss DSM962 system without the external IBAS control computer.
Three Dimensional Light Microscopy (3D LM)

Selected embedded specimens were viewed prior to BSE-SEM using 3D microscopy to determine first the general tissue morphology of the sinus graft specimens under a fluorescence light source as the specimens were unstained. An EDGE® True-View 3D™ Head microscope (Edge Scientific Instrument Corp., Santa Monica, CA) has previously been used successfully to assess bone formation around retrieved implants (Boyde et al 1998b). This microscope was used in this application with a Plan Apochromat 20/0.75 objective which was chosen for its ability to reveal structures in 3 dimensions in thick samples over a broad field of view (Greenberg and Boyde 1997).

Transmitted Light Microscopy (LM)

Following BSE-SEM, selected specimens were repolished, and cleaned in an ultrasonic bath to remove the carbon coating prior to staining and LM. Each specimen was surface stained by covering the surface with a drop of toluidine blue in 50% alcohol, for 15 minutes after which the surface was washed with distilled water and dried. Ethidium bromide, a DNA stain, was then pipetted onto the previously stained surface and left for 5 minutes, after which the specimens were washed, dried and prepared for LM examination. Reflected and fluorescent mode photomicrographs were recorded on Ektachrome 400 ASA film.

Statistical Analysis of BSE data

The image histograms of 256 grey levels were re-scaled as detailed above. The total number of hits (pixels>0) in each image was recorded as the bone fraction of the total image area, with the ratio of pixels per bin to give the contribution of each bin as a percentage of the total bone volume. Thus the total amount of each phase of bone mineralisation density per image was recorded. The mean, median, and standard deviations were recorded for each histogram. Standard deviations (SD), were found to fall within a narrow range. The data were tested for normality using the Ryan –Joiner test for normality. The Student’s paired t-test was used to determine differences between saline and serum treatments in bilateral sinus grafts. The two sample t-test was then used to compare the columns
of pooled data from all sinuses grafted. Unless otherwise stated, the mean data and the standard error of the mean (SEMean) are presented in the graphs and tables. Statistical analysis was performed using Minitab statistical software (Minitab, State College, PA, USA).

Results

Bone volume of grafts in qBSE

Some fields of view for qBSE included variable sized regions with no bone, for example, when the field of view included the margins or periphery of the specimen. The percentage of bone volume in these fields would not be truly representative of bone volume within the specimen and so were removed from the data for bone volume analysis. A preliminary visual assessment of the bone core specimens prior to detailed qBSE and morphological analysis indicated a range of bone volume fractions within each core specimen which appeared to decrease with increasing height of the graft above the host bone bed. To test this view, the images were further classified into two sub-classes, h1 being all images that appeared above an arbitrary midway line located at no less than 5 mm above the sinus floor and divided the graft core region into near equal halves and h3 being all images that appeared below halfway and lay closest to the native alveolar bone bed. All short core images, i.e. less than 4 mm of graft, were classed as h3 images. The addition of another sub class of h2, to represent the middle region of the specimen would have been desirable, but unfortunately the variations of the lengths of bone cores did not allow for this degree of precision. This data were found to be non-parametric with large variations in means and medians. The percentages of total volume of bone within each field in sinus and mandibular grafts were analysed by Mann Whitney U test for non-parametric data.

Comparison of contralateral grafts from individuals.

Although the data from showed general shifts in mineral density distribution as shown in Figure 2-2, analysis by paired t-test within patients revealed only one subject to have an individual difference with a shift of the serum graft towards a lower mineralisation density (Table 2-3).
Table 2-3. Results of Paired t-test of mean mineralisation density distribution between left and right sinus grafts in bilateral cases.

<table>
<thead>
<tr>
<th>ID. No of patient</th>
<th>Mean</th>
<th>SE Mean</th>
<th>T</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.58</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>-0.02</td>
<td>0.23</td>
<td>-0.07</td>
<td>0.95</td>
</tr>
<tr>
<td>4</td>
<td>0.00</td>
<td>0.73</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>0.00</td>
<td>2.17</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>8</td>
<td>-6.25</td>
<td>1.61</td>
<td>-3.88</td>
<td>0.01</td>
</tr>
<tr>
<td>15</td>
<td>0.01</td>
<td>0.71</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>17</td>
<td>0.00</td>
<td>0.91</td>
<td>0.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Pooled sinus Data

The unilateral sinus graft data from 6 subjects, 3 with serum and 3 with saline treatment, were then pooled with the bilateral data, and analysed. The data is listed in Table 2-4. There was no overall significance between the saline and serum treated grafts in mineral density distribution at 6 months.

Table 2-4. Results of Two sample t-test for pooled sinus data between saline and serum treated grafts.

<table>
<thead>
<tr>
<th>Class</th>
<th>Number of patients</th>
<th>Mean grey level in Br-I range</th>
<th>SE Mean</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline graft (5)</td>
<td>10</td>
<td>155.13</td>
<td>1.7</td>
<td>ns</td>
</tr>
<tr>
<td>Serum graft (8)</td>
<td>10</td>
<td>151.06</td>
<td>1.9</td>
<td>ns</td>
</tr>
</tbody>
</table>

Possible sex differences

The number of samples was too small to enable a strict comparison based upon gender (Figure 2-3A). There was no significant difference between males and females in sinus graft bone mineral density distributions, although a trend to a greater amount of bone in bins 10 and 12 is shown in the male group.
Sinus graft compared to mandibular graft (IMCA) and unused IMCA

Again, the numbers of samples were small, but the pooled data of saline and serum grafts were tested for differences to that of the same graft material placed in the posterior mandible. The mandibular graft had been placed into extraction sockets and bone defects and delayed implants placed at approximately 4 months. Results in Table 2-5 show a shift between the bone mineralisation distribution in sinus grafts and posterior mandibular grafts at p<0.05. However the difference between the unused IMCA and the grafts is highly significant at p<0.001. Figures 2-3B, shows the relative bin distributions between the various grafts and to (unused) IMCA.

Table 2-5. Results of 2-sample t-test for mineralisation density distribution between sinus and mandibular grafts and unused IMCA (p values are in bold).

<table>
<thead>
<tr>
<th>Anatomical site</th>
<th>Number of patients</th>
<th>Mean grey level in Br-I range</th>
<th>SE</th>
<th>Pooled Sinus grafts</th>
<th>Mandibular grafts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled sinus grafts</td>
<td>14</td>
<td>152.90</td>
<td>1.31</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Mandibular grafts</td>
<td>2</td>
<td>162.96</td>
<td>3.48</td>
<td><strong>0.024</strong></td>
<td>*</td>
</tr>
<tr>
<td>Unused IMCA</td>
<td>2</td>
<td>121.21</td>
<td>4.2</td>
<td><strong>0.0003</strong></td>
<td><strong>0.0000</strong></td>
</tr>
</tbody>
</table>

Bone volume results

The results as listed in Table 2-6, revealed no significant differences between similar sub-classes for saline and serum treated grafts, but the two subclasses h1 and h3 were highly significantly different at (p< 0.0001) in percentage bone volumes. The mean bone volume occupied by the graft and new bone in the h3 group, at 30.88%, was greater than 23.51% for the h1 group. There was a wide range of values from 47.18% to 16.06% for the h3 group and 36.29% to 7.27% for h1 group, but, in general, decreased new bone formation appeared to occur with increased height above the host sinus bone bed of the graft.
Figure 2-2. Graphs of percentage mineralisation density distributions in bilateral sinus graft patients.
Figure 2-2. Graphs of percentage mineralisation density distributions in bilateral sinus graft patients.

Figure 2-3. Graphs of mineralisation density distribution by sex (A) and site (B).
A.  
B.
<table>
<thead>
<tr>
<th>Location by site</th>
<th>No of patients</th>
<th>Mean</th>
<th>SE</th>
<th>Median</th>
<th>h1 sinus</th>
<th>h3 sinus</th>
</tr>
</thead>
<tbody>
<tr>
<td>h1 sinus bone</td>
<td>12</td>
<td>23.5</td>
<td>1.0</td>
<td>24.5</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>h3 sinus bone</td>
<td>12</td>
<td>30.9</td>
<td>1.1</td>
<td>29.6</td>
<td>0.0001</td>
<td>*</td>
</tr>
<tr>
<td>Mandibular graft bone</td>
<td>2</td>
<td>38.7</td>
<td>5.0</td>
<td>39.4</td>
<td>0.0013</td>
<td>0.0475</td>
</tr>
</tbody>
</table>

Table 2-6. Results of Mann Whitney U test and Percentage bone volume data for all graft patients. Mann Whitney p values in bold.

When the pooled sinus graft data were tested against the native mandibular graft, the mandibular graft had significantly higher \((p<0.05)\) percentage bone volume, with a mean at 38.7\%, than the h3 group at 30.9\%. But when tested against the h1 group the differences became highly significant \((p<0.0001)\), with the h1 group mean of 23.5\%.

**Morphology of unused irradiated mineralised cancellous allograft (IMCA: Figures 2-4 to 2-5) and bone formation in IMCA grafts (Figures 2-6 to 2-19)**

The features of IMCA were consistent and plainly obvious from one field to another and from one patient to another. The processing procedures did not appear to have changed the character of the cancellous trabecular nature of the material. Plates, rods and fine longitudinal structures were the common scene in all graft images. Many plate like particles exhibited half moon bays with two extended rod-like extensions leading out from the bays (Figure 2-4). Others were angled structures with thicker dimensions at the roots of the angles with legs leading out to fine tapered processes, often terminating with formed rounded ends rather than the jagged appearance of a fractured one.

Rod like structures were measured up to 1.5 mm in length. Isolated particles were sometimes dumb-bell in shape with larger rounded ends with robust short central joining struts. Cross sectional views showed the structures to be round but more often ovoid, ranging from 70 μm to over 350 μm in diameter. Larger rounded
structures cross sectioned by the plane of preparation were more triangular, but invariably all such sections appeared with a clean periphery, sometimes interrupted by small rounded notches and sharper tangential sections that broke the continuity. However, it was rare to find a fully intact cancellous plate with round or oval openings. An irregular finding was a knob like section occurring at the junction or end of a rod. These appear bulbous with a number of darker areas indicating the bone to be of low mineralisation density and recent remodelling events (Figure 2-4 and 2-5).

**Fragmentation and micro-cracks**

Fractures of the material, from a few microns to over 300 microns in length when not directly in a traumatic cross sectional cleavage direction, appeared to occur preferentially along old cement lines indicating that the interface between new and old bone was mechanically weaker than the original graft bone. Many of these longitudinal cracks had margins of very much lighter grey scale, including the cementing interface material. However this appearance can also be an edge artefact owing to extra BSE escaping from the crack. Micro-cracks that occurred in a transverse direction across the trabeculae were mainly small and limited to under 50 microns (Figure 2-6). The spatial positioning of the graft constituents was also consistent with very few bone structures being greater than about 500\( \mu \)m distant from one another, with no compacting of graft being evident in any sections. Each fragment of graft appeared close to, but not compressed against, adjacent ones.

**Cement lines**

BSE-SEM shows very clearly the regularity of the cement lines of this material. They vary in mineral density and always align in the axial directions of each rod or strut, often overlapping and intersecting each other. In the half moon bays, these lines followed the contours of the half circles delineating clearly the underlying lamellar bone formation of the original cancellous trabecular plate and rod structures. Rods in cross section exhibited cement lines as continuous circumferential lines in successively larger radii, each one representing the layer of lamellar formed in close succession to the next. Cement lines are seen in Figure 2-7 and 2-8.
Cellularity

BSE-SEM revealed all graft material to exhibit normal trabecular bone structure with osteocyte lacunae lying along cement lines. These were very few in number and tended to be scattered with each osteocyte sitting some distance from the next. The material was low in cellularity.

Light Microscopy

Light microscopy of stained specimens revealed islands of bone within the marrow stroma infiltrated by many blood vessels. Regions could be observed where the stained osteocytes were aligned over sections of bone without stained osteocytes. These represented new bone tissue formed over an IMCA trabecular surface whose osteocyte lacunae did not take up stain (Figure 2-9). IMCA was easily identified by the empty osteocyte lacunae which appeared almost translucent without any cellular staining. In vital bone, osteocytes with nuclei were observed to be plentiful with their associated canaliculi interconnecting other osteocytes in all directions. Osteoblasts were observed lining the bone surfaces. An abundance of adipocytes was regularly observed in bordering the bone margins of both vital and non-vital graft bone.

3D Light Microscopy

The images that the Edge 3D head produced allowed for an increased depth of field and visualisation of the connective tissue stroma with a wide field of view. Stereo pair images showed graft bone invested in fully differentiated stromal connective tissues, with many blood vessels. It was not possible to distinguish between IMCA and vital new bone, nor the cellular nature of the bone. There appeared to be many islands of bone with few interconnections. The discontinuities contrasted sharply with the large volume and number of interconnections seen in the samples derived from the anterior native maxillary alveolus. The stromal structures were well defined and organised with webs of thickened reticular connective tissue filling all spaces between islands of bone (Figure 2-10).
**Other Features from sinus specimens**

Within the sinus graft groups, three patients were included for revisions following failures using DFDB and thermo-ashed bone mineral granules (45-500μm: Pacific Coast Tissue Bank, LA). Specimens from two of these patients revealed the remnants of hydroxyapatite (HA) granules embedded within the host bone. The surrounding bone matrix of Haversian systems and interstitial lamellae indicated that active bone remodelling was occurring around these fragments. The granules reveal penetration by blood vessels with up to three Haversian systems seen within the body of the HA. At high magnifications small fragments of HA appear as to have been disseminated at the margins of the HA and blood vessels, with no signs of osteoclastic resorption. The interface between granules and the bone forming front appears as a distinct appositional process of bone formation with the cement line being difficult to distinguish due to its similar high mineral content. The interface between the bone forming within the granules is seen to be both similar to the outer interface on one aspect whilst on the opposing aspect the interface is granular with speckles of HA being dispersed into the forming bone substance. The HA granules showed numerous small cracks possibly due to the embedding process when the brittle nature of the material is less likely to flex under stress. In one specimen an HA granule has been lost leaving its footprint in the specimen. The clinical history revealed that these fragments had been in situ for over 2 years. These features are seen in Figures 2-11 and 2-12.

**Mineralised osteocyte lacunae in sub-sinus maxillary alveolus**

Within two specimens, qBSE revealed fields where unusually high mineralisation was visible in host alveolar bone beneath sinus grafts. These areas were located in the crestal cortical bone beneath the alveolar crest. The field viewed was part of a larger region of similarly highly mineralised cortical crestal bone. White patches showed distinctly with smaller white spots that represent mineralised osteocyte within their lacunae. These white patches represent interstitial lamellae formed for many years that became starved of nutrient sources leading to cell death and mineralisation. In the middle of this region, bone of more recent formation and Haversian systems appear to be slowly remodelling and removing
some elements of the mineralised lacunae. This is shown in both grey and pseudo-colour images in Figure 2-13.
Figure 2-4. IMCA in unused state

Figure 2-4a. 20kV BSE-SEM. 33x Field width 2.7 mm. Micrograph of irradiated mineralised cancellous allograft embedded straight from the container. The trabecular gross morphology is depicted very well by the intact plate with four trabecular “rods” and a central oval opening. The other trabecular particles show different states of fragmentation with angled sections, semi-lunar shaped sections previously formed as plates, and isolated small fragments of irregular sizes.

Figure 2-4b. 20kV BSE-SEM. 100x. Field width 900μm. Higher magnification field from Figure 2-4a above. Note the semi-lunar shaped bays leading out to the rod formations with a distinct darker shade of grey indicating a lower mineralised lamellar bone than the more central portions. There is a noticeable paucity of osteocyte lacunae. At the root of the upper trabecular rod, the plane of cross section has exposed areas of different formative stages in the history of this structure, each having its preferential direction of collagen formation. To the left of this area are two localised deep Howships lacunae with characteristic notch like internal surfaces which records the osteclast resorption phase of remodelling.
Figure 2-5. IMCA II in unused state

Figure 2-5a. 20kV BSE-SEM. 100x. Field width 900μm. Another view of the inherent vertebral trabecular morphology. The plane of section of this structure indicates that this trabecula may have performed a completely different structural function to that seen in Figure 2-4b. It has an almost straight linear section on the left margin of the image without an intersecting rod or strut whilst on the right there is an extension leading out to a terminated rod or plate. Note the many cement lines.

Figure 2-5b. 20kV BSE-SEM. 100x. Field width 900μm. This structure differs from the other images with its high cellularity with many osteocytes lying along the planes of lamellar, and the presence of a haversian canal in the upper right of the image. The darker low central region is a section across a recent remodelling event containing much larger osteocyte lacunae that are also poorly mineralised.
Figure 2-6. BSE-SEM micrograph of microcracks and woven bone in sinus graft

Figure 2-6a. 20kV BSE-SEM 44x. Sinus IMCA from a 64 year old male. The general picture shows new bone formed on most exposed surfaces of IMCA, with almost complete coverage of some trabeculae. The growth of woven bone appears exhuberant between trabecular elements, but less in thickness over the extensive flat surfaces of individual trabeculae. Most concavities and cracks between adjacent fragments have become bone forming sites.

Figure 2-6b. 20kV BSE-SEM 56x. Part of the above field showing cracks within trabeculae filled with new bone. The same has occurred between adjacent elements in close proximity seen in the upper and lower left of the field where apparently ‘V’ shaped clefts are filled with wedges of new bone.
Figure 2-7. BSE-SEM micrographs of sinus grafted IMCA

Figure 2-7a 20kV BSE-SEM 58x. IMCA graft from a 49 year old male where there was new bone formation (11 level), appearing darker and more cellular attached onto some of the surfaces of the graft. A rod shaped trabeculum located towards the middle, lower right, has been sectioned transversely to expose the successive “growth rings” of lamella bone. The internal structure is distinctively delineated into two zones by a reversal line of higher mineral density than the body of the rod. The outer region is made up of multiple lamella. The small dark spot is dirt. The long trabecule on the right side of the image is 1500μm long using the scale bar at the bottom.

Figure 2-7b. 20kV BSE-SEM 100x. A higher magnification image of sinus graft and new highly cellular bone in female patient of 44 years. Both graft trabeculae illustrate the axial alignment of the coincident lines of lamella bone formation with small transverse microcracks. The lamellae are able to be viewed due to the polishing relief that occurs in sample preparation.
Cleavage as a result of the trauma of retrieval and processing. It appears that the bonding between the woven bone and the cementing layer was stronger than that between the cement and the graft bone surface. Note how small the contact surface is on the right of the field between the column of woven bone and the graft trabeculae.

An unusual isolated section of cementing layer in the middle of the field that appears to have maintained its integrity but has split off from both bone surfaces.
Figure 2-9a. Light micrograph of new woven bone from a sinus graft, stained with toluidine blue (and ethidium). The whole field shows high cellularity (osteocytes) in keeping with the disorganised structure of more immature (woven) bone. The shadowy cells are those osteocytes that lie below the surface, out of focus, and beyond contact reach of the surface staining procedure. Note osteoblasts lining the bone surface in the upper left corner.

Figure 2-9b. Abrupt end to the osteocyte layers lying over a region devoid of cells, which is almost certainly graft material.
FIGURE 2-10. Three Dimensional Light micrographs. Images are one of a stereo pair, other not shown.

Figure 2-10a. Flourescence light micrograph of trephine core prior BSE-SEM imaging. The trabeculae appear as lighter islands of bone structures embedded in the darker background of blood vessels and other marrow elements. Edge 3D head, objective 40X, NA = 0.95

Figure 2-10b. Flourescent Light at a higher magnification and different light filters. The stromal nature of the connective tissues are very nicely revealed in this image.
Figure 2-11. Incorporated Hydroxyapatite I.

Figure 2-11a. 20kV BSE-SEM 24x.
Hydroxyapatite granules are shown with many totally incorporated into the host bone bed. The larger irregular dark holes in the bone are regions previously filled with HA that has been lost during processing of the specimen.

Figure 2-11b. 20kV BSE-SEM micrograph 100x.
Part of the above field showing a Haversian system close to the HA, and lamellar bone formation on the surfaces of the HA. Note the ingrowth of bone at the left aspect of the HA which is shown at a higher magnification in the next figure 2-12.
Figure 2-12. BSE-SEM micrograph of HA-bone interface

Figure 2-12a. 20kV BSE-SEM 1000x.
A higher magnification of Figure 2-11 that reveals the variable appearance of the HA-bone interface. The right and top part of the HA surface is of an irregular speckled nature, whilst the HA-bone interface on the left is without irregularities. Three osteocyte lacunae with canaliculae are seen in the centre of the bone formed within the HA.

Figure 2-12b. 20kV BSE-SEM 2000x. A very high magnification of the HA-bone interface. The granular nature of the HA and its porosities are very evident. The centre of the field shows penetration of the HA surface by fine capillaries and canaliculi of the osteocyte. There does not appear to be any resorption bays along the interface.
Figure 2-13. BSE-SEM micrograph of hypermineralisation.

Figure 2-13a. 20kV BSE-SEM micrographs of mineralised osteocytes. This grey scale image of a sub-sinus bone specimen was an unexpected finding. The small white spots represent the mineralised osteocyte lacunae which are in turn embedded in regions of more highly mineralised bone showing as lighter patches than the surrounding cellular bone of darker greys containing osteons.

Figure 2-13b. Pseudo-colour coded image of the above. Note the highly mineralised regions especially the cement lines well displayed in red.
**Discussion**

*Bone Volumes*

Bone volume fractions were found to decrease with increased height above the sinus floor and that mandibular graft volumes were greater than of the sinus grafts (Figures 2-14 and 2-15). This result was not surprising as it confirms the anatomical differences of each site. The sinus graft site is essentially a 5 walled osseous defect, of large dimensions, but is not well contained and poorly protected at its superior surface by the sinus membrane. This membrane is a fragile, thin, poorly vascularised tissue (see chapter 3) whereas the mandibular graft is held inside a much smaller 5 walled osseous defect by an overlying mucoperiosteum that is a resilient protective tissue and highly vascularised. These results confirm the effectiveness of IMCA in permitting or encouraging new bone to form on its surfaces, especially as the maturation time of the mandibular graft was less than that of the sinus grafts.

*Irradiated mineralised cancellous allograft donor history*

IMCA is procured from the spinal column from selected cadavers, which determines the homogenous nature of this allograft. Cancellous vertebral trabeculae has been described (Galante et al 1970; Whitehouse et al 1971; Jayasinghe et al 1993) as a complex three dimensional network of plates and rods, or semilunar units with the trabeculae interconnected as a honeycomb pattern to maximise its mechanical properties. This region has attracted intense interest from investigators in all fields because of osteoporosis, a progressive debilitating condition where the affected bones especially vertebrae, show diminishing bone mass and strength by a thinning of the inner and outer cortices.

These morphological and structural changes in cancellous bone have been related to age and hormonal imbalances by its dual function in skeletal support and as a biochemical reservoir in Ca homeostasis (Raisz and Shoukri 1993). Osteoporosis can occur in multiple skeletal sites with bone fractures as an end point. The endosteal envelope of cancellous bone which is greatest in the vertebra is only 20% of the total skeleton whilst the other 80% is formed of cortical bone.
Upper section of core at height $h_1$ above the sinus floor. Note the poor bone structure and its paucity.

Mid-section of core

New bone formed at sub class $h_3$, height with new woven bone sprouting off the surfaces of allograft trabeculae.

Original sinus floor approximately 3 mm in thickness.

Figure 2-14. 20kV BSE-SEM, montage field width 2.7mm. The image was formed by merging consecutive images of the core specimen to demonstrate the use of a colour coded image using a predefined, “Look-up Table”, (LUT), shown on the left of the montage, with the 256 levels being divided into 8 bins where 0 = black or no bone, 1-32 dark blue, 33-64 blue, 65-96 green, 97-128 yellow, 129-160 orange, 161-192 red, 193-224 pink, and 225 and above as grey. New bone of low density appears as blue/green or yellow. Red indicates higher mineralisation density bone.
Figure 2-15. BSE-SEM micrograph of mandibular IMCA graft

Figure 2-15a. 20kV BSE-SEM. Field width 2.7mm. IMCA mandibular graft from the posterior mandible at approximately 4 months. Note how fairly homogeneous the distribution of the graft particles have occurred, which is much closer and uniform that that seen in the sinus graft site. There is complete incorporation of all graft surfaces with interconnections by new woven bone formation.

Figure 2-15b. 20kV BSE-SEM 100x. A higher magnification of the top right portion of 15a. This view shows clearly the highly cellular nature of woven bone at the forming front on the surfaces of the IMCA at the top left of the field. Centre field, two primary osteonal systems can be seen. Top right is an unusual appearance of a smaller graft particle being fragmented with smaller pieces dispersed into the newly formed adjacent bone.
The cancellous portion is considered to exhibit a higher metabolic activity (Parfitt 1983) which is evident in the characteristic multitude of cement lines seen in vertebral trabeculae. Both sexes are affected, but especially post menopausal females (Eriksen et al 1985; Vesterby 1990), in whom oestrogen levels are decreased with a resultant increased bone turnover but reduced bone deposition.

Vertebral fractures occur as a crush phenomenon as a result of the thinning of trabeculae (Figure 2-16) and in severe cases complete perforation and disconnection of trabecular formations (Jayasinghe et al 1993). It is self evident that the donors for IMCA will come from a variety of situations, but it is also expected that the majority will come from the older age group in whom age changes will be inherent in the derived IMCA. An evaluation of another commercially available allograft, demineralised freeze dried bone (DFDB: Schwartz et al 1996), exposed the variable quality of DFDB procured from six different Tissue Banks. They assessed its ability to form new bone on its surfaces and its assimilation into host bone, and found its response to be highly variable and dependent on the sources of supply. Some bone banks (for example, LIFENET, USA), in recognition of the age changes in bone composition, have modified and applied age exclusion criteria to improve on the quality of their products. A parallel situation may be present in the use of this material especially when the anatomical site of retrieval is known to be highly correlated with detrimental structural and hormonal changes with increasing age. However, on balance, donor age may not be an important factor in the use of IMCA as it is in the use of DFDB, which relies on the assumption of BMPs being present in the allograft, when the morphological features of this material are considered.

**Bone matrix and tissue engineering**

The intricacies of biological extracellular matrix and its role in providing optimal surface energy, topography and architecture for chemotaxis, and adsorption of attachment molecules for cells has been under investigation for many years (Slavkin 1972) and is now considered of primary importance in tissue engineering (Gomi and Davies 1993; Jones et al 1986; Ross 1998). The preferential response of bone cells to certain types of topography *in vitro* and *in vivo* has been
Figure 2-16. BSE-SEM Micrograph of trabeculae that appear osteoporotic

Figure 2-16a. 20kV BSE-SEM 100x. Sinus IMCA from a 56 year old female. A single donor trabeculum exhibiting advanced osteoporotic features. On the right rod element a large and deep osteoclastic resorption bay is evident on its top surface which has been infilled with new bone from the host. In the centre top of the image there is a marked extended scalloped area of resorption, between the trabecular body surface and the patients own new bone which fills the defect At far right is a nearly complete perforation which is partly filled with new bone.

Figure 2-16b. 20kV BSE-SEM 110x. Graft and new bone in a female patient of 44 years. The centre field trabeculum exhibits two deep resorption bays on opposing surfaces, in close proximity.
demonstrated by many investigators. Gray et al (1996), using rat cells in vitro on specified substrate topography, revealed preferential bone formation in deep grooves and cracks. They confirmed the quality of the substance formed to be true bone by immunologically locating connexin-43 gap junctions in osteocytes within a mineralised matrix. Gotfredsen et al (1995) found that TiO2 blasted implants, had significantly higher removal torques in rabbit tibia after 3 weeks than normal machined implants. Histology confirmed a greater bone mass and contact on the TiO2 blasted surfaces than on machined surfaces. In contrast to DFDB and other allografts which are mechanically fragmented into particles of variable sizes and shapes, from 75μm to in excess of 1000μm, IMCA retains its original trabecular morphology even though it is fragmented as seen in Figures, 2-4, and 2-5. This three dimensional generic honeycomb structure (even fragmented) ensures that spaces remain between fragments when compacted together as a graft. Percentage bone volume statistics from qBSE show that, within the sinus, grafted IMCA in the h1 sub class (very little new bone) had a mean percentage bone volume of 23.5%. This percentage bone volume is similar to that found by Schenk (1994), 20–30%, to be optimal in porosity to promote osteogenesis.

A common occurrence observed in IMCA was the formation of micro-cracks within the trabecular structure and on its surfaces. These were noted to vary from a few microns to over 300 microns in length, and progress in an axial direction along cement lines. Micro-cracks in cancellous trabeculae have been investigated and found to be occur in human vertebral cancellous bone under normal physiologic loading conditions (Wenzel et al 1994). Projections have been made as to the value of micro-cracks in vivo, as structural safety factors that act as signals for normal remodelling and repair in response to mechanical fatigue (Choi and Goldstein 1992). In this study the existence of such cracks may be a beneficial processing artefact, as distinct from those caused by the subsequent laboratory embedding process which occur across new bone formations. Thus micro-cracks that ended with the outer surface of the trabeculae were often observed to be in-filled with new bone when adjacent surfaces were not. Most of the micro-cracks that occur in a transverse direction across the trabeculae were small and limited to under 50 microns. These micro-cracks were within the
body of the trabeculae and not made available for new bone to in-fill. Studies on substrate topography in osteogenesis provide evidence that it is highly likely that the topographic nature of the micro-cracks were responsible for their in-fill of bone as a preferential site rather than the flat surfaces (Gray et al 1996). This may have the effect of accelerating subsequent remodelling and replacement of IMCA by the host tissue.

The main constituent of cancellous bone matrix is collagen type I, which is a suitable substrate for the adsorption of many cell types and plasma proteins. The first response of biological tissues to injury is the formation of a blood clot by the protective actions of thrombin and fibrinogen. This is also true in grafting where the blood product of cross-linked fibrin pervades and binds grafted tissue as a globular mass and also acts as an attachment of the mass to the supporting substrate of the bone bed (Mosesson 1992). The fibrin strands, by their interconnection within and without the graft mass, provide pathways for perivascular cells to migrate into the graft. Thus IMCA will have an inherent advantage in its collagen type I makeup to provide favourable surface properties for the attachment of fibrin as a provisional matrix for cellular migration when compared to non-collagenous materials. This then provides for the delivery of pluripotential mesenchymal cell populations to the graft to differentiate and form bone cells.

Cell adhesion to an extracellular matrix (ECM) is essential for cellular differentiation, growth and development of tissues. Cells attach to ECM by forming focal adhesion complexes with ECM proteins or ligands (Pløpper 1993). Fibronectin is one of these which contains the first known integrin binding sites consisting of the Arg-Gly-Asp (RGD) sequence in its alpha five beta one (α5β1) and alpha four beta one (α4β1) fibronectin receptors (Brown and Juliano 1985; Guan and Hynes 1990). The role of ligands and integrins appears to allow a two way control of cytoskeletal behaviour via the integrin's cytoplasmic element directly connecting to actin cytoskeleton stress fibres (Dalton et al 1995). One of the known cellular response actions when either fibroblast, or endothelial cells are bound by fibronectin to ECM is an elevation of cytoplasmic pH, which occurs with cell spreading and growth. Fibronectin and other essential ligands that have
preferential attachments for the collagen type I substrate of IMCA will facilitate attachment of the earliest arrivals of endothelial and bone progenitor cells to differentiate and develop new osseous tissue ((Weiss and Reddi 1981; Winnard et al 1995). Bone matrix is known to be a reservoir of growth factors produced by bone cells, including BMPs, Insulin-like growth factors, TGF-β, acidic and basic fibroblast growth factors, and PDGF (for a full review see Burwell 1994). Once osteoid is laid down the differentiated osteoblast cells, the cementing interface that mineralises as a cement line is non-collagenous, but becomes bonded to the new bone by fusion to the collagen (Davies 1996; Zhou et al 1994). The interfacial strength of this cementing interface appears to be dependent on both material and topographical characteristics of the substrate (de Bruijn et al 1995). Both the physical and chemical entities of IMCA have properties that are known to promote osteogenesis.

Graded Regions of bone formation
Detailed images of the sinus cores indicate new bone formation occurred in all IMCA grafts in all patients treated, but not in a homogenous manner as previously described. Whether this consistency is owing to the surface characteristics noted above or to the substance of the graft is not known, but it is most likely to be a positive synergistic action of both factors for osteogenesis. The only regions with no new bone formation on allograft surfaces occurred at the top of the sinus graft at the greatest distance above the sinus floor. This observation was confirmed statistically by the preceding quantitative analysis of bone volumes, but the significance must be balanced against the fact that the maturation time selected for this study was approximately 6 months from the time of grafting, which is only 2 months longer than that required for autogenous bone grafts (Boyne et al 1985; Raghoebbar et al 1997) before they are exposed for placement with implants. In comparison Becker et al (1996) found that demineralised and mineralised freeze dried bone allografts remained as non-vital particles within fibrous connective tissue after 13 months in extraction sockets and implant sites. Smiler et al (1992) found that at 6 months sinuses grafted with demineralised freeze dried cortical bone had an insufficient volume of new bone to accommodate dental implants.
One limitation of the present study of core biopsies is that the specimens are derived solely from the central internal structure of the alveolus, the superior aspect being often in excess of 15 mm above the sinus bone bed, and more than 5 mm from its closest bone bed, the lateral sinus wall. This did not allow for assessment of the possible role of the lateral bony walls of the sinus to graft development in this study. BSE-SEM images of lateral sinus wall specimens (e.g. Chapter 3) show full marrow cavities with active endosteal lamella bone formation.

Together with the rich vascular blood supply (Solar et al 1998) it is expected that the sinus lateral wall would contribute to the development of new bone in the graft. The observations of bone formation at the superior aspect (hl level) in this study shows no evidence of this, possibly due to the physical constraints of distance and short maturation period. In those specimens where new bone formed at this level, it was not possible to determine the radial direction of bone formation, i.e. whether from the mesial, distal, medial, or lateral aspects. However, new bone was observed in specimens taken from the most anterior aspect of the sinus region, where the superior aspect of the core specimens were in close proximity and often included native bone that forms as an outcrop of the premaxillary region of the canine eminence and which forms part of the anterior wall of the sinus.

Jensen and Sennerby (1998) have recently reported on retrieved micro-implants of 2 mm diameter by 6 mm length from the lateral walls of sinuses grafted with IMCA and autogenous bone to determine the percentage of bone to implant contact areas. The positioning of these micro-implants equates well with the h1 sub-class specified in this study (approximately 15 mm above the ridge) as do their results. At 6 months they observed 50 to 75% of bone to be non-viable in loose connective tissue with very sparse bone formation on a few allograft requires a conduction medium to direct its growth into the graft. In contrast, within the same maturation time, their autogenous graft micro-implants exhibited viable trabecular lamellar bone with resorption and active bone formation on graft surfaces, this latter description is similar to that observed in the present study in the h3 sub-class, that is in the lower half of the core specimens. All the sinuses
grafted in the Jensen and Sennerby (1998) study had a residual ridge of 4 - 6 mm, compared to 20 out of 24 grafts included in this thesis falling in the 1 to 3 mm range. New bone formation in the h3 level indicates similar rates and volumes of new bone formed to that of autogenous graft directly above the sinus floor, but deriving from a reduced alveolar bone bed. Both graft sites are similar in height from the host bone bed, with IMCA producing results similar to an autogenous graft placed on an optimal host site from a compromised bone bed. However, there is no evidence of differences in the osteogenetic potential of a reduced height of sub-sinus ridge at 1 to 3 mm to that of a more robust one at 4 to 6 mm. Jensen and Sennerby (1998) retrieved one micro-implant from one IMCA grafted patient at 14 months. More new bone was observed, with lamellar bone growing on surfaces of graft particles, some of which showed sites of resorption. The 11 to 12 month autogenous specimens showed more trabecular bone with total incorporation of graft to host bone.

The analysis of the limited number of bone cores examined in this study shows that this surgical scrap material may be usefully investigated. At least, we may conclude that the site response to wounding can be assessed at the 6 months allowed before re-operation for implant placement.

Unprocessed bone cores taken at 8 and 9 months maturation time have been found to be consistently more robust at the superior aspect on retrieval, but time did not allow for the laboratory analysis to be included in this thesis. Irradiated mineralised cancellous allograft does appear to be conducive to both woven and lamellar new bone formation on its surface. At h3 levels trabeculae were regularly observed to be completely encapsulated within a matrix of new host bone.

Osteoinduction and its meaning for irradiated mineralised cancellous allograft
The quality of bone grafts in implant dentistry has been evaluated by the ability of a graft material to either induce or conduct new bone formation. Osteoinduction was defined by Urist et al (1967), who classed inductive materials as those that would induce bone formation in ectopic sites, for example, in intramuscular pouches. This phenomenon has now been directly related to the bone morphogenetic proteins. Urist demonstrated that lyophilised cortical bone,
demineralised in hydrochloric acid, when placed in muscle pouches, can become
stimulate woven bone formation eventually this being remodelled with lamellar
bone. The new bone was observed to form via a cartilaginous phase. It is not
known whether IMCA used in this study is osteo-inductive in the precise nature
of Urist's definition, but his previous assessments of irradiated bone were that the
process severely reduces its bone inductive potential (Buring and Urist 1967;
Urist et al 1975), but Wientroub and Reddi (1988) found evidence contrary to this
opinion. Irradiation may not affect the allograft BMPs as they are not accessible
unless exposed by demineralisation. However current evidence specifies a broad
spectrum of requirements for osteogenesis (Bruder et al 1994) of which an
inductive capacity of a matrix may be a minor component.

Mechanical implications
Mechanical characteristics of graft materials are important considerations
throughout the treatment phase and life of a functioning dental implant. The ideal
material should possess properties that are similar to the host tissues. IMCA is
produced from vertebrae of cadavers and processed for use without a loss of
mineral. In this respect it is expected to retain much of its mechanical properties
whilst demineralised allografts can not. Choi et al (1990) studied trabecular
micro-specimens in mechanical and structural terms and found that the modulus
increased with increasing mineral content. Reimer (1994) investigating age
differences in trabecular architecture and mechanical modulus with a four point
bending model, samples from his 75-85 age group, were significantly stiffer than
those from a 55-65 year group. IMCA from older individuals may be more highly
mineralised than from donors of a younger age. The qBSE mean mineralisation
density for unused IMCA was 121.21 (bin 8.4) which is lower than the mean of
that of extraction sites at 143.31 (bin 9.4) of predominant woven bone formation
(Chapter 3). The mechanical properties (particularly the modulus of elasticity) of
IMCA (qBSE mean = 152) would be similar to that of the adjacent bone tissues
and be valuable in stabilising the implant. Demineralised particles, however, are
noted to be slow in their rate of replacement by host bone and, if loaded within a
similar matrix of new bone, will not offer the same mechanical advantages as the
mineralised material and possibly buckle under mechanical loading. Because it is
mineralised, IMCA will be resorbed by osteoclasts and eventually replaced by new bone.

The maturation process and normal bone physiology within a bone graft is dependent on a vascular supply that can provide the necessary nutrients for bone formation. One critical requirement is ionic calcium for both physiological and mineralising functions. The use of DFDB from cortical bone is based on the assumption that BMPs, TGF-β and other growth factors are exposed and made available by the demineralising process to induce bone formation. This material is entirely devoid of calcium which may be a factor responsible for its poor performance when used alone. Thus from a biochemical and mechanical standpoint, the rate of replacement of IMCA by host bone may not be a critical factor for its success in an implant bed, as it is for DFDB, and may offer some explanations for its observed success as a grafting material in this and other studies (Tatum et al 1993; Chanavaz 1996). Another important mechanical perspective is that of structural orientation. Although bone is considered to be anisotropic, the sinus graft is an ectopic bone mass which is initially isotropic. No preferential orientation or direction of bone formation was observed. Woven bone was observed to bridge graft and native bone islands in all directions—vertical, horizontal and oblique and not always by the most direct route. Some fields showed complete connectivity across the whole field of view. This was especially true in the mandibular grafts, where many graft trabeculae were interconnected by substantial woven bone bridges. Connectivity and bone volume fraction are primary indices in the assessment of mechanical competence of trabecular bone (Compston 1994). A similar importance would be assumed for this material when it ultimately becomes the supporting implant bed. The cross-sectional design of this study did not allow for a progressive assessment to be made in the IMCA, but the appearance of Haversian systems and lamellar bone indicate that further maturation would follow normal remodelling with function and time to form an increasingly anisotropic structure.

**Histogenesis in IMCA**

Histogenesis is the study of the origin and differentiation of tissues. Intramembranous bone (IM) forms directly within a condensation of
mesenchymal tissue membrane bones and includes the jaws. Endochondral bones (EC) form via cartilaginous anlage and include the limbs, pectoral and pelvic girdles and the vertebrae. Membranous onlay bone grafts in rabbits were found to vascularise earlier than endochondral bone grafts using microangiographic techniques (Kusiak et al 1985). Scott and Hightower (1991) have shown demineralised EC bone matrix to induce bone formation via cartilage and IM bone matrix to produce bone directly. Isaksson and Alberius (1992) noted no discernible differences in bone regeneration using EC and IM grafts in skull defects in rabbits. Moskalewski et al (1990) could not discern any histological differences in woven bone formation or in osteoblasts between cells derived from scapulae and vertebrae.

This present study provided a host bone bed of IM origin with an allograft of EC origin. The bone formed on all graft surfaces is the result of direct bone formation and showed normal bone tissue with osteoblasts lying on bone surfaces, connective tissue stroma with blood vessels and no chondrocytes were observed. Adipocytes were seen in large numbers. Urist et al (1997) believe lipids to be closely associated with non-collagenous proteins and observed composites of recombinant bone morphogenetic proteins-2 and acetone-soluble lipids to induce larger deposits of bone than implants of recombinant bone morphogenetic proteins-2 without acetone-soluble lipids. It appears that lipids may serve an important intermediary or supporting function for BMP activity. The sinus graft is a region of intense activity with volumes of up to 15 cubic centimetres of new bone being synthesised. The discovery of a predominance of adipocytes under light microscopy in these sinus graft specimens of EC origin but IM route of bone formation contradicts the observations of Moskalewski et al (1986): in which lipids were reported to be associated with endochondral bone formation but were not present when bone formed from parietal bone cells of IM origin but supports the concept of increased numbers of adipocytes in a region of increased bone anabolic activity.

The rate of bone formation
This avenue of investigation was unfortunately discontinued for two reasons. The use of fluorochrome labelling was dependent on accurate timing between markers
and the amounts required to enable these to be observed for analysis (Boyde and Reid 1983; Frost 1960). Patients given written and verbal instructions to take tetracycline labels at set time periods forgot and took them on different days. The use of standard fluorescence microscopy requires large doses. In view of the voluntary nature of the participation of patients, it was decided that such regimes would not be ethically acceptable and a minimal prescription of one 250 mg capsule of tetracycline was prescribed per day on a 1-7-1: 7 regime (Frost 1983a). Very few forming fronts were found, and this avenue of investigation was discontinued. However, a general overview is possible from the BSE-SEM micrographs. The majority of new bone appeared as woven bone, and markers are only useful for studying appositional lamellar bone formation. Mature lamellar bone could be distinguished on graft surfaces close to native bone beds. The discovery of lamellar bone on graft trabeculae indicated that remodelling of the new bone had occurred with new Haversian systems often developing within the graft material. In normal modelling, woven bone forms bridges, ridges, and beams of bone close to vascular channels and the osteoid mineralises very quickly, within approximately 2 to 3 days (Neuman and Neuman 1958). Woven bone growth can be fast and eventually encircle budding blood vessels to form primary osteons. Similar stages of development have been recognised here with the additional encapsulation of graft trabeculae by woven and lamellar bone being repeatedly observed adjacent to sites of blood vessels. The rate of bone growth via lamellar formation in the normal adult in remodelling is much slower and is known to occur at a rate of about 1 to 1.5 μm per day, although it can be much faster. New bone formation, varied from a few microns up to approximately 500μm in length or breadth of bone bridges formed between graft trabeculae, often emerging as isolated structures growing towards another particle or island of bone. The height of the sinus graft to support implants is 12 to 18 mm including the residual alveolus. When the minimal requirements are met with a 1 to 3 mm residual sinus floor and 10 mm of graft formed above it over about 180 days, simple calculations show that the rate of the bone formation front must advance at a minimal rate of 50 μm per day which is well within known the formative rates of woven bone. This infers another limiting process was in play that has retarded the bone forming at the higher levels in the graft. Light microscopy of these regions show normal stromal tissue with blood vessels but no records were made.
of their relative numbers or distribution. Results from Doppler Laser Flowmetry (to follow in Chapter 4), confirm the presence of blood vessels at the h1 levels investigated. However there was no evidence of necrosis. Thus the blood supply must have been at least good enough to ensure some life in the whole graft region.

The most likely reasons for the poor bone formation at the top of the graft are the short maturation period and poor quality of nutrient supply. The combined effects of the gaseous respiratory exchange and intra-sinal pressures on the overlying sinus membrane and how it may affect the graft directly below is unknown.

**Safety**

Safe use of an allograft is dependent on the non-transmission of active infectious bacterial or viral disease. In a study of the infection status of non-infected bone derived cells exposed to HIV virus, Campbell et al (1996) found no signs of infection over 8 weeks of examination by reverse transcriptase activity, microscopy, and immunofluorescence. Analysis by polymerase chain reaction showed less than 0.1% of bone derived cells to be infected. It appears that for HIV infections, blood is the greatest viral reservoir. The suppliers of IMCA (Rocky Mountain Tissue Bank, Colorado, USA) warrant that the screening of donors is strict and must be negative for active infectious disease, malignancies, degenerative neurological disease, and diseases of unknown aetiology. They state that blood samples are tested for HIV 1 / 2, Hepatitis B surface antigen, Hepatitis C antibody, HTLV 1 and Syphilis (STSBDR/RPR), using test kits licensed by the Food and Drug Administration (USA).

Gamma radiation has been used to both sterilise and remove the antigenic properties of bone allograft (Urist et al 1975). Campbell et al (1994) found that bone infected with HIV (classed as HTLV-III bone allograft) and frozen at -70°C was sterilised with a minimal dose of 2.5 megarads of radiation. The same regime has been found to successfully remove the antigenic properties inherent in allograft materials. IMCA processing procedures include freezing allograft at -70°C followed by irradiation at 2.5 to 3.8 megarads.

The final requirement is the acceptable sterility of storage, handling and despatch, and necessary documentation that would enable a tracking system to record its
recipient for any unexpected complications in its use. Receipt of IMCA is accompanied by a tracking form which details the lot number expiration date and the size of the sample. This form is completed by the clinician and it is expected that it be returned to the suppliers with details of the recipient. The clinician is contacted if the relevant forms have not been returned by the expiration date of any despatched allograft samples. Directions on use and contraindications are also included in the documentation. Although being a member of the American Association of Bone Banks does not guarantee complete compliance by any of its members, membership infers integrity and collective quality control and maintenance of adequate standards.

The packing characteristics of IMCA, due to its morphology, appear to enable this material to maintain an ideal pore size between particles. Even when new bone formation is not included, the volume fraction of IMCA (mean of 25%) exceeded that of previously reported bone volume fractions for trabecular posterior maxillary bone, i.e. 17.1% for females and 23.4% for males (Watzek 1999). This means that when new bone formation does take place, as in the mandibular graft and h3 sub-class, the newly formed maxillary bone volume in the sinus graft has a greater volume fraction than the bone normally found in this region.

A similarly increased bone volume was reported by Moy et al (1993) when autogenous bone was mixed with HA. The universal truth is that all bone allograft surfaces will have been formed by bone cells, and hence offer an inherent architecture designed for bone cells for the species (Davies and Baldan 1997). IMCA retains these features that make it an excellent matrix for bone neogenesis. No adverse antigenic or unusual infection events were observed in this study, with all patients exhibiting new bone forming on IMCA surfaces. The graft qBSE shift in grey level mean of 121.21 (8.44) to a higher mean of 152.93 (10.11), indicated an increase of mean mineralisation density which was close to that found in posterior maxilla sub sinus native sites (see Chapter 4).

Finally, the quality of the woven bone bed observed is intuitively ideal for mechanical and tissue engineered stimulation remodelling by placement of implants.
This study at 6 months has shown IMCA to be a suitable substrate for new bone formation when grafted in the maxillary sinus. Only regions below 5 mm height above the original sinus floor produced new bone in sufficient quality and quantity to support dental implants. The newly formed sinus graft had equivalent mineralisation density distribution (quality) to that found in native maxillary cancellous bone, with normal bone histology. The regions of poor bone formation may require a greater healing time for bone to form.
Chapter 3

Evaluation of bone mineralisation density distribution and percentage bone volumes of native implant sites.

Introduction

In this Chapter, I describe the use of the methods outlined in the previous Chapter to study native core biopsy specimens representative of anatomical sites in both maxillary and mandibular sites where dental implants might be placed, excluding the pterygoid plates and extra-oral sites which are the domain of the specialist maxillo-facial surgeon. This data was analysed together with that from sinus specimens. Bone quality is further discussed with a view to formulating a scientific quantitative methodology to reflect bone microstructure.

In clinical terms, bone quality is cited as a primary factor in implant success second only to bone quantity. Both factors have been popularly described in the dental implant field in empirical descriptive terms for ease of clinical understanding. At the present time there is no single quantitative scale of reference, by which bone quality can be assessed, that encompasses bone mineralisation, bone volume, and the connectivity of the bone volume under scrutiny. Compact bone is the optimal bone type for both structural and biomechanical function. Its apparent density (mass per unit volume), and its mineralisation density are optimised for mechanical loading, but in addition its vital cellular response via remodelling enables this bone type to adjust its structural mass and mineralisation density distributions to withstand functional mechanical loading as in occlusal function. The inner core of the alveolar bone is primarily of a cancellous nature except in the anterior symphysis of the mandible where over 95% success rates over 25 years have been achieved with dental implants. Although evolution has selected this to be best bone bed for the natural dentition with a periodontal interface, this is not the case for dental implants as
defined by bone quality. Cancellous bone structure is formed as a network of plates and struts interconnected to provide a local mechanical load bearing role. Some believe that the cancellous bone compartment is also a metabolic reservoir of calcium ions for systemic calcium homeostasis. The degree of connectivity and thickness of trabeculae together with the mineralisation density distribution of the structure determines their mechanical integrity as functional units. Two of these three factors have been quantified by the qBSE technique for bone sections viewed under BSE-SEM. The combined values of mineralisation density and bone volume together with an index of connectivity would provide the basis for a quantitative scale of bone quality. Such a scale would then be applicable for the quantitative assessment of any bone bed by BSE-SEM.

Present classification systems for bone quality in implant dentistry do not include certain markers of bone quality, including mineralisation density, bone volume fraction, and structural connectivity at the microscopic scale. The internal jaw structure of dental implant sites has not been previously evaluated by qBSE for mineralisation density.

**Materials and methods**

Specimen preparation and analysis was the same as that described in Chapter 2, for qBSE and LM.

**Results**

The data from all qBSE runs were examined and classified as per Table 2-2. All images were re-examined after the qBSE analysis to deselect any images that included non-bone regions in the field of view. Once the selections were completed, the data recorded as percentage bone volumes per bin were recalculated into pixels per bin to allow for summation of data per bin for each specimen. Mean grey scale values and percentage bone volumes were then calculated for each individual and anatomical site as defined by Table 2-2. Because of the number of classes to be analysed, frequency distributions were calculated to allow for direct inter-class analysis.
Mean mineralisation density

A preliminary graphical analysis for each class was performed to visually assess the distributions within each class. The distributions representative of the main anatomical sites were near normal distributions, except for the non site class 9, of bone shavings. There was a spread of mineralisation density distributions by frequency within each class. These are illustrated in Figure 3-1 to 3-5. The mean grey scales per class data are listed in Table 3-1. The highest mineralisation density value and also the highest mean, was found in class 4 the posterior mandible at (198.89 and 168.82), whilst class 7, the sinus wall had the smallest range of grey scales, between (160.42 and 177.15). The lowest mean (113.24) occurred in class 9, (bone shavings), whilst class 12 (unused IMCA) was (121.21), below that of class 11, (extraction sites) at (143.32) and class 6, (sub-sinus alveolus) at (154.27). The mean of extraction sites is of limited value due to the variable increasing rates of mineralisation density that occurs with increasing time after extraction.

Table 3-1. Mean mineralisation density values (grey scales in Br-I range) by class.

<table>
<thead>
<tr>
<th>Classification by site</th>
<th>No of patients</th>
<th>Mean</th>
<th>Median</th>
<th>SE Mean</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>class 1 ant max</td>
<td>10</td>
<td>161.00</td>
<td>165.71</td>
<td>1.73</td>
<td>126.99</td>
<td>179.15</td>
</tr>
<tr>
<td>class 2 post max</td>
<td>13</td>
<td>163.28</td>
<td>165.06</td>
<td>1.55</td>
<td>136.97</td>
<td>178.44</td>
</tr>
<tr>
<td>class 3 ant mand</td>
<td>9</td>
<td>164.96</td>
<td>164.07</td>
<td>1.38</td>
<td>125.92</td>
<td>190.78</td>
</tr>
<tr>
<td>class 4 post mand</td>
<td>8</td>
<td>168.82</td>
<td>168.53</td>
<td>2.73</td>
<td>135.1</td>
<td>198.89</td>
</tr>
<tr>
<td>class 6 sub sinus</td>
<td>23</td>
<td>154.27</td>
<td>153.95</td>
<td>1.61</td>
<td>107.58</td>
<td>182.62</td>
</tr>
<tr>
<td>class 7 sinus wall</td>
<td>3</td>
<td>167.54</td>
<td>165.04</td>
<td>4.99</td>
<td>160.42</td>
<td>177.15</td>
</tr>
<tr>
<td>class 9 shavings</td>
<td>7</td>
<td>113.24</td>
<td>117.26</td>
<td>5.67</td>
<td>55.79</td>
<td>156.09</td>
</tr>
<tr>
<td>class 10 mand graft</td>
<td>2</td>
<td>162.96</td>
<td>162.87</td>
<td>3.48</td>
<td>144.65</td>
<td>175.72</td>
</tr>
<tr>
<td>class 11 ext sockets</td>
<td>10</td>
<td>143.32</td>
<td>148.12</td>
<td>2.69</td>
<td>100.44</td>
<td>166.98</td>
</tr>
<tr>
<td>class 12 IMCA</td>
<td>2</td>
<td>121.21</td>
<td>123.07</td>
<td>4.16</td>
<td>108.27</td>
<td>134.22</td>
</tr>
</tbody>
</table>
Figure 3-1. Mineralisation density frequency distributions of bins by Class (where each series number represents a patient).
Figure 3-2. Mineral density frequency distributions of bins by Class (where each series number represents a patient).
Figure 3-3. Mineral density frequency distributions of bins by Class (where each series number represents a patient).

Class 9 Bone shavings

Class 10 IMCA mandible graft

Class 11 Healing extraction sites
Figure 3-4. Mean mineralisation distributions in bins by Class

Figure 3-5. Boxplot of mean grey scale by class

Figure 3-6. Boxplot of mean grey scales by sex.
The data were then organised to allow for analysis by One-way Analysis of Variance (ANOVA), for both sex and class. There were no differences noted between male and female data for mean grey scale values (Figure 3-6).

Between classes only small differences occurred between anterior and posterior regions in both maxilla and mandible. Greater differences were exposed between the sub-sinus and all the other classes. The data were then analysed by the Students 2 sample t-test between classes. Results are listed in Table 3-2. There were no statistical differences between the anterior maxilla and other sites except the extraction sockets (11). All sites had significant shift of mean distributions towards higher bins when compared to the extraction sites, except for the sub sinus (6), and mandibular graft (10). Both posterior maxilla (2), and anterior mandible (3), had significantly higher mean distributions than the sub-sinus (6), at \(p < 0.0004\), and \(p < 0.0052\) respectively.

Table 3-2. Results of two sample Students t-test in mineralisation density distributions between classes with p values and degrees of freedom as df.

<table>
<thead>
<tr>
<th>Classification</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>7</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-anterior maxilla</td>
<td>*</td>
<td>0.23</td>
<td>0.082</td>
<td>0.49</td>
<td>0.063</td>
<td>0.21</td>
<td>0.79</td>
<td>0.015</td>
</tr>
<tr>
<td>n=10</td>
<td>df=18</td>
<td>df=16</td>
<td>df=10</td>
<td>df=15</td>
<td>df=4</td>
<td>df=1</td>
<td>df=15</td>
<td></td>
</tr>
<tr>
<td>2-posterior maxilla</td>
<td></td>
<td>0.5</td>
<td>0.99</td>
<td>0.001</td>
<td>0.57</td>
<td>0.56</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>n=13</td>
<td>df=19</td>
<td>df=9</td>
<td>df=26</td>
<td>df=3</td>
<td>df=1</td>
<td>df=13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-anterior mandible</td>
<td></td>
<td>0.74</td>
<td>*</td>
<td>0.006</td>
<td>0.84</td>
<td>0.48</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>n=9</td>
<td>df=8</td>
<td>df=6</td>
<td>df=3</td>
<td>df=1</td>
<td>df=12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-posterior mandible</td>
<td></td>
<td>0.092</td>
<td>0.68</td>
<td>0.092</td>
<td>0.68</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=8</td>
<td>df=8</td>
<td>df=8</td>
<td>df=1</td>
<td>df=13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-sub sinus alveolus</td>
<td></td>
<td>0.09</td>
<td>0.78</td>
<td>0.09</td>
<td>0.78</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=33</td>
<td>df=2</td>
<td>df=1</td>
<td>df=11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-sinus wall</td>
<td></td>
<td>0.47</td>
<td>*</td>
<td>0.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=3</td>
<td>df=1</td>
<td>df=6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-MCA mandible</td>
<td></td>
<td>0.47</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=2</td>
<td>df=1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-extraction sites</td>
<td></td>
<td>0.47</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=10</td>
<td>df=1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant p values are in bold.
Bone Volumes

The qBSE results, of mean percentage bone volume for each anatomical site are presented in Table 3-3. Both the maximum and highest mean value was found in the posterior mandible (class 4), at 72% and 57%, respectively. The lowest value at 32% occurred in the sub-sinus alveolus (class 6), and similarly in the posterior maxilla (class 2), at 33%. The percentage bone volumes were collated and tested by one way analysis of variance (ANOVA), for sex and then for class. The results showed males to have a greater percentage bone volume at 42% compared to the females at a mean of 38%, but the differences were not significant. When analysed by class, there was a significant spread of mean values, at p=0.0001. These are illustrated in the box-plots in Figure 3-7 and 3-8.

<table>
<thead>
<tr>
<th>Classification</th>
<th>No of patients</th>
<th>Mean</th>
<th>SE</th>
<th>Median</th>
<th>Max</th>
<th>Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-anterior maxilla</td>
<td>3</td>
<td>43</td>
<td>7</td>
<td>47</td>
<td>53</td>
<td>30</td>
</tr>
<tr>
<td>2-posterior maxilla</td>
<td>7</td>
<td>33</td>
<td>3</td>
<td>32</td>
<td>46</td>
<td>23</td>
</tr>
<tr>
<td>3-anterior mandible</td>
<td>6</td>
<td>50</td>
<td>4</td>
<td>46</td>
<td>65</td>
<td>42</td>
</tr>
<tr>
<td>4-posterior mandible</td>
<td>6</td>
<td>57</td>
<td>4</td>
<td>55</td>
<td>72</td>
<td>42</td>
</tr>
<tr>
<td>6-sub sinus alveolus</td>
<td>15</td>
<td>32</td>
<td>2</td>
<td>33</td>
<td>41</td>
<td>20</td>
</tr>
<tr>
<td>10-IMCA mand graft</td>
<td>2</td>
<td>51</td>
<td>12</td>
<td>51</td>
<td>63</td>
<td>39</td>
</tr>
</tbody>
</table>

Table 3-3. Percentage Bone Volumes for each Classification

The percentage bone volume data were then analysed by Students 2 sample t-test for differences between the classes. Students t-test results are listed in Table 3-4. No significance occurred between class 1, and any other of the classes. Class 2, however, was highly significantly different to class 3, at p< 0.008, and class 4, at p< 0.001. Similar results were seen for class 6. There was a greater difference noted between the anterior and posterior of the maxilla than in the mandible, but the differences were not significant within each jaw.

An additional non-parametric test was then applied to the data for differences between classes. Mann Whitney U-test results verified those found by Student t-test analysis.
Figure 3-7. Boxplot of Percentage Bone Volume by Sex.

Figure 3-8. Boxplot of Percentage Bone Volume by Class.
<table>
<thead>
<tr>
<th>Classification</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-anterior maxilla</td>
<td></td>
<td>0.26</td>
<td>0.48</td>
<td>0.19</td>
<td>0.26</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>df=3</td>
<td>df=3</td>
<td>df=3</td>
<td>df=2</td>
<td>df=1</td>
</tr>
<tr>
<td>2-posterior maxilla</td>
<td></td>
<td>*</td>
<td>0.008</td>
<td>0.001</td>
<td>0.93</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>df=10</td>
<td>df=10</td>
<td>df=8</td>
<td>df=1</td>
</tr>
<tr>
<td>3-anterior mandible</td>
<td></td>
<td></td>
<td>0.22</td>
<td></td>
<td>0.005</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>df=9</td>
<td>df=6</td>
<td>df=1</td>
</tr>
<tr>
<td>4-posterior mandible</td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
<td></td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>df=6</td>
<td>df=1</td>
</tr>
<tr>
<td>6-sub sinus alveolus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>df=1</td>
</tr>
<tr>
<td>10-IMCA mandi graft</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

Table 3-4. Students 2-sample t-test for Percentage bone volume between classes showing (p) values and degrees of freedom (df).

Morphology
There were large variations in the morphological appearance of specimens, best seen in the montages of contributing images from the same specimen. This was true for all anatomical sites.

Normal jaw bone structure
In addition to those features discussed in Chapter 2, Part II, the following sections describe the broad spectrum of morphological variations that can be found in jaw bone across the age range of 32 to 86 years in male and female patients. The normal bone structural features are those that are generally equated with "young adult bone". Such normal bone can be, and is still, observed within older bone. This is true of this study where "normal bone structure" has been observed in all specimens examined (albeit variable in quantity) and are described below.

Cortical bone was clearly distinguished by its large continuous surface area of fine striations due to the successive bone lamellae which were interrupted by secondary osteons with osteocyte lacunae aligned along the lines of previous lamellar bone forming fronts. Of particular interest is the variety of osteonal
alignments well represented by the different ovoid shapes of each Haversian system sectioned by the specimen preparation process. Around each osteon distinct concentric lamellae could be seen due to the successive changes in collagen fibre alignment. Interstitial lamellar regions cut off by later forming osteons were a regular feature indicating that a dynamic process of constant renewal had been taking place. These features are illustrated in Figure 3-9.

**Sinus wall**

Three sinus wall sections from patients 17, 22, and 29, were examined (Figure 3-10). All sections were highly cellular, i.e. many osteocytes are evident. Signs of remodelling, could be observed with the most active secondary osteonal activity occurring in the section derived from a patient who had a recent extraction of the second maxillary premolar in the same quadrant. This was also the thickest sinus wall section, at over 2 mm, which was almost three times that of the thinnest specimen at about 700μm.

In the thickest section (Figure 3-10), three large, distinct and closely aligned new osteons can be observed lying adjacent to the endosteal surface region of which is a darker grey, indicating a more recently formed bone surface. A total of 19 osteons were counted in this specimen. The next smallest sample (Figure 3-10b), had only 8 osteons in the field of view, of which 4 were equal in size to those of the previous major osteons. A lamellar bone forming front could be seen on one surface, whilst directly opposite there was an enlargement of the sinus wall formed as a “hump”. There were patches of darker grey, indicating that remodelling was taking place around the osteons. In the thinnest specimen (Figure 3-10a), the small number and size of osteons was very obvious when compared to the other sections. Although present, dark grey patches were smaller and less distinct than in the other specimens. The contours of the outer surfaces were very flat and almost parallel, indicating a very stable state and low cellular activity. This specimen derived from a patient who had been edentulous for over 20 years with a basal posterior maxillary ridge height of less than 1 mm thickness observed on a panoramic radiograph.
Figure 3-9. Normal lamellar bone structure

Figure 3-9a. Maxillary cortical bone from the premolar region of a 58 year old male. The interstitial lamellae can be seen distinctly cut off by later formed Haversian systems that appear darker.

Figure 3-9b. Posterior maxillary cortex from the sub-sinus region of a 57 year old female. This specimen shows larger Haversian canals, each one much closer to adjacent ones. These are at an early stage of formation, and may represent a site of intensive remodelling and high turnover.
Figure 3-10. BSE-SEM of lateral sinus wall specimens

Figure 3-10a. 20kV BSE-SEM, field width 2.7mm. This image derives from a female patient 61 years of age who had been edentulous for over 20 years. Although there are a few small osteons present, the field is one of low cellular activity.

Figure 3-10b. 20kV BSE-SEM, field width 2.7mm. Compared to a, this image shows bone remodelling activity with a number of new large Haversian systems at an early stage of lamellae formation. Note that the lower surface of the image shows an even profile of lower mineralisation density. The patient a 46 year old male had lost his maxillary teeth about 5 years earlier.

Figure 3-10c. 20kV BSE-SEM, field width 2.7mm. This image derives from a 51 year old male who had the second maxillary premolar removed 8 weeks prior to the biopsy. The field shows intense remodelling activity. The wall is substantially thicker than a and b.
Maxilla and Mandible

The anterior maxillary cores were consistently of a robust appearance with greater bone volume than in any other class. Trabeculae were thick and spanned small spaces as struts between other bone surfaces which also indicated a high volume bone fraction. Although all bone is connected, few islands of bone appeared disconnected within the fields of view. Cortical bone was very evident especially at the apical regions which derive from the nasal floor (Figure 3-11).

In complete contrast, the sub-sinus class was consistent in the predominance of smaller, finer trabecular elements often in apparently disconnected arrays and with a low bone volume. Even when connected within the field of view in the plane of section, many trabeculae were very thin and long compared to those seen in the anterior maxilla. Although present, cortical bone was obvious by its paucity. The sub-sinus alveolus in these specimens was very thin at 3 mm or less in bone thickness (Figures 3-12).

The other anatomical sites showed a diverse range of bone structures and bone volumes within each class. The maxillary premolar regions (class 2), produced some specimens similar to that of the anterior maxilla, but others were completely dominated by the marrow spaces with a mix of apparently short (more transversely sectioned) and some very long fine trabeculae that were often aligned vertically rather than across the field of view (Figure 3-13).

The anterior mandible specimens showed the greatest bone volume and generally solid compact cortical bone structure. Different cores showed different morphologies. The structural diversity was very apparent between specimens taken within the same region from the same individuals (Figure 3-14). One example consisted entirely of dense lamellar bone with many scattered patches of more densely mineralised tissue. These regions of higher mineralisation density are represented in red, less well mineralised patches are mainly associated with osteons, indicating recent remodelling activity. A matching core specimen contained large marrow cavities equal to the width of the adjacent specimen core.
Figure 3-11. BSE-SEM Anterior maxillary Cores

Fig3-11a. 20kV BSE-SEM, field width 2.7mm
A maxillary bone core from the central incisor region of a 50 year old male. Note the compact cortical bone at the top of the image.

Figure 3-11b. 20kV BSE-SEM, field width 2.7mm. Maxillary core from the central incisor region of a 50 year old male. There is a similar bone morphology to that of ‘a’ at the top of the image.
Figure 3-12. 20kV BSE-SEM, field width 2.7mm. Sub-sinus maxillary cores.

Figure 3-12a and 3-12b. Two sub-sinus core images from a 43 year old female from different locations following a sinus graft. Note the height of the sub-sinus basal bone in the lower image is similar to the field width. The cortical structure has become extremely thinned and of low mineralisation density. The upper images show the sinus graft directly above the residual sub-sinus bone.

Figure 3-12c. Images of a core specimen from a 67 year old male.

Figure 3-12d. Images of a core specimen from a 46 year old male.
Figure 3-13. BES-SEM Montage of a posterior maxillary core

Figure 3-13. 20kV BSE-SEM, field width 2.7mm. This montage illustrates the fine thinned internal structure of the posterior jaw that can be found in premolar regions. Note the near vertical alignment of the trabeculae and the very open marrow spaces in this example from a 77 year old male.

Level of Cortical bone at the crestal region
of approximately 2 mm. These morphological differences are easily seen in the montage of BSE-SEM images in Figure 3-14.

Developmental variations in mineralisation density

As was noted in Chapter 2, highly mineralised regions were observed in the posterior maxilla (Figure 2-13). Similar regions were observed in the mandibular specimens. These took the form of small areas of localised hypermineralisation within which smaller very dense spots occurred representing mineralised osteocyte lacunae. Three specimens showed mineralised Haversian canal contents.

Most notable was that many of these regions seemed to also show highly mineralised cement lines as though these were also increased in mineralisation density concurrently with the regions they enclosed. One common factor was that these hypermineralised areas appeared to be more common in samples of patients over 50 years of age. Another feature seen was a brush-like effect representing more recently formed packets of bone cut nearly in the plane of the collagen. This appearance is due to the normal variation of collagen orientation within the formative surface (Figure 3-15).

Extrinsic fibres (Sharpey fibres)

The incidence of extrinsic fibres in the specimens examined was found to be limited to those patients with more recent tooth loss, even though the remaining dentition in some cases were examples of chronic and progressive periodontal disease. An interesting feature within this bone type was the formation of new Sharpey fibres to interconnect the old bony surface and the new woven bone. This was not seen at the lamellar bone forming fronts. The insertions of these fibres are easily seen in Figure 3-16.

Woven bone

The formation of woven bone was seen in specimens from sites of recent tooth removal, trauma such as the sinus floor elevation and representing every anatomical region of the mouth suitable for dental implant reconstructions. Although woven bone was always formed, not all regions exhibited the same qualitative and quantitative response. The most robust appearance was found in
late stage healing extraction sites of over 125 days and is discussed in the next section. Woven bone is shown in Figure 3-17.

*Extraction sites and IMCA*

Of particular interest from the samples analysed were the sequential and periodic nature of the specimens containing portions of alveolus that were healing extraction sites. These samples provided an opportunity to quantify the progression of extraction wound bone healing in a manner not previously investigated. Bone core biopsies were taken at intervals of 62, 64, 66, 88, 125 days, and one at 8 months post extraction. In all fields examined the formation of woven bone in the healing socket appeared to rise from small sprouting nodules from which the woven bone branches in all directions beyond the original bone surface. In some images, lamellar bone was developing within the woven bone.

Extraction site images were found unsuitable for a controlled analysis by qBSE as all images contained both alveolar bone bed and new bone formation. They would have provided inconclusive results on mineralisation density distributions and the percentage bone volumes. This was due to the fact that the site of trephine biopsies was solely determined by the requirements for the best position of the implant in relation to the final prosthetic result rather than the site of extraction.

Seven patients provided 17 bone specimens that included extraction sites, from which 33 fields were examined for new bone formation characteristics. The mean grey levels associated with each period of healing is tabulated in Table 3-5, together with the anatomical site.

There was found to be a general trend of increasing mean mineralisation density distribution with increasing time for post extraction healing. However, for the relative time change of 185 days, between 64 days and the longest healing period at 249 days, the increase in mineralisation density was small. The limited sample size and range of periods of healing do not allow for any quantitative evaluations.
Figure 3-14. BSE-SEM montages of anterior mandibular cores from same patient.

Two anterior mandibular core images derived from biopsy specimens taken from adjacent implant sites either side of the midline within the same patient, a 60 year old female. The montages provide a vivid contrast between the left image of virtually all compact bone and the right where large marrow spaces dominate with long fine trabeculae. The width of these marrow spaces appear almost equal to the width of the core on the left.
Figure 3-15 BSE-SEM micrographs of mineralisation variations and extrinsic (Sharpey) fibres.

Figure 3-15a. Image is of mandibular cortical bone from a 55 year old male. The field shows highly mineralised cement lines and hypermineralisation of osteocyte lacunae at the lower centre of the field.

Figure 3-15b. The most unusual feature in this field is the brush-like appearance of woven bone that has its collagen fibres sectioned across the plane by the angle of the cut surface. The osteocyte lacunae within the woven bone show highly mineralised margins. Unmineralised centres of Sharpey fibres in the bundle bone dominate the centre field but characteristically terminate at the interface with lamellar bone on the right of centre.
Figure 3-16. BSE-SEM micrograph of extrinsic (Sharpey fibres) fibres

Figure 3-16a. 20kV BSE-SEM 110x. This image is derived from the mandibular core of a 55 year old male who had a clearance of chronic periodontally diseased teeth 3 months prior to implant placement. The extrinsic fibre centers show as numerous dark diagonal lines of varying lengths of which the longest in the field are about 200µm. Centre field shows the interface between the original jaw bone and the later formed bone which is undergoing remodelling. Of great interest is the continuity seen at the interface of Sharpey fibres penetrating the later formed bone which appears to be a regular feature in this image. In contrast, this is not evident in the lamellar bone interface in the right of the image.

Figure 3-16b. This image from the same patient as 16a above, shows the Sharpey fibres cross-sectioned by the plane of the cut surface of the specimen. The change in alignment of the various layers of Sharpey fibres can be seen in the lower right of the image.
Figure 3-17. BSE-SEM micrograph of woven bone formation

Figure 3-17a. The morphological differences between the highly cellular and irregular nature of woven bone and the ordered structure of lamellar bone is easily seen in this image. The older lamellar bone on the right show osteocyte lacunae in their typical elliptical form. The woven bone formed on the left of the field has four primary osteonal systems at different stages of formation and has lamellar bone formed on its free surface.

Figure 3-17b. A higher magnification image of woven bone. The osteocyte lacunae are characteristically large and of an irregular shape. This field illustrates the robust branching and multidirectional formation that is typical of de novo bone formation.
The formation of new woven bone in the extraction socket appears to achieve rapid mineralisation very early in the healing period: in this study, a mean grey level of 153 by 64 days. A very much slower consolidation by increasing mineralisation density follows, in this instance, to a mean grey scale value of over 163 after about 4 months. The morphological pictures show this progression very well (early bone formation = lighter colours, later healing periods assume orange to red colours in Fig 3-18).

Table 3-5. Mean mineralisation density values (grey scales in Br-I range) of healing extraction sites by period and site.

<table>
<thead>
<tr>
<th>Anatomical site</th>
<th>Number of days</th>
<th>Mean grey level in Br-I range</th>
</tr>
</thead>
<tbody>
<tr>
<td>anterior maxilla</td>
<td>62</td>
<td>147.36</td>
</tr>
<tr>
<td>anterior mandible</td>
<td>64</td>
<td>139.20</td>
</tr>
<tr>
<td>posterior mandible</td>
<td>64</td>
<td>151.04</td>
</tr>
<tr>
<td>posterior mandible</td>
<td>64</td>
<td>153.28</td>
</tr>
<tr>
<td>anterior maxilla</td>
<td>66</td>
<td>113.60</td>
</tr>
<tr>
<td>anterior maxilla</td>
<td>84</td>
<td>150.56</td>
</tr>
<tr>
<td>anterior maxilla</td>
<td>88</td>
<td>153.76</td>
</tr>
<tr>
<td>anterior mandible</td>
<td>125</td>
<td>163.68</td>
</tr>
<tr>
<td>posterior mandible</td>
<td>125</td>
<td>162.56</td>
</tr>
<tr>
<td>anterior maxilla</td>
<td>249</td>
<td>171.20</td>
</tr>
</tbody>
</table>

*Regional acceleration phenomenon (RAP)*

This behaviour of bone to form callus and accelerate new bone growth for healing and at a site distant to that of the site receiving treatment has been observed consistently in experimental animals and in fractures. It was described by (Frost 1964, Frost 1983b) and is considered a normal accompaniment for the
normal course of bone healing. Although the mechanism of its action are unknown, the rates of cellular response of all stages in healing are accelerated. Examples were seen in this study. In the sinus structures, the response from the sinus floor to the trauma of membrane dissection and elevation could be seen in the direct outgrowths of robust trabecular bone into the overlying graft. The sinus wall specimen (Figure 3-10c) exhibited extensive remodelling, the location of which was distant to the site of a tooth extraction some months before. From the mandible, a sample of fractured posterior buccal cortical plate in cross section from the left mandibular second premolar region showed fine trabecular formations on both its endosteal and periosteal surfaces even though this region had not been traumatised. The patient had the mandibular anterior teeth removed 88 days before biopsy (Figure 3-19).
Figure 3-18. BSE-SEM micrograph of bone formation in extraction sites

Figure 3-18a. 20kV BSE-SEM, field width 2.7mm. The new woven bone formed in this field at 64 days post extraction appear as fine trabeculae with a very low bone volume fraction. The mean mineralisation density was 140 (Br-I scale). Image is of a specimen from the anterior mandible of a 50 year old female.

Figure 3-18b. 20kV BSE-SEM, field width 2.7mm. An example of extraction socket healing at 125 days post extraction from the posterior mandible of a 50 year old male. Although the woven bone trabeculae appear long and fine there is a greater bone volume and higher mean mineralisation density of 163 (Br-I scale).

Figure 3-18c. 20kV BSE-SEM, field width 2.7mm. This image of extraction site healing in the anterior maxilla of a 42 year old female had the longest healing period of 249 days. The extraction wound in the field has been totally filled with new bone of high volume fraction and mean mineralisation density of 171 (Br-I).
The above six BSE-SEM fields were imaged at 20kV, field width 2.7mm. This montage of a mandibular buccal cortical plate show a new bone forming response on the inner endosteal and outer periosteal surfaces even though this region close to mental foramen) was not subject to any direct treatment prior to dental implant placement. Extractions of the lower incisors had taken place 8 weeks previously.
Quantitative bone quality - the scale design concept

The design concept of a bone quality scale is conceived as a matrix of three parameters that are related to bone strength: connectivity, mineralisation density and its vitality, that is its marrow space. The last two have been quantified above, but connectivity representing a 3D structure has yet to be quantified. For the purposes of this discussion connectivity is assigned an arbitrary quantitative scale between 0 and 10, where 10 represents compact bone without any trabeculae.

These three parameters were then represented as three individual scaled axes designated x, y, and z, of a three dimensional figure. One horizontal axis y, was designated as the mean mineralisation density distribution of the specimen, the other horizontal axis x, became the mean percentage volume of the specimen, and the vertical axis z, was designated the connectivity scale.

All three axes cross at their origins of zero value. The values of the parameters x, y, and z are scaled so that the maximum is also the optimal value for each. For any three values of z, x, and y, the lines that join and intersect all three points forms the boundary of a triangle in space. The area of the triangle indicates quality. This quality value is designated ‘q’, in lower case. The calculation of the area of the triangle so formed is derived as below:

The area of the triangular surface will make up the quality rating ‘q’.
The three quality factors that make up the overall quality rating, mineralisation density, bone volume and connectivity, give the three co ordinates for the triangular surface (ABC).

In the Cartesian Co-ordinate form (x, y, z)
A = (Bone volume, 0, 0)
B = (0, 0, Mineralisation density)
C = (0, Connectivity, 0)
This is shown in Figure 3-20

![Figure 3-20. Three dimensional graph to derive the area representing Bone Quality](image)

O is the origin and M is the point on the line AB that gives a perpendicular to O.
θ is the angle OAB
OA = x bone volume
OB = y mineralisation density
OC = z connectivity

Using Trigonometry
\[ \tan \theta = \frac{y}{x} \]
\[ \therefore \sin \theta = \frac{y}{\sqrt{x^2 + y^2}} \]

For triangle OAM
\[ \sin \theta = \frac{OM}{x} \]
\[ \therefore OM = x \sin \theta \]
\[ \therefore OM = \frac{xy}{\sqrt{x^2 + y^2}} \]

For triangle OCM
\[ MC = \sqrt{OM^2 + z^2} \]
\[ \therefore MC = \sqrt{(\frac{xy}{\sqrt{x^2 + y^2}})^2 + z^2} \]

For triangle ABC
\[ AB = \sqrt{x^2 + y^2} \]
Height = MC = \sqrt{\left(\frac{xz}{\sqrt{x^2 + z^2}}\right)^2 + y^2}

Area = \frac{1}{2} \text{ (height x width)}

= \frac{1}{2} \text{ (MC x AB)}

So quality \quad q = 0.5\left\{ \sqrt{\left[ \frac{(xy)}{\sqrt{x^2 + y^2}} \right]^2 + z^2} \cdot \sqrt{x^2 + y^2} \right\}

Equation 1.

The value \( q \) can be represented as a percentage of the maximum quality, that is, when all the parameters are optimal, that is when they all equal 10.

The percentage value of bone quality ‘\( q \)’ was designated as ‘\( Q \)’.

Thus to calculate \( Q \), is as follows:

\( x = y = z = 10 \)

\[ \therefore \quad q = 86.6 \]

\[ \therefore \quad Q = \frac{q}{86.6} \times 100\% \]

Reference parameters for the Bone Quality Scale

The techniques utilised for the preceding sections provided the two parameters of bone mineralisation density distribution and the percentage bone volume within a field of view. The mean mineralisation density \( qBSE \) scale were found for the internal alveolus of the anterior mandible (class 3) to be 10.92 (165). The suitability of this region as the optimal anatomical site for dental implant placement has been discussed previously and is undisputed. Percentage volume results placed class 4, the posterior mandible, as having the highest mean percentage volume of 57 % with a maximum of 72%. The anterior mandible, however, only reached a mean of 50 % with a maximum of 65%.

Because this data is derived primarily from the internal structure of the jaw, it does not include many of the regions of known high mineralisation density and
high percentage volume that are known to be found in the supporting cortical walls and buttresses of both jaws (Kingsmill and Boyde 1998b). Instead, the contents examined by core biopsy reflect accurately the bone structure that directly adjoins and supports the implant structures actually placed.

The combined factors of the anterior mandibles historical success as an implant site and the observed success of posterior mandibular bone grafts to give rise to high bone volume fractions gives some justification for the use of these results as baseline optimal quantitative values relevant to implant requirements.

The value selected for the optimal mineralisation density on the 16 bin scale was chosen to be that of anterior mandible (class 3), rounded up to an integer figure of 11. The figure chosen for percentage bone volume was the mean of posterior mandible (class 4), at 57%. Examination of the images within this group showed normal cortical bone structure as shown in Figures 3-14 and 3-15.

The scales of mean mineralisation density distribution and percentage bone volume have an optimum value to recognise optimal bone properties from each factor. However, this optimal factor does not equate to the maximum value on each scale. Mineralisation density distribution ranges from 0 to 16 bins, whilst it is 0 to 100 for percentage bone volume. It was considered to be overly complex to integrate three scales of measurements from which the maximum did not represent the optimal value. To overcome this problem, the two scales were recalculated by piecewise linearisation, such that values above the optimal value assumed the same relative values as those below. By this method, the maximum values became the optimal values for both factors. This is explained below:

**The Mineralisation Density**

The optimal mean mineralisation density by qBSE lay in bin 11. The peak of the scale was placed at bin 11.

For simplicity's sake a linear scale was used where bin 1-11 equal Quality 1-10.
So at bin 11 the quality will be 10.

This will give an equation in the form $y = mx + c$

where

$y =$ Quality $= Q_b$

$x =$ Bin no. $= B$

$m =$ Gradient

$c = 0$

The equation derived is

$$Q_b = \frac{10}{11}B$$  
For bins 1-11

For bin numbers 11-16 the gradient was negated for consistency and the quality at bin 11 will be 10.

The equation derived

$$Q_b = 20 - \frac{10}{11}B$$  
For bins 11-16

The graph of Quality vs. Bin no. is shown below in Figure 3-21.
Mean Percentage Bone Volume

The mean percentage bone volume for each image was obtained from qBSE analysis. The optimal percentage bone was chosen as 57%. The mean percentage bone volume was dealt with in the same way as the mineralisation density, using two linear equations to give a quality rating. The equation derived for mean percentage bone volume, 0-57% is as follows:

where

\[ Q_v = \text{Quality} \]
\[ V = \text{BV}\% \]
\[ Q_v = \frac{10}{57}V \]
The equation derived for mean percentage bone volume 57-100% is:
\[
Q_v = 20 - \frac{10}{57}V
\]
For bone vol 57-100%

The graph of Quality vs. % Bone volume is shown below in Figure 3-2.

---

Connectivity of bone

The third factor required to describe the quality of the bone under observation was the degree of connectivity. For the purposes of this study, a simple 2-D quantitative measure of bone structure continuity obtainable from a micrograph was required, but as discussed previously no evaluation of connectivity can be made sensibly from derivations that rely on single 2-D representation (Odgaard 1998).
Gundersen et al (1993) provided an unbiased connectivity measure, the ConnEuler, using two pairs of 2-D images. Odgaard and Gundersen (1993) and Odgaard (1998) provide a derivation based on topological theory for quantifying connectivity using the Euler characteristic ($\chi$). Extensive mathematical and theoretical assumptions showed that $\chi$ for cancellous bone would always assume a negative figure and that connectivity was equal to one minus $\chi$. Because of the constraints of time and mathematical resources it was not possible to derive connectivity values. An arbitrary value was assigned to the scale of connectivity in a sample of 20 images.

Values of the three parameters for each image were obtained from the qBSE data. Connectivity is assigned values on a scale of 0 to 10 relating to the general connectivity of the field for demonstration purposes only. The quality factor 'q' was then calculated by reference to Equation 1. The results were listed in Table 3-6. To demonstrate the concept, bin numbers are used, grey scale values follow in brackets. Four of these images were selected and a Quantitative Bone Quality factor Q derived and is demonstrated in Figure 3-23.
Figure 3-23. Graph of Quantitative Bone Quality Scale

Bone Quality 1
Bone Quality 2
Bone Quality 3
Bone Quality 4

Anterior Mandible
Sinus floor
Sinus floor
Posterior maxilla

Connectivity
Mineral Density
% Bone Volume

85.54%
43.60%
58.77%
23.24%
Table 3-6. Quality data for 20 sample images

<table>
<thead>
<tr>
<th>Image ID</th>
<th>Anatomical Position</th>
<th>Mean bin and Br-I range</th>
<th>Mean Percentage Bone Vol</th>
<th>Connectivity</th>
<th>q Value</th>
<th>Q %</th>
</tr>
</thead>
<tbody>
<tr>
<td>05-066</td>
<td>Postmaxilla</td>
<td>9.88 (158)</td>
<td>34.8</td>
<td>2.00</td>
<td>20.12</td>
<td>23.24</td>
</tr>
<tr>
<td>05-069</td>
<td>Postmaxilla</td>
<td>9.89 (158)</td>
<td>38.0</td>
<td>3.00</td>
<td>24.63</td>
<td>28.44</td>
</tr>
<tr>
<td>04-060</td>
<td>Sinusgraft</td>
<td>8.47 (135)</td>
<td>39.1</td>
<td>4.00</td>
<td>24.96</td>
<td>28.82</td>
</tr>
<tr>
<td>04-063</td>
<td>Postmaxilla</td>
<td>9.04 (144)</td>
<td>59.2</td>
<td>4.00</td>
<td>34.41</td>
<td>39.73</td>
</tr>
<tr>
<td>05-026</td>
<td>Subsinus</td>
<td>9.78 (156)</td>
<td>61.0</td>
<td>3.00</td>
<td>34.47</td>
<td>39.81</td>
</tr>
<tr>
<td>04-016</td>
<td>Postmaxilla</td>
<td>9.65 (154)</td>
<td>58.9</td>
<td>4.00</td>
<td>36.32</td>
<td>41.94</td>
</tr>
<tr>
<td>04-017</td>
<td>Postmaxilla</td>
<td>9.94 (159)</td>
<td>58.9</td>
<td>4.00</td>
<td>37.27</td>
<td>43.03</td>
</tr>
<tr>
<td>05-014</td>
<td>Subsinus</td>
<td>8.07 (129)</td>
<td>57.6</td>
<td>6.00</td>
<td>37.76</td>
<td>43.60</td>
</tr>
<tr>
<td>05-033</td>
<td>Sinusgraft</td>
<td>9.11 (145)</td>
<td>82.0</td>
<td>2.00</td>
<td>39.31</td>
<td>45.40</td>
</tr>
<tr>
<td>04-048</td>
<td>Sinusfloor</td>
<td>9.22 (147)</td>
<td>54.1</td>
<td>6.00</td>
<td>40.64</td>
<td>46.92</td>
</tr>
<tr>
<td>03-040</td>
<td>Subsinus</td>
<td>8.81 (140)</td>
<td>59.4</td>
<td>6.00</td>
<td>41.25</td>
<td>47.63</td>
</tr>
<tr>
<td>04-050</td>
<td>Sinusgraft</td>
<td>9.47 (151)</td>
<td>63.7</td>
<td>5.00</td>
<td>41.50</td>
<td>47.92</td>
</tr>
<tr>
<td>03-043</td>
<td>Postmandible</td>
<td>9.86 (157)</td>
<td>50.3</td>
<td>7.00</td>
<td>45.99</td>
<td>53.10</td>
</tr>
<tr>
<td>03-068</td>
<td>Antmandible</td>
<td>9.17 (146)</td>
<td>87.3</td>
<td>4.00</td>
<td>47.37</td>
<td>54.70</td>
</tr>
<tr>
<td>04-064</td>
<td>Sinusfloor</td>
<td>8.47 (135)</td>
<td>73.6</td>
<td>7.00</td>
<td>50.13</td>
<td>57.89</td>
</tr>
<tr>
<td>04-023</td>
<td>Sinusfloor</td>
<td>9.92 (158)</td>
<td>80.0</td>
<td>5.00</td>
<td>50.90</td>
<td>58.77</td>
</tr>
<tr>
<td>03-038</td>
<td>Antmandible</td>
<td>9.53 (152)</td>
<td>78.9</td>
<td>8.00</td>
<td>62.18</td>
<td>71.80</td>
</tr>
<tr>
<td>03-034</td>
<td>Antmandible</td>
<td>8.37 (133)</td>
<td>93.7</td>
<td>8.00</td>
<td>63.75</td>
<td>73.61</td>
</tr>
<tr>
<td>03-061</td>
<td>Postmandible</td>
<td>9.62 (153)</td>
<td>63.8</td>
<td>10.00</td>
<td>65.35</td>
<td>75.46</td>
</tr>
<tr>
<td>03-041</td>
<td>Antmandible</td>
<td>9.63 (154)</td>
<td>86.8</td>
<td>9.00</td>
<td>71.73</td>
<td>82.83</td>
</tr>
<tr>
<td>03-037</td>
<td>Antmandible</td>
<td>9.80 (156)</td>
<td>87.8</td>
<td>9.00</td>
<td>73.22</td>
<td>84.54</td>
</tr>
</tbody>
</table>
Discussion

The values for the different anatomical sites for both bone mineralisation density and percentage bone volume reveal the great disparity that separates the sub-sinus region Class 6, from the other regions: it has both the lowest anatomical site mean mineralisation density and percentage bone volumes. Both of these two factors and the morphological assessments reflect the high turnover and hence metabolic rate within this region and provide further evidence of the excessive alveolar bone loss observed in both volume and height in this region compared to other sites. This result is similar to that arrived at by DEXA (Devlin et al 1998). The low value of mean mineralisation density distribution indicates that the bone turnover occurred at such a rate that the packets of new bone formed did not remain undisturbed for periods long enough for final maturation phase to take place. However, a few isolated packets of highly mineralised bone were noted in some specimens.

The range of qBSE values within all anatomical sites excluding extraction and graft sites was large, with the lowest at 107.58 in Class 6, to 198.89 in Class 4. It was surprising that no significant correlation was established between the anterior maxilla and any other anatomical site. One possible explanation may be related to the anatomy of the premaxilla which is generally narrow and bounded by robust cortical plates on the palatal and labial aspects.

The cancellous space would then be narrow with shorter distances to be spanned by trabeculae between cortical bone beds. Razavi et al (1995) evaluated the maxillary alveolus of 17 edentulous cadavers for bone quality and quantity by histology and observing trabeculation patterns and found that the anterior maxilla showed an increase in trabeculation and a thicker cortex than the posterior regions.

A standard procedure in treatment planning allows a healing time of approximately 8 weeks for soft tissue closure following extractions before placement of implants. The values seen in the 84 and 88 day healing extraction
sites show a qBSE of 150 and 153, which are very close to the mean value observed in the sub-sinus sites. Unfortunately, the percentage bone volume could not be determined. However, the mandibular graft value of 51% at approximately 4 months gives some indication of the percentage bone volume achieved in alveolar woven bone formation.

From these two values, it is very clear that a maturing extraction site with exuberant woven bone forming with high apparent density, mineralisation density and bone volume characteristics would be an excellent implant site due to the high rate of bone formation already taking place. Although shorter periods of extraction site healing were not available in this study, the mean mineralisation density was 153, at 88 days, compared to 163 at 125 days. The similar value of 162 for IMCA graft in the mandible was not visibly different from the surrounding alveolar bone in radiographs.

The qBSE mandibular results were not very different from that of the other maxillary sites. The mean mineralisation density distribution of the anterior mandible was similar to that observed by Kingsmill and Boyde (1998b) on cadaver specimens with the percentage bone volume being substantially greater than that of the maxillary sites, and significantly different to the posterior and sub-sinus sites.

Overall, the qBSE results show that the internal structure of the dento-alveolus differs in both quality and quantity of bone within sites and within individuals, but intra individual variations appeared to reduce the inter-site differences within each jaw.

The present arbitrary value scale for connectivity used to demonstrate a concept is obviously unsatisfactory. Connectivity is currently a focus of research and its relevance to the mechanical properties of cancellous bone has yet to be quantified (Odgaard 1998). The application of a quality scale was applied to a number of images with arbitrary connectivity values for illustration purposes only, which indicated a trend of increasing quality that was expected from known anatomical
sites that is, the highest Q value arose from the anterior mandible images and the lowest from the sinus graft images. This Q scale would allow for quality of bone to be rated by measurable properties once a useable bone connectivity methodology is devised. Application of the concept demonstrates that quality ‘Q’ of bone may be quantified for comparative bone studies of bone behaviour, and be developed as a useful index of bone quality as an extension of the qBSE technique.
Chapter 4

Laser Doppler Flowmetry for clinical detection of blood flow as a measure of vitality of sinus bone grafts.

Introduction

The successful development of a bone graft is dependent on the establishment of a blood supply to the graft, since only then will osteogenesis, modelling and remodelling be possible. If this does not occur, the graft may remain as a non-vital filler material that is not capable of achieving an osseointegration healing response when prepared as a recipient site for a dental implant. Studies by Becker et al (1996) found that demineralised freeze-dried bone allografts may remain unchanged at 15 months after placement. A non-vital graft will be vulnerable to infection during the surgical procedure and/or via the internal sinus membrane. If the sequelae of infection do not destroy the non-vital graft prior to the placement of the implant, then the discovery of the failure of the implant may not be made until 6 months after placement, when the implant is exposed for loading.

The problem

The height of a maxillary sinus can be as much as 34 mm from the sinus floor, with volumes as great as 27 ml being recorded (Anagnostopoulou 1991). Sinus grafts can often in-fill the sinus so that the original sinus cavity is almost totally obliterated. Dental implants over 20mm long are available and are utilised in this region. The difficulties in assessment of a graft in situ is that the surgeon is unable directly to view or to explore the superior aspect of the graft. One method is to probe the deepest regions of the graft and to evaluate the hardness by tactile feedback through an instrument. This method is not ideal since a lateral movement at the tip of a probe of 1-2 mm in diameter in an osteotomy site of less than 3.75 mm in diameter and up to 20 mm in depth is severely limited, as is the force that can be applied to test the hardness. Assessment by direct vision or reflected light is impossible due to the continuous bleeding of the usually more mature cancellous bone in the inferior aspect of the graft and the jaw bone, masking any non-vital sinus graft bone lying above it. Radiography provides grey levels that may be interpreted only as guesswork as to the density and volume of the graft. The major
handicap with normal dental panoramic or intra-oral radiographs is that the linear extent of the volume represented in a 2D projection cannot be properly measured. CT scans overcomes this problem to a certain extent, but then poses other problems that need to be addressed: the high radiation dose and the limited resolution of bone structure within a 2 mm "slice" and the lack of information about the vitality of the structures. Autografts, allografts, xenografts, and some alloplasts provide particular radiographic problems since the radiodensity of the graft is often very similar to live native bone.

**Laser Doppler Flowmetry**

One clinical method of providing the diagnosis of vitality is to detect and measure blood flow within the bone graft by a small pilot drill hole. This can be achieved at the time of implant placement by Laser Doppler Flowmetry. The use of Laser Doppler Flowmetry (LDF) was first demonstrated in clinical applications to register the blood flow in the skin and other soft tissues (Holloway 1977; Nilsson et al 1980). The Doppler effect describes the frequency shift in radiation emanating from a moving object. For reflected light this shift is proportional to the difference in velocity between light at the source and that returning from the object. Light beams interacting with stationary objects do not show the Doppler effect. Modern techniques utilise a beam of low power, near infrared laser light, for example, 780 – 820 nm, directed into the tissues by a fibre optic source. The photons are scattered by moving blood cells and adjacent tissues, but only the moving blood cells will cause a frequency shift to the monochromatic laser light according to the Doppler principle. The data observed are the blood flux expressed in arbitrary units (AU) described by the function Equation 3-1; This is the first moment of the power spectrum which is proportional to the product of the average speed of blood and the concentration of moving cells Equation 3-2 is computed by the laser Doppler processor (Nilsson 1984).

\[
LaserDopplerFlux = K \int_{\omega_1}^{\omega_2} \frac{P(\omega)d(\omega)}{dc^2} - noise
\]
Equation 3-2

\[ \text{Laser Doppler Flux} = [bc] \times \text{Average speed of bc} \]

Where:

- K is a constant used to scale the raw output to a predetermined calibration point
- \( \omega \) is the Doppler shift frequency
- \( P(\omega) \) is the power at frequency \( \omega \)
- \( d_c \) is the light intensity, used to normalise the signal to help eliminate the effects of gross variations in scattered light intensity (due to the beam angle of incidence).
- \( \omega_1 \) is the lower cut-off frequency used to help eliminate movement artefacts.
- \( \omega_2 \) is the upper cut-off frequency; the bandwidth is limited to reduce the overall noise of the system.
- bc blood cells in a sample volume under the light source.

noise encompasses both the ‘dark’ and ‘shot’ noise components of the system.

**Literature review**

**LDF in Bone**

Hellem et al (1983) utilised 17 young pig mandibles as an experimental model and established correlation between LDF measures of blood flow in the cancellous apical regions of alveoli as determined by histological examination of the tissues. Handley et al (1990) constructed a laboratory model by perfusing cancellous bone at the distal end of a fresh bovine metatarsal with a peristaltic pump. They recorded the flow rates at room temperature of an initial volume of heparinised Ringer's solution, followed by human blood, and established real-time oscillations of the LDF signal synchronous with the alterations of the rate of peristaltic pumping.

LDF has been shown to be successful in the clinical assessment of vitality of bone in surgical treatment of osteomyelitis (Swiontkowski et al 1989; Duwelius and Schmidt 1992). Kirkeby et al (1994) successfully observed the microcirculation of corticocancellous allografts in rats with LDF. Hoke et al (1994) recorded intra-oral, forehead and dorsal surface readings of the right hand with LDF, and reported no difference between hand held and stent stabilised probes. At the time of the present investigation, the author was not aware of any published studies in the use of LDF to examine the blood supply of sinus grafts and adjacent anatomical structures.
The objectives of this investigation were to apply LDF techniques to record the relative blood flow in
1. Sinus grafts and implant recipient sites and
2. The sinus membrane from the periosteal surface.

**Materials and Methods**

Six patients were studied in this investigation. Special equipment consisted of a 2 Channel DRT4 Laser Blood Perfusion Monitor with laser Doppler probes (Moor Instruments Ltd, Axminster, UK; Figure 4-1). The DRT4 is a dual channel machine capable of recording from two separate probes simultaneously, together with a temperature sensor probe. The DRT4 bandwidth was set to the range 20Hz to 15 kHz. The data acquisition rate was set at 40 data points per second with an integration time of 0.1 second. Although the DRT4 is able to store recorded data, it was more convenient for the data to be recorded via a cable connection directly to a PC. The temperature probe was placed in the sublingual region prior to and during all recording sessions.

A round headed endoscopic probe (DP6b), diameter 1.35mm, with flexible cable was used to take readings from the exposed intact sinus membrane and internal sinus membrane on elevation. A needle probe (DP4sd/T), diameter 1.3mm, with right angled delivery of light within 1mm of the tip, enabled graduated measurements to be taken from inside the graft via a pilot drill hole up to 15 mm in height from the crest of the alveolus. All probes were hand held. Probes and cables were scrubbed with 0.2% chlorohexidine gluconate (Hibiscrub), washed with saline and then scrubbed with 70% medical grade alcohol before being packaged into sterile instrument tubing ready for use.

Blood flow or flux was expressed as arbitrary units (AU). The DRT4 was calibrated using a 0.5% suspension of 0.5μm polystyrene microspheres (sub-micron diameter spheres in water), supplied by the manufacturer. The calibration signal derived from the particles undergoing Brownian motion. At each site, measurements of separate recordings were displayed and stored as a continuous graph. Five seconds was chosen as a standard noise free period for recording that would not interfere with the
The data recorded from the DRT 4 laser Blood Perfusion Monitor was downloaded directly to the laptop computer running DRTWIN software in Windows 95.

The smaller needle probe has a right angled delivery window within 1 mm of the tip. The larger end reading probe was used to record exposed sinus membranes after the bone window was dissected.
normal dental implant procedure and an average flux was calculated using DRTsoft software (Moor Instruments Ltd, Axminster UK).

**Interpretation and selection of data and its validity**

As a control, observations were made in 4 native edentulous maxillary sites in one patient who was also a subject for the sinus graft observations. In addition, buccal mucosal and sinus membrane observations allowed for further comparative analysis. Statistical evaluation was performed on Minitab Statistical software on a PC.

**Clinical Procedures**

Patients were undergoing the surgical placement of implants 6 months after the sinus graft procedure utilising irradiated cancellous allograft. All surgical procedures were conducted under intravenous sedation using Midazolam. Monitoring of patients status was by pulse oximeter (Kontron UK) with both flashing digital display and sonic alarm when \( pO_2 \) (haemoglobin saturation) was below 80% and or the heart rate exceeded 130 or fell below 50 beats per minute. The local anaesthetics administered were lignocaine with 2% adrenalin. Observations were taken when possible before the administration of local anaesthetics and sedation. Patients were treated in the supine position.

**In sinus graft patients using DRT4, n=5 with 24 sites measured**

Intra-osseous readings were taken from the graft with a 20 mm long lateral viewing probe of 1.3 mm diameter, with an optical window 1 mm from the end. A flat on the handle of the probe indicated the direction of the optical window. A temperature probe was placed sub-lingually during all observations. 5 subjects were included in this group. All subjects were treated under the same conditions. Within the group, 9 sinus grafts were investigated from which 24 sites were observed. Four subjects had undergone bilateral sinus grafting, and one patient had a unilateral sinus graft. All subjects were treated with irradiated cancellous allografts procured from Rocky Mountain Tissue Bank, USA. Of the 24 sites, 4 were taken in native edentulous jaw. A total of 240 intra-osseous and intra-graft readings were analysed at levels h1, h2, and h3 in the graft.
Measurements of blood flow in sinus grafts 6 months after initial grafting procedure and native maxillary alveolus, at the deepest, midpoint and shallowest

1. Once the full periosteal flap had been raised by a crestal midline incision to expose the maxilla, sites of implant placement were located by use of a surgical stent and marked with a small round marking bur. A pilot hole of 1.5mm diameter was then drilled into the graft to a depth of 15mm at the angulation and orientation that was chosen for the final position of the dental implant.

2. A side reading probe was then inserted to the full depth of 15 mm or 13 mm depending on the graft height. 4 radial readings were recorded at 90 degree rotations, namely mesial, buccal, distal, and palatal. A second set of similar readings was taken at the 6 mm below the first depth. Then a final set of readings taken within 3mm of the alveolar crestal surface.

3. This procedure was repeated for each implant recipient site.

Figure 4-2. Diagram of LDF measurements in implant sites within maxillary alveolus and grafts.
Teeth \( n=3 \) and Osseointegrated Maxillary Implants \( n=3 \)

In one of the subjects observations were made by LDF using the lateral viewing probe on the labial mucosa directly overlying three anterior maxillary teeth and overlying three osseointegrated implants without the administration of local anaesthetics. The level \( h_1 \) was 15 mm sulcal to the point of greatest curvature of the labial gingival margin of the restored implant or crown of the tooth. The midsection level \( h_2 \) was 6 mm gingival to \( h_1 \) and \( h_3 \) was measured at 3 mm below the gingival margin. This was coincident with the lower border of the gingival cuff in the tooth measurements. The crowns of teeth and prostheses attached to implants both served to stabilise the probe.

Measurements of sinus membrane \( n=1 \)

One subject gave consent for LDF to be utilised whilst undergoing bilateral sinus graft operations. Routine removal of the bony access window for the sinus graft procedure was modified so that the cortical window could be completely dissected from the underlying sinus membrane. An end viewing probe was placed on to the exposed membrane structure and data recorded following the procedures described above. Once the sinus membrane was dissected and elevated, a round-ended LD probe (Moor Instruments, DP6b) on a flexible cable allowed access to the pseudo-sinus cavity which had been created and readings were taken at random points over the surface of the raised membrane structure.

Subjects

The subjects were participants in the study of sinus grafts described in Chapter 2. Of the 6 subjects in the study, one exhibited restless body movements during the surgical procedure. As a consequence the 5 second quiet periods required for data recording could not be obtained without jeopardising the implant placement procedure. The data from this subject was excluded from the study. The remaining subjects provided data from 7 sinus grafts and 20 implant sites, of which 4 were in native maxillary alveolus. All procedures were completed without incident. Patient #8 had a failed implant that became mobile after exposure and loading. Treatment on the remaining implants proceeded to completion without complication.
The clinical situation in which dental implants are placed under sedation in the general practice scenario restricted the time that recordings could be made, yet still allowed the procedure to be completed as routine.

**Results**

Graphical displays of recordings demonstrated pulsatile effects in all the different tissue types sampled, namely buccal mucosa, attached gingiva, cortical alveolar bone, cancellous maxillary alveolus, periosteum, sinus membrane, and sinus cancellous allograft. The strongest pulsatile flow signal was found in the mucosal sites and the weakest in the sinus membrane sites. Vasomotion was strongest in the buccal mucosal readings while those from grafts and native sites were less obvious, and not detectable in some graft sites. The most regular example of vasomotion was observed from the buccal mucosa overlying standing teeth. Examples of blood flow are illustrated in Figure 4-3.

Native alveolus
The means, medians, maxima, minima and SEM's of the LDF data for the 4 implant sites in native alveolus are given in Table 4-1.

The readings at levels h1, h2, and h3 were very similar the highest at h1, and the lowest at h2, with h2 at mid-value between h1 and h3. There was no significant difference between the three levels.

Table 4-1. LDF data on native alveolus taken at deepest h1, h2, and shallowest h3 levels.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of sites</th>
<th>Mean</th>
<th>Median</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>h1</td>
<td>4</td>
<td>34.90</td>
<td>38.70</td>
<td>3.48</td>
</tr>
<tr>
<td>h2</td>
<td>4</td>
<td>28.52</td>
<td>30.45</td>
<td>2.05</td>
</tr>
<tr>
<td>h3</td>
<td>4</td>
<td>32.20</td>
<td>27.85</td>
<td>3.56</td>
</tr>
</tbody>
</table>
Figure 4-3. Doppler graphs of blood flow.

Bone graft at position H2

Bone graft at position H1

Jaw mucosa
Mucosa over tooth

Native bone

Sinus membrane through an access window
Sinus membrane measurement of patient with hiccoughs.
Frequency of hiccups approximately 0.3Hz

Sinus graft
The mean, median, max, min, and SEM of the LDF data for the 16 implant sites placed in sinus grafts are given in Table 4-2. The mean at level h1 is very much lower than that of h1 in native sites.
Table 4-2. LDF data on sinus graft taken at h1, h2, and h3 levels

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of sites</th>
<th>Mean</th>
<th>Median</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>h1</td>
<td>16</td>
<td>24.04</td>
<td>20.20</td>
<td>1.86</td>
</tr>
<tr>
<td>h2</td>
<td>16</td>
<td>30.90</td>
<td>23.85</td>
<td>2.70</td>
</tr>
<tr>
<td>h3</td>
<td>16</td>
<td>33.89</td>
<td>30.10</td>
<td>1.95</td>
</tr>
</tbody>
</table>

The data was transformed by converting to natural logarithms \( \ln \) and tested for normality by first producing a histogram of h1, h2, and h3. The Ryan-Joiner correlation based normality test was performed on the transformed data. R values were \( h1=0.9950 \), \( h2=0.9952 \), \( h3=0.9918 \).

Sinus grafts of saline and serum protocol

Results were collated into two classes, one of saline treated grafts and the other for serum treated grafts. Both were tested for significant difference between the two classes using the paired students t-test. The data is presented in Table 4-3.

Table 4-3. Descriptive Statistics for \( \ln(h1) \), \( \ln(h2) \), and \( \ln(h3) \) in saline and serum grafts

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of sites</th>
<th>Mean</th>
<th>SE Mean</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (h1)</td>
<td>7</td>
<td>3.016</td>
<td>0.116</td>
<td>3.073</td>
</tr>
<tr>
<td>Serum (h1)</td>
<td>9</td>
<td>2.964</td>
<td>0.113</td>
<td>2.944</td>
</tr>
<tr>
<td>Saline (h2)</td>
<td>7</td>
<td>3.29</td>
<td>0.122</td>
<td>3.198</td>
</tr>
<tr>
<td>Serum (h2)</td>
<td>9</td>
<td>3.1991</td>
<td>0.0986</td>
<td>3.1302</td>
</tr>
<tr>
<td>Saline (h3)</td>
<td>7</td>
<td>3.4415</td>
<td>0.0917</td>
<td>3.4717</td>
</tr>
<tr>
<td>Serum (h3)</td>
<td>9</td>
<td>3.4067</td>
<td>0.0742</td>
<td>3.3347</td>
</tr>
</tbody>
</table>

The results from this data set showed no significant differences between the control and the test grafts. From this information, the data from all graft sites were then pooled and analysed as a single data set. Table 4-4.

A one-way analysis of variance was performed to test whether differences were present between the means of groups h1, h2, and h3. Descriptive statistics are tabulated in Table 4-4 and results in Table 4-5.

Table 4-4. Descriptive Statistics (\( \ln \)) h1, h2, h3 pooled sinus graft data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of sites</th>
<th>Mean</th>
<th>Median</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \ln(h1) )</td>
<td>16</td>
<td>2.9866</td>
<td>3.0057</td>
<td>0.0808</td>
</tr>
<tr>
<td>( \ln(h2) )</td>
<td>16</td>
<td>3.2389</td>
<td>3.1718</td>
<td>0.0766</td>
</tr>
<tr>
<td>( \ln(h3) )</td>
<td>16</td>
<td>3.422</td>
<td>3.4043</td>
<td>0.0575</td>
</tr>
</tbody>
</table>
A two sample t-test was performed to test whether differences were present between the means of the h1, h2, and h3 groups. The results show a significant difference between the blood flow at h1 and h2 and h3 at \( p < 0.05 \). There was no significance between h2 and h3. The results are shown in Table 4-5.

<table>
<thead>
<tr>
<th>Location</th>
<th>(ln)h1</th>
<th>(ln)h2</th>
<th>(ln)h3</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ln)h1</td>
<td>*</td>
<td>0.025</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>df = 125</td>
<td>df = 113</td>
</tr>
<tr>
<td>(ln)h2</td>
<td></td>
<td>*</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td></td>
<td>df = 116</td>
<td></td>
</tr>
<tr>
<td>(ln)h3</td>
<td></td>
<td></td>
<td>*</td>
</tr>
</tbody>
</table>

**Teeth and Implants**

The small numbers of observations on mucosa around natural teeth and osseointegrated implants does not allow for statistical inferences to be made. However the limited data shown in Table 4-6 show similar trends in mean LDF values between teeth and implants at the h1, h2, and h3, with the greatest values at level h2.

Table 4-6. LDF data in arbitrary units of labial mucosa of maxillary incisor teeth and osseointegrated implants at h1, h2, and h3 levels.

<table>
<thead>
<tr>
<th>Implant levels</th>
<th>h1</th>
<th>h2</th>
<th>h3</th>
<th>Tooth levels</th>
<th>h1</th>
<th>h2</th>
<th>h3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Implant 11</td>
<td>50.6</td>
<td>50.1</td>
<td>73.1</td>
<td>Tooth 13</td>
<td>92.3</td>
<td>151.4</td>
<td>108.7</td>
</tr>
<tr>
<td>Implant 12</td>
<td>72.3</td>
<td>106.2</td>
<td>87.8</td>
<td>Tooth 22</td>
<td>43</td>
<td>136.6</td>
<td>64.8</td>
</tr>
<tr>
<td>Implant 21</td>
<td>63.8</td>
<td>94</td>
<td>75.7</td>
<td>Tooth 23</td>
<td>41</td>
<td>98.3</td>
<td>57</td>
</tr>
<tr>
<td><strong>mean</strong></td>
<td>62.23</td>
<td>83.43</td>
<td>78.87</td>
<td><strong>means</strong></td>
<td>58.77</td>
<td>128.77</td>
<td>76.83</td>
</tr>
</tbody>
</table>

**Sinus membrane**

The results of readings recorded from bilateral sinus membranes are listed in Table 4-7. Data was transformed to natural logarithms and tested for normality. The left and right data was tested for differences, with no significance found.

<table>
<thead>
<tr>
<th>Location</th>
<th>Mean</th>
<th>Std</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rw1</td>
<td>35.5</td>
<td>4.9</td>
<td>23.6</td>
<td>53.0</td>
<td>34.5</td>
</tr>
<tr>
<td>Rw2</td>
<td>22.4</td>
<td>9.9</td>
<td>12.4</td>
<td>60.7</td>
<td>19.9</td>
</tr>
<tr>
<td>Rw3</td>
<td>20.8</td>
<td>5.9</td>
<td>12.4</td>
<td>47.0</td>
<td>19.7</td>
</tr>
</tbody>
</table>
Table 4-7. LDF values in arbitrary units from sinus membrane. RW, LW=left or right intact membrane observed through bony window. Re, Le=left or right elevated membrane readings from inside the pseudo-sinus.

<table>
<thead>
<tr>
<th></th>
<th>lw1</th>
<th>lw2</th>
<th>lw3</th>
<th>lw4</th>
<th>Re1</th>
<th>Re2</th>
<th>Re3</th>
<th>Re4</th>
<th>Le1</th>
<th>Le2</th>
<th>Le3</th>
<th>Le4</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDF</td>
<td>24.3</td>
<td>18.0</td>
<td>23.5</td>
<td>20.6</td>
<td>12.8</td>
<td>14.1</td>
<td>22.1</td>
<td>20.1</td>
<td>13.8</td>
<td>14.9</td>
<td>36.1</td>
<td>11.0</td>
</tr>
<tr>
<td>LDF</td>
<td>10.9</td>
<td>4.9</td>
<td>4.4</td>
<td>10.1</td>
<td>6.1</td>
<td>10.3</td>
<td>11.3</td>
<td>5.4</td>
<td>2.4</td>
<td>7.7</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>LDF</td>
<td>13.2</td>
<td>10.1</td>
<td>15.6</td>
<td>5.7</td>
<td>3.9</td>
<td>2.3</td>
<td>9.5</td>
<td>10.0</td>
<td>4.4</td>
<td>20.1</td>
<td>20.1</td>
<td></td>
</tr>
<tr>
<td>LDF</td>
<td>80.2</td>
<td>45.0</td>
<td>43.4</td>
<td>82.9</td>
<td>38.1</td>
<td>69.7</td>
<td>46.6</td>
<td>48.1</td>
<td>25.5</td>
<td>46.6</td>
<td>64.7</td>
<td></td>
</tr>
<tr>
<td>LDF</td>
<td>21.6</td>
<td>16.5</td>
<td>22.7</td>
<td>19.8</td>
<td>17.9</td>
<td>12.1</td>
<td>19.3</td>
<td>13.1</td>
<td>13.3</td>
<td>32.5</td>
<td>32.5</td>
<td></td>
</tr>
</tbody>
</table>

The data indicates that there was no difference between left and right sinus readings and there was no significance between intact membrane and elevated membrane observations.

**Discussion**

*Pulsatile waveform and Vasomotion*

Pulsatile waveforms recorded in this study indicated that microvasculature was present in the tissues observed. Vasomotion is a cyclical variation that is observed in blood flow that is secondary to the rhythmical dilation and constriction of arterioles. Graphical examples of vasomotion 4 to 5 cycles per minute were recorded in mucosal and bone sites, but this was not a consistent finding in bone sites. This may be due to the short time scale that clinical reasons allowed in recording at each site. All mean flux readings from the graft sites fell within 32% of the mean flux value from native maxillary cancellous sites, but all bone flux values were substantially lower than values recorded from mucosal sites.

*Angiogenesis and the sinus membrane*

New bone formation requires successful infiltration of the graft mass by endothelial cells to form a microvasculature with which mesenchymal osteogenic precursors can be transported to differentiate and proliferate into the osteogenic lineage. The investigations in Chapter 2 demonstrated that the amount of new bone formed in an irradiated cancellous allograft at 6 months depends on the height above the sinus floor. The present results show that the blood flow in the bone deficient region was poor. This
observation accords with the anatomical observations of Solar et al (1998), who studied 18 maxillary specimens to elucidate the anatomical location and distribution of the major blood supply vessels responsible for nutrient supply to the edentulous maxillary alveolus and sinus after tooth loss. Solar et al (1998) observed that there were almost twice as many branches caudally as cranially from the main vessels anastomosing on the lateral sinus wall. The present observations confirm that the sinus floor is well endowed with microvasculature compared to the adjacent sinus walls, and infer that the sinus floor is the primary source of the blood supply that infiltrates the graft placed on it.

The antral mucosa derives its vascular supply from all the adjacent structures. The posterior, middle and anterior superior dental, greater palatine and sphenopalatine branches of the third part of the maxillary artery all contribute to the antral mucosa (McGowan et al 1993b), with additional blood supply from the facial, infraorbital, and greater palatine vessels (Williams et al 1989). The sinus membrane readings from the single subject in this study indicate that the blood supply was similar to that observed in the graft directly beneath the membrane and substantially less than that of oral mucosa and alveolar bone. It is possible that the low blood flow may be explained by the fact that membrane being measured had been elevated from the host bone bed and hence all vascular supply from the intra-osseous vessels would have been severely restricted. However, blood flow readings of the exposed sinus membrane through the dissected sinus osseous window, when not elevated, were not significantly different to those of the membrane when elevated. From these observations, it can be inferred that the sinus membrane in this study showed an inherently poor blood flow. This deficiency of blood supply will have a detrimental effect on the natural regenerative capacity of the sinus mucosa, as described by McGowan et al (1993b), and it may be the reason for its poor contribution to the graft healing beneath it. Observations by antroscope show the membrane to be traversed by many small blood vessels, but that it is not as vascular as the oral or nasal mucosa (Pogrel 1985).

**Conclusion**

This study produced a positive diagnosis of vitality at all sites within the grafts investigated. The mean laser Doppler blood flow values (flux) in the graft sites showed
a consistent trend with flux at the superior aspect (h1), was 30% less than the flux near basal alveolar bone (h3) whilst the mid section (h2) being 10% less than that at (h3). This contrasts with the flux values observed in the native sites, where the highest flux was at (h1), with (h2) 19% less than (h1) and (h3) was 8% less than (h1). The flux values of the region directly below and closest to the sinus membrane were similar to those observed at (h1) in the grafts, that is.

The results of this study show that the sinus membrane was a poor contributor to vascularisation of a sinus graft and that LDF can be applied successfully as a clinical diagnostic tool in the evaluation of blood flow or vitality within a developing sinus graft. The detection of blood flow in all regions of the graft is an indication that the first phase of graft development, i.e. angiogenesis, has taken place. In the event that blood flux readings from a graft site were negative in both pulsatile and vasomotion effects then this would be an important clinical indicator that the graft was bereft of blood flow. As a consequence the treatment schedule for such a patient and or the use of the graft materials might need to be reassessed for successful implant osseointegration. Further investigations are required to extend the use of LDF as a diagnostic aid in dental implantology.
Chapter 5

Thermodynamics of temperature in dental implants,
a hypothetical model

Introduction

Modern man has developed dietary habits within which the temperatures of the ingested food can range from iced beverages to scalding hot soups. This chapter is a theoretical examination of how normal dietary and behavioural habits might affect endosseous dental implants. Dental implant reconstructions are predominantly of metal, and provide a continuous connection between the deep core of the jaw bone through which the fibrous soft tissue layers of periosteum, connective tissue, and mucosa are exposed to the thermal, oral environment. Oral temperature is synonymous with sublingual temperature, and is often clinically referenced as body ‘core’ temperature (Haffajee et al 1992) and has a typical value of 36.9°C or 98.4°F. This is always within 1°C of the temperature measured at the axilla, whilst both oral and axillary temperatures generally lie within 1°C of the rectal temperature (Emslie-Smith 1988) which is again often referred to as “core” temperature. Because of this narrow range of fluctuations 37°C is considered the ‘normal’ body temperature in this thesis.

Surface temperatures of the intra-oral structures have been recorded to range between 0 - 67°C during eating and drinking of food as beverages and solids (Palmer et al 1992). The extent of heat transfer from foods and drinks to the mucosa, tongue and exposed teeth or prosthetic substitutes will depend upon the temperature of the ingested material and the time over which it remains in contact. Typical thermocycling considerations in the evaluation of dental materials range, for example from 10°C for 2 seconds, 36°C for 4 seconds to 60°C for 4 seconds over 500 cycles (Yap 1998), to 5°C, 37°C, and 60°C for 15 seconds for 2000 cycles (Theodoridou-Pahini et al 1996).

Literature review on heat conduction in dental implants

A study by Rams et al (1993) measured clinical oral temperatures related to functional osseointegrated dental implants in vivo. They found no significant differences between
sulcus temperatures adjacent to natural teeth and implants in anatomically similar positions. In a study using two dimensional finite element calculations (Moroi et al 1993) reported on the heat conduction from hydroxyapatite (HA) coated titanium and pure uncoated titanium implants when temperatures were lowered from 36° to 0° C.

The thermal effect of drilling bone to receive a dental implant produces potentially high denaturing temperatures (Abouzgia and James, 1997; Bragger et al 1995; Watanabe et al 1992). These studies have led to refinements in the surgical preparation, instrumentation and drilling techniques in implant treatment. Eriksson and Albrektsson (1983) observed temperature induced bone injury in the rabbit tibia using a titanium bone growth chamber at 50° C for one minute, 47° C for five minutes and 47° C for one minute. They concluded that 47° C was the critical temperature for heat induced tissue injury. In a further study, Eriksson and Albrektsson (1984) studied the effect of heat on bone regeneration, quantifying bone in-growth into a 1mm slit inside their experimental chamber. They found that 50° C exposure for one minute almost eliminated bone regeneration. The effects were reduced at 47° C for the same duration, whilst 44° C for one minute caused no observable disturbance in tissue regeneration. They concluded that the one minute temperature threshold for impairment of bone regeneration measured at a distance of 0.5mm from the implant was in the range of 44° C to 47 °C.

**Statement of the problem**

The effects of normal, but high, oral temperatures on the dental implant/bone interface has not been investigated.

**Objective**

A theoretical model is proposed to determine thermal conduction along the length of a titanium implant at normal oral temperatures, and the possible effects that a temperature transient may have on the bone-implant interface.

**Method**

**Heat Conduction**

The transfer of heat along a metal rod or bar is treated in thermodynamics as thermal conduction (Rogers 1992). The thermal conductivity, k, is defined as the rate at which
heat is transferred through a material resulting in a temperature change of $\Delta T$ across surface area $A$. Then

$$k = \frac{Q A}{\Delta T},$$

where $Q$ is the quantity of heat transferred (Watts), $A$ is the area ($m^2$) and $\Delta T$ is the change in temperature ($^\circ K$).

A more useful measure of the potential for a material to transfer heat as a transient phenomenon is its thermal diffusivity, $\alpha$. This is defined by

$$\alpha = \frac{k}{(\rho c_p)},$$

where $k$ is the thermal conductivity ($W m^{-1} ^\circ K^{-1}$); $\rho$ is the density of the material ($kg m^{-3}$) and $c_p$ is the specific heat ($kJ kg^{-1} ^\circ K^{-1}$).

The value for the diffusivity, $\alpha$, has been calculated for a number of materials (gold, titanium, bone, and dentine) in Table 5-1.

The Dental Implant

Dental implants can have diverse morphologies, but the most studied is that of a solid screw of pure titanium or titanium alloy passing the external cortical plate of the mandible or maxilla into the inner trabecular bone, and ideally seated into an opposing cortical endosteal surface to achieve bicortical fixation. The implant may be 3.3 to 6 mm in diameter and 7 to 20 mm in length. Even after apparent osseointegration has taken place, there is only a partial contact between the implant screw thread and the adjacent bone which varies from 25% to 72% bone to implant contact at any time (Misch 1993a; Wilson et al 1998). Together with bony ingrowth into machined recesses on the implant body, the screw threads provide the mechanism for retention of the implant and a ‘heat sink’ via which excess heat in the implant may be dissipated to the adjacent bone and soft tissues by conduction or convection to the local vasculature. This implant is connected to a prosthetic superstructure in the oral cavity by a titanium abutment connection.

The Hypothetical Model: Constraints

The model discussed here considers the implant to be a semi-infinite solid, cylindrical in shape, which is initially at a uniform temperature of 37$^\circ$ C. Heat is applied as a constant heat source to one end surface for a period of 10 seconds. No heat is lost to the
surroundings by induction, convection, or radiation. Under these conditions we may apply the equations derived from thermodynamics (Rogers and Mayhew 1992).

\[ T(x,\tau) = T_{Init} + T_{App} \left[ 1 - \frac{x}{2\sqrt{\alpha \tau}} \right] \]

Where \( T(x,\tau) \) is the temperature after a time \( \tau \) at a distance \( x \) from the implant surface and the temperature \( T_{App} \) is applied to a material of thermal diffusivity \( \alpha \) whose initial temperature was \( T_{Init} \). The value of the function \( \text{erf}(\cdot) \) may be calculated for the values of \( x \), \( \tau \) and \( \alpha \) and its value derived from published tables (Wolfe 1983). A computer program was written in PASCAL to vary the applied stimulus \( T \) over values 50, 55, 60, 65 and 70°C. The thermal change for the resulting temperatures in the implants after 0.2s, 0.5s, 1s, 2s, 5s and 10s were calculated. The results are shown graphically in figure 5-1 for 50°C, 60°C and 70°C stimulus, applied for a period of 5 seconds.

**Results**

From the data predicted by this model, a nominal temperature of 47°C will be reached at a distance of 1.29mm, 2.60mm, 3.45 mm, 4.07mm, and 4.54 mm down the implant after an applied temperature pulse of 1 second with a heat source at 50°C, 55°C, 60°C, 65°C and 70°C respectively. The data from this hypothetical model suggests that 47°C may occur regularly down the surface of an implant structure embedded in bone from unexceptional oral temperatures.

Table 5-1. Physical properties of dental materials. Thermal conductivity, specific heat, density and thermal diffusivity.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Thermal Conductivity ( k ) W m(^{-1})°K(^{-1})</th>
<th>Specific Heat ( c_p ) kJ kg(^{-1})°K(^{-1})</th>
<th>Density ( \bar{\rho} ) kg m(^{-3})</th>
<th>Thermal Diffusivity ( \alpha ) m(^2) sec(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold (Weast 1986)</td>
<td>319</td>
<td>0.129</td>
<td>19300</td>
<td>0.128</td>
</tr>
<tr>
<td>Titanium (Weast 1986)</td>
<td>22.4</td>
<td>0.523</td>
<td>4540</td>
<td>9.434x10(^{-9})</td>
</tr>
<tr>
<td>Cancellous bone (Clattenburg et al 1975)</td>
<td>0.30</td>
<td>1.44</td>
<td>1920</td>
<td>0.109x10(^{-9})</td>
</tr>
<tr>
<td>Dentine (Craig 1961)</td>
<td>0.63</td>
<td>1.17</td>
<td>2100</td>
<td>0.256x10(^{-9})</td>
</tr>
<tr>
<td>Enamel (Braden 1964)</td>
<td>0.92</td>
<td>0.75</td>
<td>2900</td>
<td>0.423x10(^{-9})</td>
</tr>
</tbody>
</table>
Figure 5-1. The temperature gradient down a simulated titanium implant when (A) stimulus is 50°C, (B) stimulus is 60°C, (C) stimulus is 70°C. The columns show how far any given temperature rise has advanced at 0.2, 0.5, 1, 2, and 5 seconds in time.
Discussion

This hypothetical model of heat conduction within a dental implant predicts that, within the ideal conditions of the model, a temperature of 47°C will be reached 2 mm below the top of the implant within 2 seconds, 0.3 seconds and 0.2 seconds after the applications of heat sources of 50°C, 60°C and 70°C respectively.

Alkaline phosphatase is an enzyme that is detected in the membrane of osteoblasts and is often accepted as a useful primary marker of osteoblastic activity. Although it can maintain its biological activity over a wide temperature range, the protein becomes denatured above 50°C and is irreversibly damaged (Lundskog 1972).

Collagen type I is the major fibrillar component of all fibrous connective tissue, including periodontal ligament and bony tissues, and its typical configuration is a long triple stranded helical structure formed by the intertwining of three separate collagen polypeptide α chains into a super helix. These molecules assemble into ordered fibrils 10-300 nm in diameter, which in turn aggregate into the larger collagen fibre bundles that are up to several microns in diameter. The organised deposition of collagens in osteoid provides the extracellular matrix for the seeding of calcium phosphate crystals. Although collagen fibres have been shown by Kronick and Cooke (1996) to become thermally stabilised on mineralisation, collagens and collagen fibrils in the free state prior to mineralisation are more sensitive to temperature and can be denatured by elevated temperatures. Schmid and Linsenmayer (1984) found the denaturation temperature of the helical structure of short chain cartilage collagens to be 47°C. Hayashi et al (1979), found that both procollagen and collagen type I have denaturation temperatures of 42°C in neutral buffers.

Garetto et al (1995) recorded a continual state of bone turnover within 2.0 mm of the implant to the bone interface in human and animal studies, such that some new osteoid patches would be present at the implant interface throughout the life of an implant. In vivo, the percentage of bone to implant interface at any one time may be highly variable and depend on the clinical history of that particular implant, the type of implant coating and the structure of the bone bed. Histomorphometric and other studies (for example,
by Johansson and Albrektsson 1991; Lum et al 1991; and Sagara et al 1993) demonstrate that the degree of osseointegration, that is, intimate bone-implant contact, is highly variable in the life of an implant. Misch (1993a) provided histological evidence for D-4 bone to have 25% initial bone to implant contact, D-3 bone about 50%, D-2 about 70%, and D-1 dense cortical about 80%. Johansson and Albrektsson (1991) found a 44 % bone to implant contact with cp titanium implants in rabbits after a healing period of 3 months. Wilson (1998) who studied implants placed in extraction sites, analysed retrieved human material after 6 months and found a mean bone to implant contact of 50%, with a maximum of 72% in a non-loaded implant. Temperature surges may affect the quality and quantity of the bone to implant interface, and may cause loss of osseointegration, especially in cases when the percentage of bone contact is low.

The crestal bone, by being closest to the oral source of heat, will be the most “thermocycled” element of alveolar bone. Early cratering or saucerisation (the crestal loss of bone around the coronal collar of the implant after exposure and or loading), and thread exposure at the crestal bone circumscribing the dental implant is a continuing problem with dental implants. Mechanical forces of exposure, abutment connection and loading in the oral environment have been considered as possible causes (Bidez et al 1992; Esposito et al 1993; Rangert et al 1997). Perel (1994) describes this phenomenon of apical migration of the crevicular epithelium to be a biological adjustment by the tissues to restore the concept of "biological width" to combat bacterial infection. Pham et al (1994) further confirmed that the crestal bone loss phenomenon also occurred in non-submerged single stage dental implants.

Jaffin and Berman (1991) considered poor bone quality and short implant length as significant factors predisposing to implant failure. Current opinion is that such failures are due to the reduced flexural and structural strength of the shorter implant and physiological overload on the implant-bone interface supporting the occlusal loads in mastication. The present model predicted that the critical temperature of 47°C exceeded 7mm along the implant with a 5 second heat exposure at 70°C. Short implants would be more affected than long implants by temperature transients. The distant apical regions in longer implants for example 15 mm, would not be affected by heat conduction.
Conclusion

Temperatures greater than 50° C encountered in normal oral function are conducted rapidly down the hypothetical implant. Although the calculated temperature of 47°C in this model may occur at a particular distance down an implant structure after a set time, regions closer to the heat source will obviously be at a higher temperature. The coronal end of the implant structure will have a steep temperature gradient, above 47°C rising up to the stimulating temperature at source. The implant heats up rapidly. Oral temperature changes can take up to 10 minutes to return to equilibrium (Leithead et al 1964). The duration of exposure to heat in normal oral function is not quantified. This model suggests that normal high oral temperatures may, in certain situations, affect bone quality and quantity in turnover at the bone-implant interface.
Chapter 6

Osteoclast resorption assay with temperature.

Introduction

In vitro studies using cell and tissue culture provide clues as to cell and tissue function within controlled conditions without animal experimentation. The bone resorbing cell, the osteoclast, has been extensively studied by cell culture using the osteoclast assay developed by Jones et al (1984). Functional osteoclast physiology, morphology and the osteoclast’s interactive role in developmental modelling and remodelling of the osseous tissues have been observed. Mammalian, avian and murine osteoclastic cells have been examined. The experimental protocol in the osteoclast assay is standardised at 37° C as the incubation temperature of the tissue culture, which is accepted as the normal human body temperature. Chapter 5, indicates that high normal oral temperatures may impact on bone cells at the bone-implant interface. This chapter explores the effects of temperature on the avian osteoclast in vitro.

Literature review

Jones et al (1984) demonstrated osteoclasts to be capable of independent of species or substrate specificity in resorbing mineralised tissues. Arnett and Dempster (1986) established pH of the medium as a controlling factor in osteoclast function. Taylor et al (1990) studied the effects of fluoride. Horton et al (1991) demonstrated that osteoclast function can be disrupted by low concentrations of the anti-vitronectin receptor antibody, with osteoclast cell adhesion being consistently inhibited in an Arg-Gly-Asp (RGD)-dependent manner. Retinoic Acid effects were observed to decrease the resorptive activity of chick osteoclasts in vitro (O’Neill et al 1992). The number of nuclei in osteoclasts has been studied in relation to osteoclastic volume (Piper et al., 1992). This study confirmed that larger osteoclasts made larger pits in vitro. Vesely et al (1992) observed cell-cell contact behaviour and networking between avian osteoclasts with osteoblasts. They also noted that co-cultures of avian osteoclasts with rat osteoblasts resulted in the death of the osteoclasts beyond 1-2 days from the time of primary culture. Biphosphonates were found to reduce the volume, area and pit depth resorbed per nucleus per osteoclast using the avian osteoclast assay (Piper et al 1994).
Potentiation of avian osteoclastic bone resorption activity by inhibition of nitric oxide synthase was reported (Kasten et al 1994). Hill et al (1994) using a rat osteoclast assay, concluded cathepsin B to have an intracellular action whilst, cathepsins L and/or S were involved extracellularly in osteoclastic resorption. Cheung et al (1995) observed increased resorption in avian osteoclasts exposed to ethanol. The rate of osteoclastic destruction of calcified substrates has been investigated by Jones et al (1995) in cultured avian osteoclasts. Resorptive activity was observed on three calcified tissues, enamel, dentine, and cementum. The results indicated an inverse proportionality between the rate of resorption and mineralisation density of the substrate, the demineralising phase was found to be the rate limiting step in osteoclastic resorption.

All prior osteoclastic assay investigations appear to have been conducted at 37°C.

**Statement of the problem**

The normal body temperature of the hen is 41.5°C. 37°C may not be the optimal temperature for function of avian osteoclasts.

**Objectives**

The aim of this preliminary study was to determine the effects of different incubation temperatures on the osteoclastic activity of chick osteoclasts on a homogeneous calcified tissue substrate.

**Materials and Methods**

**Culture of Osteoclasts**

Osteoclasts were obtained from the long bones of 19-day-old prehatch chicks. The shafts of long bones were excised and freed of periosteal soft tissue and cartilage, (the epiphysis’s discarded) and washed twice in phosphate buffered saline solution. The bones, free of adherent non-bony tissues, were then sectioned into small fragments in Eagle’s minimum essential medium (EMEM, GIBCO BRL Life Technologies), combined with 10% heat-inactivated Foetal Calf serum (FCS), and 2 mM of L-Glutamine and Gentamycin (50 gg/ml). The fragments were gently agitated with an oscillating action inside a plastic pipette to aid in the release of osteoclasts from the bony fragments. The bone fragments were allowed to settle, and the cell suspension was
then seeded as aliquots to carefully prepared 1-cm-square, 250μm thick slabs of sperm whale dentine (SWD). The cells were allowed to settle for 60 minutes at 37°C and in 5% CO₂ to allow the cells to attach to the substrate. The slices were then gently rinsed with fresh medium to remove non-adherent cells and sufficient fresh medium approximately 3ml was added, to ensure that the slices were covered. Four slices were seeded in each dish, one dish for each test and each control. Dishes were then transferred into their respective incubators. Incubators were set to the required temperatures for at least 45 minutes before incubation of experimental specimens to achieve a steady state.

Substrate
The substrate chosen for this study was sperm whale dentine (SWD). SWD was the substrate of choice due to its size, availability, and its uniformity in its mineralised structure. Dentine is, compared to bone, homogeneous an ideal biological mineralised substrate that can enable meaningful comparative studies. 250μm slices were prepared by using a water-cooled diamond wafering saw (Beuler Isomet). The slices were cleaned by ultrasonication and sterilised by immersion in, and drying from, absolute ethanol. Dentine slices are prone to distort and curl during the seeding stage if dehydrated. This was overcome by keeping them moist, but not wet, to allow for ease of seeding: the cell suspension would spill off the slice if it is wet, due to the loss of surface tension.

Culture Temperatures
Experimental cultures were incubated at 35, 39, 41, and 43°C for 24-hours in humidified incubators held at steady state temperatures, using 5% CO₂ to maintain the culture medium pH of approximately 7.0. Four separate experiments were run in series. Two incubators were used, one control at 37°C and the other at the experimental temperature. Both control and test cultures were treated identically. At 24-hours after incubation the cells were swept off the substrates, which were then washed thoroughly and air dried. The slices were viewed by a Bausch Lomb reflected light microscope to determine which surface was resorbed, after which they were mounted on glass slides with resorption pits uppermost for measurement. A random coding, which was only broken after measurements were completed was assigned by another colleague.
Method used to record osteoclast activity

Boyde et al (1985) applied reflection confocal scanning microscopy for measurements of pit depths. The resorption lacuna volume represents the work done by the osteoclast. The technique in particular for this study is that approach of 3D analysis detailed by Boyde and Jones (1995). The pits excavated by the osteoclasts were measured using a Confocal Video Rate Laser Scanning Microscope specially adapted for surface mapping (Lasertec Corporation 1LM21, Japan). This system utilises a He-Ne red laser beam $\lambda=633$ which scans the field at a full video-rate by an acoustico-optic controller. Focus is motorised to give fine focus steps of $0.01\mu m$. The 1ZC1 Z-axis controller enables the operator to establish two outside focus levels. Two images are acquired automatically: the max (maximum signal intensity at each pixel during the through focus series) and the map (focus level at each pixel at which the maximum signal intensity was recorded). The map image is digitised to 256 height levels.

All imaging was obtained using a Nikon 40/0.95NA dry objective with cover-slip correction to which a 170µm glass cover-slip had been cemented. Two methods of determining the volume of an osteoclastic pit were available. In the first, a tracing is made around the periphery of the osteoclastic pit by use of a mouse and screen cursor, to mark the surrounding reference surface, the area and volume is calculated by the software. The second method “levels” using heights determined in three areas outside the pit. Thresholding establishes the border between the surface of the substrate and the periphery of the pit.

Dedicated software (SIS, Munster, Germany) calculates the area and volume below the surface enclosed within that area. The mean depth is calculated as volume/area for each pit. Pits were located on a raster scan of the whole of each dentine slice. Volumetric and area data were logged from each SWD slice within each culture. Over 150 observations of each of 4 slices of SWD were recorded. Pits greater than 20,000µm$^3$ were excluded from the analysis (Dataset A) because they represent cumulative effect over several resorption cycles of one or more osteoclasts. Pits less than 10,000µm$^3$ were designated as Dataset B. This set is more representative of the pits produced by single osteoclasts. Finally, all pit volumes less than 5,000µm$^3$ were collated into Dataset C to represent the smaller pits.
The objective was to record at least 100 pits from each slice for analysis. However, experiments 1 and 4, produced slightly less than this number of pit observations. The data distribution was skewed rather than normal. One-way analysis of variance and the Mann-Whitney U-Test were selected for statistical analysis (Minitab, State College, PA, U.S.A.).

Set A. The data from pits with volumes of pits < 20,000µm³.

Set B. The same as for Set A except all pit volumes were less than 10,000µm³.

Set C. The same as for Set A, except that all pit volumes were less than 5,000µm³.

**Results**

The means, medians, and SEM of Volume, Area, and mean depths (V/A) at the experimental temperatures and each related control are shown in Table 6-1. Mann-Whitney U-test results between test and control are also tabulated in Table 6-1.

**Experiment 1  35°C.**

The areas and volumes of the pits were not statistically significant from the control at 37°C. However, the mean depths (V/A), of the pits at 35°C were significantly shallower than the control at 37°C (p < 0.0000). Similar significance was found in Dataset B, and Dataset C.

**Experiment 2  39°C.**

No significant differences were shown between test and control, for all Datasets.

**Experiment 3  41°C.**

The areas of the pits in this group were very similar to the control group, but both the volume and mean depth (V/A) means were greater (by a factor 1.26 and 1.38) than the controls. Both were significantly different at p < 0.0000. Dataset B and C were all highly significantly different for both volume and mean depth.
Experiment 4 43° C.

At the highest temperature test, areas, volumes and mean depths were all significantly different to the control. The mean areas in the test were 1.43 times greater than control, while the mean volume of the test was 1.36 times greater than the control (significance was at p< 0.0001 for both area and volume). Although the mean depth (V/A) of the test was greater than control, this variable was only significant at  p< 0.05. Dataset B, and C repeated the significance for area and volume as for Dataset A, but there was no significant difference for mean depth.

Numbers of Pits

Numbers of pits in the 43° C group were approximately 20% less than the control at 37° C. The 35° C group had approximately 6% less than controls. This small difference in numbers remains similar in all datasets. Pit numbers for both 39°, and 42° C, were similar to controls in all datasets. However this data can be disregarded because the experiments were not synchronous and the cells did not derive from the same animals at the same time.

Discussion

The experiments have shown that osteoclasts from pre-hatch chicks excavate deeper and larger volumes of lacunae in mineralised dentine at 41°C and 43°C, than at 37°C. The normal incubation temperature for the domestic chicken eggs is 39°- 40°C (Whitow 1976), the body temperature reaching adult levels after about 3 weeks at 41.5°C (Richards 1970).

The volume resorbed by an osteoclast in a single event is determined by the initial demineralisation phase (Jones et al 1995). The increase of volumes of pits in this experiment may be achieved by an increased rate and or timing of proton production, or a more efficient removal of the demineralised matrix: some decalcified material is always left over after the osteoclastic event.
Table 6-1. Experiment 1. Test temperature 35°C

<table>
<thead>
<tr>
<th></th>
<th>35°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=369</td>
<td>n=392</td>
</tr>
<tr>
<td>Area of pits</td>
<td>245.1</td>
<td>187.8</td>
</tr>
<tr>
<td>Volume of pits</td>
<td>947</td>
<td>755.8</td>
</tr>
<tr>
<td>V/A of pits</td>
<td>2.583</td>
<td>3.166</td>
</tr>
</tbody>
</table>

Significant difference of test to control (Mann-Whitney test). *p<0.0000

When volume < 10,000  #p<0.0001

Table 6-2. Experiment 2. Test temperature 39°C

<table>
<thead>
<tr>
<th></th>
<th>39°C</th>
<th>37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=400</td>
<td>n=400</td>
</tr>
<tr>
<td>Area of pits</td>
<td>334.2</td>
<td>267.9</td>
</tr>
<tr>
<td>Volume of pits</td>
<td>1376</td>
<td>1088</td>
</tr>
<tr>
<td>V/A of pits</td>
<td>2.997</td>
<td>2.9488</td>
</tr>
</tbody>
</table>

No significant difference of test to control (Mann-Whitney test).

Table 6-3. Experiment 3. Test temperature 41°C

<table>
<thead>
<tr>
<th></th>
<th>Test Temperature of 41°C</th>
<th>Control Temperature of 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=400</td>
<td></td>
</tr>
<tr>
<td>Area of pits</td>
<td>290.9</td>
<td>20.4</td>
</tr>
<tr>
<td>Volume of pits</td>
<td>1449</td>
<td>121</td>
</tr>
<tr>
<td>V/A of pits</td>
<td>3.955</td>
<td>0.106</td>
</tr>
</tbody>
</table>

Significant difference of test to control (Mann-Whitney test). ***p<0.0000

When pit volume < 10,000  #p<0.001, ##p<0.0000

Table 6-4. Experiment 4. Test temperature 43°C

<table>
<thead>
<tr>
<th></th>
<th>Test Temperature 43°C</th>
<th>Control Temperature 37°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=321</td>
<td></td>
</tr>
<tr>
<td>Area of pits</td>
<td>298.7</td>
<td>27.3</td>
</tr>
<tr>
<td>Volume of pits</td>
<td>1002.5</td>
<td>97.1</td>
</tr>
<tr>
<td>V/A of pits</td>
<td>2.632</td>
<td>0.0992</td>
</tr>
</tbody>
</table>

Significant difference of test to control (Mann-Whitney test). *p< 0.05, ***p< 0.0000

When pit volume < 10,000  # p<0.0000
The results indicate that chick osteoclasts function optimally at higher temperatures closer to and or greater than the species specific body core temperature. The use of restrictive temperatures for selection of biochemical activity is demonstrated in nature in the determination of sex in developing reptilian embryos, and in research in which mutant genes are encoded to activate at a permissive temperature.

The results of this study are limited by two factors. Ideally, the incubators for this study would be identical. Each test and control culture would be run in separate incubators in a single run. Only two incubators were available. The ideal control for this study would have been to run all the test and control cultures concurrently, with cells derived from the same source. This was not possible.

The data suggest that resorbing efficiency is increased at higher temperatures close to and slightly above the normal body temperature.
CONCLUSIONS

This thesis has focussed on various bone responses that may affect the success of endosseous dental implants. The concept of quality of the osseous implant bed, and the various factors that contribute to quality has been addressed within each of the chapters 2, 3, 4, 5, and 6.

The main findings are:

1. The hypotheses that trephine cores taken from sinuses grafted with allograft to create an implant bed would provide practical information was indeed proven. The topographical and morphological characteristics of cancellous trabeculae were observed to be mainly preserved in Irradiated Mineralised Cancellous Allograft. This allograft consistently formed new woven and lamellar bone on its surfaces when grafted in the sinus. At 6 months new bone formation was noted to be more consistent and greater in volume within 5mm height above the basal bone bed (original sinus floor), than that formed above this height.

2. Although no statistical inferences can be concluded due to the small sample size in this study, serum was found not to have any beneficial effect on new bone formation in the IMCA sinus graft as compared to saline.

3. Bone quality was found to be poorest in sub-sinus implant sites, for both bone mineralisation density and bone volume. Results for the mandible, at the ages studied, confirm its better characteristics in respect of both high mineralisation density and bone volume, for dental implants. Other sites were not greatly different in quality. Healing extraction sites achieved high mineralisation densities and volume fractions within 60 to 88 days.

4. A concept for quantitative bone quality scale was conceived, and demonstrated with an arbitrary scale of connectivity and values for a number of images.
5. The clinical application of Laser Doppler Flowmetry was able to detect positive blood flow in all regions of sinus grafts, dental implant recipient sites, and gingival and mucosal tissues of adjacent oral structures. Importantly, it also revealed the sinus membrane of the edentulous posterior maxilla to be very poor in vascularity.

6. A theoretical model of heat conduction predicted that at higher normal oral temperatures, heat transfer down titanium implants may produce temperatures in excess of 47°C at the bone-implant interface.

7. An osteoclastic resorption lacuna volume, area, and V/A assay at incubation temperatures of 41° and 43° C, showed that chick osteoclasts produce deeper lacunae, with increased volumes, than those formed previously and normally at the culture temperature of 37° C.

The collective results provide new information on the quality and quantity of jaw bone structure, Irradiated Mineralised Cancellous Allograft, and the effects of higher than normal temperatures on bone function that may have direct benefits for bone grafting and implant treatments in clinical practice.
Bibliography


sites implanted with intraoral autologous bone grafts or allografts. 15 human case reports. *Journal of Periodontology* 67: 1025-1033.


International Congress on Tissue Integration in Oral, Orthopedic, and Maxillofacial Reconstruction, Mayo Medical Centre, Rochester, Minnesota, 321-333.


174


176


Richelle L (1967) Contribution à l'é'tude du Mé'tabolismeMineral de l'os Chez le rat., PhD, Lie'ge, Lie'ge.


Slavkin HC (1972) The Comparative Molecular Biology of Extracellular Matrices. University of Southern California Maine Biological Laboratory, Santa Catalina Island U.S.A.


183


All donors have been screened and shown negative for the presence of active infectious disease, malignancies, degenerative neurological disease, and diseases of unknown etiology. Blood samples have been tested for HIV 1/2, Hepatitis B surface antigen, Hepatitis C antibody, HTLV 1 and Syphilis (STSBDPR/RPR) utilizing F.D.A. licensed test kits.

1. In the Rocky Mountain Tissue Bank processing protocol, every effort is made to ensure the safety of our tissue. However, current technologies may not preclude the transmission of infectious agents.

2. The use of this tissue is limited to hospitals, physicians, dentists or other qualified medical professionals.

3. Allograft has been irradiated.

4. Do Not Resterilize.

5. Store at Room Temperature.

6. It is the responsibility of the transplant facility and the clinician to maintain the allograft in the appropriate storage condition prior to transplantation.

7. Transport Tube should be opened and inner sterilized vial placed in sterile surgical field.

8. Once the container seal has been compromised, the tissue shall be either transplanted or discarded.

9. No Special Handling is required.

10. No additional preparation is necessary for transplantation, such as diluting or reconstitution.

11. For Single Patient Use Only.

**ADVERSE OUTCOMES ATTRIBUTED TO THE TISSUE MUST BE REPORTED PROMPTLY TO ROCKY MOUNTAIN TISSUE BANK.**

**A PATIENT TRACKING FORM ACCOMPANIES EVERY VIAL OF TISSUE. COMPLETE AND RETURN IT TO ROCKY MOUNTAIN TISSUE BANK IMMEDIATELY AFTER TISSUE IS TRANSPPLANTED.**

**IT IS THE RESPONSIBILITY OF THE TRANSPLANT FACILITY AND THE CLINICIAN TO MAINTAIN RECIPIENT RECORDS FOR THE PURPOSE OF POST-TRANSPLANT TRACING.**

***THANK YOU FOR YOUR COOPERATION***

(303) 337-3330  •  FAX (303) 337-9383  •  1 (800) 424-5169

Revision date: 07/11/96
Appendix 1(b)

ROCKY MOUNTAIN TISSUE BANK
2993 S. Peoria St., #390
Aurora, CO 80014
(303) 328-3330

PROCEDURE NO.: 120.01.05
ISSUE DATE: 11/01/95
SUPERSEDES: none

PAGE 1 OF 1

DONOR SELECTION POLICY

POLICY STATEMENT:

Rocky Mountain Tissue Bank ensures that organizations under contract for procurement of donated tissue provide those services in compliance with current donor assessment criteria as specified in the AATB Standards and the Federal regulatory requirements.

The following specific criteria will be considered for the acceptance of donated tissue:

Type of Tissue: RMTB requires donated human allografts from which cancellous bone tissue can be isolated.

Age Criteria: Male and Female donors between 12 and 75 years of age.

The American Association of Tissue Bank describes specific guidelines which include criteria of the US Public Health Service for the EXCLUSION OF DONORS whose tissues present high risk of disease transmission. The guidelines list the following factors for DONOR EXCLUSION:

- Infection or sepsis, by history, physical examination, and laboratory testing.
- History of intravenous drug use.
- History of hepatitis, syphilis, slow virus infection, AIDS, AIDS-related complex or high risk of AIDS.
- History of Autoimmune disease.
- Positive serology tests.
- Toxic substance in potentially toxic amounts in tissues to be collected.
- Evidence of serious illness of unknown cause.
- Death from unknown cause.

Other factors for EXCLUSION OF DONORS include: men who have had sex with men since 1977, past or present intravenous drug abusers, certain hemophiliacs, men and women who have engaged in sex for money or drugs since 1977, and sexual partners in the last 12 months of all persons in these categories.

Also excluded are persons who in the past 12 months have had syphilis, gonorrhea, or have been exposed to known or potentially HIV-infected blood.

References: American Association of Tissue Bank Standards
Food and Drug Administration interim Rules and Regulations
Rocky Mountain Tissue Bank Policy and Procedure Manual

Executive Director

Date

President/CEO

Date

Medical Director

Date
STERILIZATION POLICY

POLICY STATEMENT:

All allografts are to be aseptically procured and surgically removed in an aseptic environment. Before final processing the allografts are preserved at -70 degrees centigrade. Allografts remain in a frozen state until they are irradiated from a Cobalt 60 source with between 2.5 and 3.8 Megarads.

**Current American Association of Tissue Banks recommendation for irradiation sterilization is 1.5 Megarads or greater. (D2.500)**

Written verification that the sterilization parameters have been achieved are to be maintained in the donor file. (D2.500)

Written verification of the sterility cultures for each donor are to be maintained in the donor file. (B2.151)

References: Food and Drug Administration Interim Rules and Regulations
American Association of Tissue Banks Technical Manual
Rocky Mountain Tissue Banks Policy and Procedure Manual

Additional Reference Material:

Appendix 1 (d)

DONOR SEROLOGY TESTING

POLICY STATEMENT:

All donor blood specimens shall be tested for the following communicable disease serological markers by tests approved for such uses by the Food and Drug Administration:

- HEP Surface Antigen - HBsAg
- HCV Hepatitis C virus antibody
- HIV-1/2
- HTLV-1
- RPR

These tests are to be performed by a laboratory certified under the Clinical Laboratories Improvement Act 1988 (CLIA)

INTERPRETATION OF SEROLOGY TESTS:

Tissue from a cadaveric donor with a repeatedly reactive HBsAg, Anti-HIV 1, Anti-HIV-2, Anti-HCV, or Anti-HTLV-1 screening test shall not be used for transplantation, regardless of the results of supplemental/confirmatory assays.

Tissue from a cadaveric donor reactive for syphilis on a screening assay shall be used only if the sample is found to be negative on a F.D.A. licensed treponemal specific confirmatory test.

Reference: Food & Drug Administration Interim Rules & Regulations
American Association of Tissue Banks Standards
Rocky Mountain Tissue Bank Policy & Procedure Manual

Executive Director

Date

President/CEO

Date

Medical Director

Date

190
The examiners for this thesis were, Professor S, Sindet-Pedersen, Head of The Department of Oral and Maxillofacial Implantology, Eastman Dental Institute, University College London, and Professor J. F. McCord, Head of Unit of Restorative Dentistry for the Elderly, Manchester University Dental Hospital.