Sol–gel based materials for biomedical applications

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Abbreviations: AAM, anodic alumina membrane; AP, 3-aminopropyl; ASO, antisense oligonucleotides; BCP, biphasic calcium phosphate; BG, bioactive glass; BMSC, bone marrow mesenchymal stem cell; cAMP, cyclic adenosine monophosphate; CaP, calcium phosphate; CHO, Chinese hamster ovary cells; CNT, carbon nanotubes; CTAB, cetyl trimethylammonium bromide; CT, computer tomography; DDS, drug delivery system; DMHA, N,N-dimethylhexadecylamine; DNA, deoxyribonucleic acid; DOX, doxorubicin; ECM, extracellular matrix; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; F127, Pluronic F-127; FAP, N-folate-3-aminopropyl; FITC, fluorescein isothiocyanate; GP, guanidinopropyl; GEGP, 3-[N-(2-guanidinoethyl)guanidine] propyl; GFP, green fluorescent protein; HA, hydroxyapatite; HCA, carbonated hydroxyapatite; INDO, intermediate neglect of differential overlap; MCM, mobile composition of matter; MBG, mesoporous bioactive glass; MNP, magnetic nanoparticle; MRI, magnetic resonance imaging; MSN, mesoporous silica nanoparticle; MTES, methyltriethoxysilane; MTMS, trimethoxymethylsilane; NC, network connectivity; NHS, N-hydroxysuccinimide; NIR, near infrared; NLR, long rod nanoparticles; NMR, nuclear magnetic resonance; NP, nanoparticle; NSR, short rod nanoparticles; P123, Pluronic® P-123; PAMAM, polyamidoamine; PAA, polyacrylic acid; PCL, poly(e-caprolactone); PDMAAm, poly(N,N-dimethyl acrylamide); PDMPS, polydimethylsiloxane; PEG, polyethylene glycol; PEL, polyethyleneimine; PET, position emission tomography; PLL, poly(l-lysine); PLLA, poly(l-lactic acid); PMMA, poly(methyl methacrylate); PS, polystyrene; PVA, polyvinyl alcohol; PVP, polyvinylpyrrolidone; QD, quantum dots; RBC, red blood cells; RNA, ribonucleic acid; ROS, reactive oxygen species; SBF, simulated body fluid; siRNA, small interfering RNA; SPECT, photon emission computed tomography; SPION, superparamagnetic iron oxide nanoparticles; TEA, triethanolamine; TEM, transmission electron microscopy; TEOS, tetraethoxy orthosilicate; TMB, trimethylbenzene; TMES, trimethylethoxysilane; TMOS, tetramethyl orthosilicate; TTCP, tetracalcium phosphate; TTP, titanium tetrabutoxide; β-TCP, β-tricalcium phosphate; 3D, three-dimensional.

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SOL–GEL CHEMISTRY OFFERS A FLEXIBLE APPROACH TO OBTAINING A DIVERSE RANGE OF MATERIALS. IT ALLOWS DIFFERING CHEMISTRIES TO BE ACHIEVED AS WELL AS OFFERING THE ABILITY TO PRODUCE A WIDE RANGE OF NANO-/MICRO-STRUCTURES. THE PAPER COMMENCES WITH A GENERALIZED DESCRIPTION OF THE VARIOUS SOL–GEL METHODS AVAILABLE AND HOW THESE CHEMISTRIES CONTROL THE BULK PROPERTIES OF THE END PRODUCTS. FOLLOWING THIS, A MORE DETAILED DESCRIPTION OF THE BIOMEDICAL AREAS WHERE SOL–GEL MATERIALS HAVE BEEN Explored AND FOUND TO HOLD SIGNIFICANT POTENTIAL. ONE OF THE INTERESTING FIELDS THAT HAS BEEN DEVELOPED RECENTLY RELATES TO HYBRID MATERIALS THAT UTILIZE SOL–GEL CHEMISTRY TO ACHIEVE UNUSUAL COMPOSITE PROPERTIES. ANOTHER INTRIGUING FEATURE OF SOL–GELS IS THE UNUSUAL MORPHOLOGIES THAT ARE ACHIEVABLE AT THE MICRO- AND NANO-SCALE. Subsequently the ability to control pore chemistry at a number of different length scales and geometries has proven to be a fruitful area of exploitation, that provides excellent bioactivity and attracts cellular responses as well as enables the entrapment of biologically active molecules and their controllable release for therapeutic action. The approaches of fine-tuning surface chemistry and the combination with other nanomaterials have also enabled targeting of specific cell and tissue types for drug delivery with imaging capacity.

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1. Introduction

In recent years, biomaterials have gained increasing interest within biomedical sciences and across various scientific disciplines. One reason for this is based on an ever increasing demand for regenerative or replacement tissues. Degenerative diseases and injuries have driven the need for more successful and cost effective treatments and this has, in turn, spurred the development of versatile methods to produce bioactive matrices and interfaces with improved functionality. It is therefore not surprising that research into biomaterials has contributed to the development of materials science as a highly interdisciplinary subject utilizing new approaches to previously exhausted synthesis routes. One key development that serves best to exemplify this fact is the use of sol–gel science for biomedical applications. This review therefore focuses on developments and the potential applications of research within this field.

Since its inception, sol–gel methods have typically involved the use of tetraethyl orthosilicate (TEOS) as a principal network forming agent. The first reason for using TEOS is that the formation of robust networks with moderate reactivity and a high degree of control are afforded through simple variations in the synthesis conditions such as pH, temperature, and additives [1–3]. For example, silica-based glass networks possess these properties and have thus provided a basis for some of the most successful bioactive substrates [4–6]. Other reasons can be found in the ease of incorporation of organic molecules or moieties, polymers, and biomolecules or cells, and this ability is due to the liquid-phase reactions that can subsequently be processed at low temperatures [2,3]. Silica and doped silica materials obtained via the solution–gelation (“sol–gel”) inorganic polymerization process are also highly functional materials with an impressive range of applications, and utilize two of the pillars of chemistry: synthesis and analysis. However, a wide range of other areas also make use of this same route. The synthesis processes can also occur under very mild conditions, so it can be used to obtain products of various sizes, shapes, formats, and, ultimately, applications.

From a purely physical perspective, sol–gel methods are particularly useful because they permit direct fabrication of multicomponent materials in different configurations (monoliths, coatings, foams, and fibers) without powder intermediates [2,3,7] or the use of expensive processing technologies, such as vacuum methods. The diversity of materials which can be obtained, has made the sol–gel method an important synthesis route in several domains of research, including optics, electronics, semi-/super-conductors, and biomaterials. In this instance, methods have no bearing on the final chemical composition of the material, rather the way that the material is obtain provides the benefit.

Sol–gel science dates back over 150 years. The term “sol–gel” was first coined by Graham in 1864 during his work on silica sols [8]. Although in 1640 van Helmont had discovered “water glass” by dissolving silicate materials in alkali and then precipitating silica gel upon acidification [9]. It was in 1846 when Ebelmen observed the formation of a transparent glass following exposure to the atmosphere of a silane obtained from SiCl₄ and ethanol [10,11] when true sol–gel experiments first began. Patrick, during his doctoral studies at the University of Goettingen in 1912–1915, to devise an economically viable and rapid sol–gel method to make silica gel from sodium silicate (Na₂SiO₃) in large quantities [12] and Kistler [13] described the first synthesis of a highly porous silica (SiO₂) form, which he dubbed “aero-gel”, by supercritically drying the gel obtained by hydrolytic polycondensation of silicic acid [Si(OH)₄]. Each of these efforts can be credited as the starting point for the modern sol–gel approach and serve to demonstrate a long history which is now coming to fruition for biomedical applications.
Sol–gel methods also enable powderless processing of glasses, ceramics and thin films or fibers, directly from solution. Precursors are mixed at the molecular level and variously shaped materials may be formed at much lower temperatures than is possible by traditional preparation methods. One of the major advantages of sol–gel processing is also the possibility to synthesize hybrid organic–inorganic materials. Combinations of inorganic and organic networks facilitate the design of new engineering materials with diverse properties for a wide range of applications. Biomedical applications invariably require the design of new biomaterials, and this can be achieved by merging sol–gel chemistry and biochemistry. The gel derived materials are excellent model systems for studying and controlling biochemical interactions within constrained matrices with enhanced bioactivity because of their surface chemistry, micro-/nano-pores and large specific surface area [5]. In biomedical applications, the coating of medical devices is an important issue. Materials used in medical devices should have appropriate structural and mechanical properties and ideally promote a healing response without causing adverse immune reactions. Medical device designers currently use various surface treatments such as coating that enhance or modify properties such as lubricity, the degree of hydrophobicity, functionalisation and biocompatibility. Sol–gel technology offers an alternative technique for producing bioactive surfaces for these applications.

Sol–gel thin film processing offers a number of advantages including low-temperature processing, ease of fabrication, and precise microstructural and chemical control. The sol–gel derived film or layer not only provides a good degree of biocompatibility, but also a high specific surface area (which can be used as a carrier of adsorbed drugs) and an external surface whose rich chemistry allows ease of functionalization by suitable biomolecules. For example, several research groups [14–17] have described the synthesis of thin, resorbable sol–gel films with controlled release of bactericidal properties on a Ti-alloy substrate, and determined the effect of processing parameters on the degradation and drug release with a close correlation between the two, suggesting the sol–gel processing can be used to control drug release.

The development of multifunctional nanoparticles that can be used as drug delivery vectors remains a significant challenge for material science. For example, superparamagnetic nanoparticles are invaluable to biomedical applications such as in magnetic resonance imaging (MRI), targeted drug delivery and magnetic separation [18,19]. These applications require intimate control of the particle size, and discrete, superparamagnetic iron oxide nanoparticles that can be prepared by the sol–gel method to lower the annealing temperatures required. Silica-based magnetic nanocomposites, formed by magnetic nanoparticles (MNPs, either \( \text{Fe}_3\text{O}_4 \) or \( \gamma\text{-Fe}_2\text{O}_3 \)) dispersed in a silica matrix, are of relevant technological and scientific interest. Here, encapsulation in silica prevents interactions between the MNPs, and consequently assures a uniform dispersion. The latter is essential for efficient performance in most applications, including diagnostic and therapeutic, where particles must display high magnetization, be stable against oxidation, and, most importantly, remain non-aggregated [20,21].

Engineering new bone tissue with cells and a synthetic extracellular matrix (ECM) represents a promising approach for the regeneration of mineralized tissues. Bone regeneration requires a scaffold material upon which cells can attach, proliferate and differentiate into functionally and structurally appropriate tissues for the body location into which they are placed. Bone is a highly mineralized tissue consisting of an apatitic mineral phase most similar to a form of carbonated hydroxyapatite (HCA), although a significant contribution is made by extraneous ions such as sodium, chloride, zinc and, to a lesser extent, fluorides. In general, HCA can be considered a model mineral for natural bone and is widely accepted as a bioactive material with excellent biocompatibility, high osteoconductivity, and reasonable mechanical strength [22–25]. For these reasons it has widely been used in tissue engineering applications, especially for bone and cartilage regeneration. However, in vivo data suggest that degradation or ion release from labile sources such as bioactive glasses (BGs) promotes new bone formation, as opposed to the relatively lower ion release that occurs as a result of HCA minerals reaching equilibrium with their surrounding medium. It appears that sol–gel methods hold the potential to apply an ever-increasing range of glass-based bioactive coating to materials, which have previously remained incompatible with alternative coating techniques. Furthermore, the versatility of the sol–gel approach is opening new doors to previously unattainable compositions [26], again increasing the potential applications of sol–gel materials as fillers to replace tissue within necrotic or defect sites.
The sol–gel microencapsulation technology and its broad application potential are now well-
known [27]. Relevant here is the use of silica for encapsulation and controlled release of both hydro-
philic and hydrophobic molecules, ensuring considerable chemical and physical protection of the
valued entrapped dopants. Given the above, it can be seen that sol–gel derived bioactive materials
hold great potential value. However, there are still significant hurdles to overcome, particularly with
respect to the economic viability of up-scaling sol–gel production. Due to the price of precursors, the
production of glass via the sol–gel process will cost approximately 100 times that of glass obtained by
conventional melting and casting. As a consequence, the production of glass via the sol–gel process
only makes sense economically when addressing high value-added products.

This review highlights the usefulness of the sol–gel route for preparing bioactive NPs, powders, coat-
ings, foams, bulks and the hybrids for biomedical applications as well as for designing more biocompat-
ible and biofunctional materials to heal and regenerate diseased and damaged tissues. The goal is that
the materials covered herein will provide a critical review of the progress, stimulate further research in
the field, and inspire for yet further applications for a method that has yet to reach its full potential.

2. Sol–gel methods and chemistry

One group of biomedical materials are those that can be classified into arising from a non-
biological source but that are ultimately applied in vivo [28]. There are currently a wide range of syn-
thesis routes that are able to produce such materials, and sol–gel is one such synthesis route. In their
simplest terms, all sol–gel methods involve two distinct phases: solution and gelation. As the name
implies, a sol is a colloidal suspension of solid particles, whereas a gel is an interconnected network
of solid phase particles that form a continuous entity throughout a secondary, usually liquid, phase.
Throughout sol–gel technology, these phases are conserved though the chemical reactions that take
place during the gel evolutions, and can be manipulated in a variety of ways; for instance, altering
the initial precursors, time allowed for gelation, catalysts, degree of solvation, gelation conditions or
physical processing of the gel itself. Sol–gel processes allow formation of solid materials through gela-
tion of solutions and can be used to produce a large number of useful morphologies (Fig. 2.1).

The processes illustrated in Fig. 2.1 are by no means limiting or exhaustive. Depending on the
specific application, these stages can be extended, altered, or with the exception of solvation and gela-
tion, removed entirely. What remains constant for the production of sol–gel derived bioactive mate-
rials are the stages that allow for hydrolysis and condensation reactions to occur. Successful
manipulation of these reactions was first achieved by Stöber et al. [29] who, by applying alkoxy precursors (both TEOS and TMOS) in the presence of an ammonium hydroxide catalyst, successfully produced colloidal suspensions of nanoscale silica particles. This work was based on earlier observations that TEOS will react to form silica and that the reaction is further favored under basic conditions [30]. Secondary to the purpose of this work, gelation was also observed [29] although this was, at the time, considered an undesirable hazard of a process that aimed to form discrete silica microspheres (i.e. for the purposes of developing compression technology, a sol–gel derived fiber binder for thermal insulation) [31]. Simple silica microspheres have since seen a plethora of applications, and today, the benefits that are provided by the gelation are well recognized. In addition, advanced processing routes have also been developed that are able to account for or exploit the “undesirable” aspects of the gelation process.

This section aims to describe the sol–gel synthesis routes that are most commonly used to produce ceramic and glass networks for biomedical applications. In addition to providing an overview of the polymerization processes, the use of classical inorganic synthesis routes and colloidal aggregation will be discussed along with adaptations to the synthesis procedures that have allowed for yet further applications. Common links between methodologies are emphasized and techniques themselves are exemplified through some of their most recent uses.

2.1. Organic precursors in sol–gel methods

Silicon alkoxides represent the main network forming agents used in sol–gel preparation methods [5,32,33]. While the sol–gel process provides key benefits, such as the low synthesis temperatures and the vast array of alkoxide precursors available, the cost associated with alkoxide precursors presents some limitations [5,34]. Nevertheless, the efficiency provided by low temperature synthesis and the accuracy with which specific compositions can be achieved have the potential to outweigh any such negative aspects of the process. Low temperature synthesis is achieved through solution-mediated formation of strong covalent bonds between elements that would otherwise require excessively high temperatures to create. For alkoxides, this requires initial hydrolysis of the alkoxy group followed by a condensation between network forming substrates. Fig. 2.2 illustrates the hydrolysis and steps involved in the formation of a basic silica glass network from an alkoxy precursor, TEOS. For the purposes of clarity, the reaction of TEOS with water is used as an example although it is important to note that several functionalised leaving groups are applicable to this process [5].

As is evident from Fig. 2.2, water is essential to the process. In order control polymerization, the medium used to produce the gel must therefore remain largely anhydrous. However, some water must also be present in order to allow the hydrolysis reaction to propagate. Once hydrolysis of the ester linkages starts, subsequent condensation reactions (Fig. 2.3) are able to generate enough water to allow the process to continue without replenishing the water lost during hydrolysis [31]. Hydrolysis and condensation therefore form a chain reaction, which is of critical importance to enabling initial
control over the process and network structural evolution, as an ability to delay polymerization allows
for the specific composition of the solution to be tailored ensuring both homogeneity and unlimited
functionality of the end product.

Referring to Fig. 2.2 again, hydrolysis itself occurs as typical $S_N^2$ reaction, whereby the addition of
$H_2O$ results in a hypervalent substrate. The central atom of the substrate (e.g., silicon in the case of
TEOS) takes on a partial negative charge, whereas the oxygen in $H_2O$ becomes partially positive. This
nucleophilic addition has important implications when considering the role of catalysts in the sol–gel
process [35]. The variation in charge causes instability in the hypervalent substrate molecule that is
immediately rectified by the transfer of a proton from the nucleophile ($H_2O$) to the ester bond of an
opposing alkoxy group. Cleavage of the single $\sigma$-bond between the opposing alkoxy (i.e., the leaving
group) and the substrate then occurs by the addition of the proton from the nucleophile itself. This
results in the formation of an available $HO^-$ group on the substrate and an alcohol from the alkoxy
leaving group. The fact that both reactions occur simultaneously defines hydrolysis of alkoxides as
$S_N^2$ reactions. Conversely, condensation does not proceed by this same mechanism.

As Fig. 2.3 shows, only one reaction occurs during condensation: the loss of an $HO^-$ group from the
substrate. This mechanism is therefore an $S_N^1$ reaction and can occur as either dehydration or dealco-
holation [36]. For the former to occur, two $HO^-$ groups must take part in the formation of an $Si$–$O$–$Si$
bond, whereas the latter results from the direct transfer of proton to the leaving group on a neighboring
substrate. Evident from these reactions, a decrease in $pH$ can promote hydrolysis through protona-
tion of the leaving groups (therefore reducing the stability of the ligand in question). Alternatively,$OH^-$ is a highly efficient nucleophilic species and electron transfer from $–OH$ groups can be facil-
itated by $H^+$ in the immediate environment. This relationship means that higher and lower $pH$ is also
able to promote condensation and hydrolysis, respectively. In silica based systems the reactions pro-
cceed by acidic catalysis at $pH < 2.5$ and basic catalysis when $pH > 2.5$, which can be explained by the
isoelectric point of silica ($pH = 2.5$) [37]. In the sol–gel route synthesis, a stepwise reaction scheme has
been undertaken to control the ratio of hydrolysis to condensation rates [38]. In general, the rate of
hydrolysis is fast compared to that of condensation in strong acidic conditions. Therefore, a well-
ordered hexagonal arrangement of mesopores (a pore structure that is commonly formed in sol–gel
silica materials) is formed at low $pH$ in acidic conditions. Meanwhile, in neutral or basic conditions
ranging from $pH 7$ to $pH 9$, the rate of condensation is faster than that of hydrolysis, and eventually

Fig. 2.3. Subsequent condensation stages of TEOS in the production of silica oligomers. Condensation between silanol groups on
two hydrolyzed TEOS molecules (a and b) and between a silanol group and an adjacent alkoxy group (c and d) result in the
production of free $H_2O$ and ethanol respectively.
the materials prepared by a single-step reaction at high pH display gel-like structure often without mesopores. It is hence an interesting attempt to synthesize ordered mesoporous materials by a two-step sol–gel route at a lower acidic pH followed by a higher pH. Up to now, however, a two-step sol–gel reaction has not been applied for the fabrication of ordered mesoporous structure, because the reaction mechanism is not yet fully understood with most well-ordered silica structures that have been achieved under strong acidic conditions. Expectedly, reaction kinetics reaches a minimum at the isoelectric point [39]. Thus the promotion or inhibition of alkoxy polymerization can be tailored to the substrate of interest by choosing a pH that provides the optimal set of reaction kinetics.

Several other silane oligomers apart from TEOS can be used to produce a similar result [5,40–45] with the prerequisite being a set of functionalised groups capable of taking part in the hydrolysis and condensation reactions. In addition to silicate, phosphate-based materials may be used for the same purpose [26,46], as can various transition metal elements [5,40,43,46–48] (as shown in Table 2.1). Network modifiers (such as magnesium and calcium) can be introduced to the network in salt form [40] or as alkoxides. However, the higher electronegativity of transition metal species in comparison to silicone and phosphorous can cause issues where condensation reactions proceed with an unfavorable bias away from the desired network composition or the end particulate structure [44,49]. Fortunately, the versatility of the sol–gel process has allowed for phenomena such as steric hindrance, chelation and selective catalysis to be exploited and enable viable production of a far greater range of materials than is possible with traditional synthesis methods alone. These benefits have led to the development of a series of methods that exploit differing chemistries to enable efficient and effective production of specifically-tailored bioactive materials.

Like silicon, phosphorous and vanadium precursors can be used as network-formers within the sol–gel process [44,46,50,51]. However, the σ–π double bond common to the latter two substrates reduces the expected coordination number and further produces repulsion between the oxygen atoms coordinated under adjacent σ bonds. This difference in network connectivity results in a more relaxed network structure when compared to silica based networks [52], as silicate is able to share all 4 oxygen atoms with neighboring cationic groups [53]. This in turn increases the range and quantity of species that are able to be included, but such flexibility comes at the expense of stability, as the high electronegativity of the =O bond leaves the network open to hydrolysis. As with conventional melt-derived materials, solubility can be controlled by the combination of network modifiers, as can the release of active agents or tailoring of other physical properties of the material in question.

Nandy et al. [43] exploited the effects of steric hindrance on basic ammonium catalysts to control the textural properties of mesoporous silica. The approach of these authors was to reduce the catalytic

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Leaving group (R–)</th>
<th>Structure</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Tetraethyloxysilane (TEOS)</td>
<td>CH₂–H₃–OH</td>
<td><img src="image" alt="Tetraethyloxysilane" /></td>
<td>Nandy et al. [43], Stöber et al. [29], Choi et al. [38]</td>
</tr>
<tr>
<td>Tetramethoxysilane (TMOS)</td>
<td>–CH₃</td>
<td><img src="image" alt="Tetramethoxysilane" /></td>
<td>Stöber et al. [29], Choi et al. [38]</td>
</tr>
<tr>
<td>di-Butylphosphate</td>
<td>–CH₂–CH₂–CH₂–CH₃</td>
<td><img src="image" alt="di-Butylphosphate" /></td>
<td>Pickup et al. [46], Foroutan et al. [26]</td>
</tr>
<tr>
<td>Titanium tetraisopropoxide</td>
<td>–CH–(CH₃)₂</td>
<td><img src="image" alt="Titanium tetraisopropoxide" /></td>
<td>Nabavi et al. [44], Foroutan et al. [26], Han et al. [54]</td>
</tr>
<tr>
<td>Vanadium O(Am⁺)₃</td>
<td>–C(CH₃)(CH₂–CH₃)CH₃–OH</td>
<td><img src="image" alt="Vanadium O(Am⁺)₃" /></td>
<td>Livage [51], Nabavi et al. [44]</td>
</tr>
</tbody>
</table>
effect of NH$_3$ on TEOS hydrolysis with the use of primary, secondary and tertiary amines. Like silanes, the addition of further organic groups on the amine reduces its capacity to act as a Lewis base. However, the steric effects of bound organic groups also prevent the nucleophilic base from entering a reactive field with the substrate; hence, sterically hindering catalytic activity. More directly, the concept that steric properties can influence nucleophilic reactions is well known in a number of fields [55]. As a result, the Tolman cone angle has been proposed as a means by which the reactivity of a complex can be predicted [56], although this is not to say that such a measure can be applied to the sol–gel process as whole. As noted above, a number of factors are able to influence the process with respect to the steric properties of the ultimate leaving groups and represent only one aspect of the system.

The solvent itself also plays an important role in determining the rate of gelation reactions [37,45,54,57]. This solvation effect can occur in two ways: through viscosity and hydration effects. For example, NiO$_2$ particle size was successfully directed by Talebian and Kheiri [45], who by employing a series of solvents with increasing viscosity, were able to increase the particle size in a controlled manner. However, the solvent was also shown to alter the NiO$_2$ crystalline structure, which serves to highlight the need for experimental confirmation as, with such a variety of avenues available to exploit, comes as set of variable that must also be controlled. The effects of viscosity, or more precisely the dielectric constant, have also been recently described by Han et al. [54]. These authors made use of non-contingent esterification between the solvent (an alcohol) and sulfuric acid to provide water for polymerization of the titanium-based network. In doing so, results were confounded through the porogenic nature of water itself. However, what was demonstrated clearly was the influence of the solvent on the physical structure of the particles produced. A more definitive explanation was put forward by Nabavi et al. [44] who identified the ability of metal alkoxide to exchange leaving groups under solvation. By altering the solvent species from that of the alkoxide itself, the substrate can become coordinated with a mixture of alkoxy groups. Undoubtedly, this would affect polymerization in a way that is dependent on specific combination of coordinated groups present on network-forming substrates throughout the solution.

2.2. Colloidal sol–gel methods

Colloidal solutions can be defined as solutions containing discrete particles that do not settle, but remain suspended for several years, unless induced to do otherwise [31]. Depending on the size of the colloidal particles, (which usually range from 1 nm to 1 µm), those with a density greater than that of the solvent can be maintained in suspension through the effects of Brownian motion alone; however, factors specific to the surface chemistry of the colloids themselves also make a significant contribution. Work within this area first began in the 1900s and was initially followed by efforts that aimed to control both particle size in absolute terms and the uniformity of the sols that could be produced. Today, methods of producing uniform particle sizes are further understood [36,48] with colloidal sol–gel methods remaining an active area of research.

Ludox was the first commercial colloidal suspension of silica available on the open market. This product offers a range of potential applications; the most pertinent to biomedical application is its ability to form condensed silica networks upon drying. This property results from condensation of –OH groups present on adjacent silica particles. The initial Si–O–Si bridges that are formed can be further strengthened by passive deposition of silica on the initial bridge as a result of the equilibrium silicic acid (Si(OH)$_4$) and the silica making up the mass of the colloids. It is important to note the delineation between this process and Ostwald ripening; the latter being influenced by a propensity to increase the thermodynamic stability of the system and thus showing a preference to enlarge the size of discrete particles. Furthermore, colloidal sol–gel methods are not limited to silica or silica-based systems [50].

The applicability of the colloidal methods is based on two key aspects of the process: stabilisation of the colloidal particles within the sol and coalescence or flocculation to form the gel. With particles that possess the same electrostatic charge, colloidal suspensions are maintained by the ζ-potential, which in turn reflects the magnitude of the electrical double layer present on charged particles in solution. As noted above, the removal of the solvent is one method by which the aggregation of colloidal particles can be achieved. Alternatively, altering the pH, salinity or temperature can induce depeptisation,
whereby the electrical double layer is reduced to a point that the \( \zeta \)-potential is no longer strong enough to prevent attractive van der Waals forces and flocculation takes place \([31,50]\).

Despite the potential of colloidal methods to provide much thicker, more structurally resistant films and deposits than methods that rely on de novo synthesis, this route has seen the least number of applications within biomedical research. This is not to say that the methods have fallen out of fashion completely. Based on the need to retain protein conformation, Liu and Chen \([33]\) explored the use of colloidal Ludox\textsuperscript{SM-30} to encapsulate cytochrome C, myoglobin, haemoglobin and catalase using an acidified solution of water glass (\( \text{Na}_2\text{SiO}_3 \)) to enhance network formation between the silica particles already present within a colloidal suspension. These authors were able to show an enhanced preservation of protein conformation in comparison to direct synthesis from alkoxide precursors. The gelation rate correlated well with the structural stability of the encapsulated proteins and served to demonstrate an ability to circumvent conditions that would otherwise damage sensitive molecules. For certain applications however, the colloidal sol–gel method does not provide the degree of protection required. For example, living cells are extremely sensitive to their immediate environment. Aside from pH, temperature, nutrient availability, and specific growth factors, salinity alone can rupture cellular walls resulting in immediate death of the organism. Colloidal methods offer a further benefit over alkoxide based systems in that the majority of the network is already present in the sol. Adaptations such as the introduction of osmoprotectants can therefore be applied without significantly interfering with the integrity of inorganic capsule itself \([58]\).

### 2.3. Metal chelation in the sol–gel methods

In aqueous solution, metal ions are coordinated by a hydration shell, the nature of which depends both on the valence of the specific metal in question and the pH of the solution \([59]\). The unique combination of the polar solvent coordination and the ability of hydrated metal cations to act as Lewis acids enable the processes of olation and oxolation to take place. This ultimately results in the formation of polymeric oxides in solution \([60]\). The exact series of reactions that take place are complex and may involve the formation of several aquo- \( [\text{M(H}_2\text{O})_n]^{\text{z}+} \), hydroxo- \( [\text{M(H}_2\text{O})_n(\text{OH})_{m-n}]^{\text{z}+} \) and oxo- \( [\text{M(H}_2\text{O})_n(\text{O})_{m-n}]^{\text{z}+} \) ligands or combination thereof. For a detailed description of the chemistry evolved in the polymerization of hydrated metal ions the reader is referred to Livage et al. \([49]\). In most basic terms, solvated metal ions \( ([\text{M(H}_2\text{O})_n]^{\text{z}+}) \) are able to displace \( \text{H}_2\text{O} \) from neighboring complexes \( ([\text{M(H}_2\text{O})_n]^{\text{z}+}) \). With the formation of a hydroxy-ligand \( ([\text{M(H}_2\text{O})_n(\text{OH})_{m-n}]^{\text{z}+}) \), \( \text{–OH} \) is able to act as a bridge between the two hydrated metal complexes. This results in the release of a proton into the aqueous medium and is followed by a subsequent deprotonation event to from a resultant \( \text{M-O-M} \) covalent bond.

From this brief description alone, the influence of pH on the process can also be deduced; an increase in pH favoring olation with lower pH inhibiting the process. As noted above however, the polymerization process seldom proceeds smoothly throughout aqueous media. Localized shifts in the immediate vicinity of the complex undergoing polymerization leading to the formation of heterogeneous ceramic aggregates, as opposed to an even dispersal that can be produced when sol–gel methods are applied \([35,36]\). For biomedical applications, sporadic synthesis is by no means ideal to the end function of the material (i.e., as biocompatible coatings, tissue scaffolds or novel composite materials).

Undoubtedly, materials composed of metal oxides exhibit a wide range of desirable properties \([5,31,32,36,61]\) and as a result, a series of methods have been developed based on chelation of metal precursors in order to control the natural polymerization processes. Essentially, metal chelation sol–gel methods employ strong chelating agents (such as citric acid or EDTA) as a means of controlling the formation of the highly reactive hydrated complexes \([36,59]\). Although discussed here in terms of chelated inorganic precursors, chelation itself is not limited to inorganic processes. Such methods can also be applied to modify the polycondensation of metal alkoxides whereby the rate of reaction is reduced following the replacement of alkoxide leaving groups with a chelating ligand in more stable conformation \([62]\).

In further discerning metal chelation methods from the alkoxide sol–gel route, the underlying principle is that polycondensation of the metal itself occurs through hydration processes described above
Fig. 2.3) as opposed to the hydrolysis and condensation steps akin to the polymerization of organo-metallic precursors. Based on a system that made use of triethanolamine (TEA) as an Fe(III) chelating agent, El Haskouri et al. [63] were able to produce mesoporous iron-phosphate–phosphonate materials via a distinct metal chelation sol–gel route. The mesoporous nature of the material was achieved with use of a surfactant system involving TEA and cetyl-trimethylammonium bromide (CTAB); however, the incorporation of Fe(III) within the material itself was made possible through the chelation of the metal with the TEA aspect of the system. Furthermore, the viability of synthesis within a phosphate based network offers a pathway for the design of biomaterials that completely degrade in aqueous media such as biological fluids. Clearly, adjustments to these procedures would require careful planning of the factors that influence the process ab initio. Nevertheless, TEA is able to form chelation complexes with a wide range of transition metal elements [64] therefore offering a plausible route for further biologically relevant substitutions within the network.

The use of epoxides as gelation agents provides another useful synthesis pathway. Typically, epoxide routes are most effective when the formal oxidation state of the dopant cation is M^{3+} [34,57] although species that possess a lower valence may also be incorporated. In this instance, the epoxide does not act as a precursor per se rather the epoxide group is able to efficiently accept protons leading to the formation of hydrated oxo-ligands \([\text{M(H}_2\text{O})_n\text{O}]^{z+}\). The pH of the reaction medium can therefore be adjusted to a range that favors the less reactive aquo-[\text{M(H}_2\text{O})_n]^{z+} coordination until deprotonation in the presence of an epoxide. Although not strictly a chelation-based method, parallels can be observed between this approach and those more typical chelation methods that aim to prevent natural polymerization processes until required.

2.4. Polymer-assisted sol–gel methods

In a natural extension from metal chelates methods are the polymeric sol–gel methods. Essentially, these methods involved the chelation of reactive inorganic gel-forming agents within an organic polymer network although, depending on the material to be produced, chelation is secondary to the stabilisation [65]. In more broader terms, gel-forming agents are maintained in a state of dispersion throughout the solution, thereby preventing the precipitation of aggregates within the sol [36]. This method does however require subsequent heat treatment to remove the organic polymer following the formation of the inorganic gel. Recently, Yang et al. [66] successfully synthesized nanoscale LiMn_2O_4 particles using Pluronic P-123 as stabilising polymer and citric acid as a chelation agent. The nanoparticle exhibited a superior degree of porosity when compared with particles that were synthesized without the presence of the polymer network. Whilst not a biomedical application, the ability to control the textural aspects that influence functionality was clearly demonstrated.

Chemical properties, such as the biomimetic molar ratios of apatites, can also be achieved with polymer assisted stabilisation [65] due to the homogeneous elemental distribution of the gel network. For example, Valliant et al. [67] applied \(\gamma\)-glutamic acid polymers ranging in molecular weight between 30 and 120 kDa to control the spatial distribution of calcium within a typical silica sol–gel network synthesized via a TEOS precursor with 3-glycidoxy-propyl-trimethoxysilane as the covalent coupling agent. Their results showed a homogenous distribution of calcium in the silica network, and the larger polymers also appeared to show reduced degradation rates. Here, a biocompatible polymer was purposefully chosen so as to remain within the network and thus avoid processing complications encountered during thermal treatment.

Inorganic networks can also be formed in situ through the polymerization of organic precursors [36]. Originally patented by Pechini [68], this method involved the formation of a three-dimensional (3D) polyester network as a result of the reaction between ethylene glycol and citric acid; the citric acid acting as a chelation agent due an available bi-dentate binding mechanism followed by an esterification with ethylene glycol (Fig. 2.4). The organic network can then be removed as with ex situ polymers described above.

This route offers a robust and effective means of synthesizing materials that disperse poorly in viscous solutions or that would otherwise form reaction products prior to assembly into the required form. This is especially useful for the calcium phosphates (CaPs) which have a tendency to form a diverse range of minerals when present at low concentration in aqueous solution [69]. This family
of minerals are extremely important from a biomedical perspective, as they allow for true hard tissue integration and bonding to take place and is utilized in orthopaedics and dentistry for direct implant bonding [48]. Effective production of both biocompatible ceramic-mineral composites [70] and apatites with predefined stoichiometry has been achieved [65,71] with polymer-assisted sol–gel methods. Such research may also have broader implications than in solving issues associated with biocompatibility as, with the advent of controlled deposition of inorganic mineral layers, abiotic hard tissue regeneration may also be within reach.

3. Sol–gel based materials

3.1. Silica-based materials

Silica-based sol–gel materials have been the subject of intense interest for the last three decades [19,32,43]. However, research on sol–gel processing of these materials is dated back to as early as mid-eighteenth century by Ebelmen and Graham [8,11,48]. These authors noticed that a solution of polysilicic or silicon alkoxide, such as TEOS is able to hydrolyze under acidic conditions and in the presence of H_2O. This yielded SiO_2 in the form of a “glass-like” material. Later works were able to obtain a clear film by spreading a colloidal solution of salicylic acid on a spinning substrate [72]. The process of reacting from the liquid allows these materials to be drawn into fibers, micro or nano-sized spheres, or as a thin film on a substrate [1,73,74]. In addition, because of the low processing temperature, there is a possibility to entrap most organic and biomolecules in the sol–gel network during the formation of the gel matrix [6,75]. Biomolecular encapsulation within sol–gel derived silica matrices was first introduced by Braun et al. [76] who successfully entrapped enzymes into TEOS matrices. Following that, Reetz et al. [77] reported sol–gel entrapped lipase enzymes with up to 100 times higher catalytic performance as compared to the free enzymes [77]. Other follow-up (?) studies confirmed the potential applications for encapsulating other biomolecules such as antibodies and phospholipids [78,79].

During the last few decades, silica-based materials have supplied successful solutions for soft and hard tissue regeneration [80]. These materials are highly biocompatible and the positive biological effects of their reaction products, make them an interesting group of materials for tissue regeneration [81]. Silica-based bioactive glasses (BGs) were first synthesized via a sol–gel technique by Li et al. [82] at lower processing temperatures compared to the melt-derived glasses. Pereira et al. [83,84] extensively researched sol–gel glasses based on the SiO_2–CaO–P_2O_5 system for biomedical applications. Silica-based sol–gel glasses exhibit many of the properties associated with an ideal material for tissue regeneration, such as high surface area and a porous structure, in terms of overall porosity and pore size that promote cell–material interactions and cell invasion [85]. Research on these glasses showed that the porous structure of these glasses brings higher surface area that exhibits higher tissue bonding rates [86]. Another study by Greenspan et al. [87] confirmed higher rates of HCA formation because of a greater release of soluble silica that nucleates HCA crystals compared to the melt-derived glasses of the same composition.

A sol–gel process, involving the foaming of a sol with the aid of a surfactant, followed by condensation and gelation reactions, has been used to prepare porous scaffolds of a few bioglasses (BGs), such as the glass designated 58S, with the composition (mol%): 60 SiO_2–36 CaO–4 P_2O_5 [88]. The
as-prepared scaffold had an overall microstructure similar to that of dry human trabecular bone, but the pore structure was hierarchical, consisting of interconnected macropores (>100 μm) resulting from the foaming process and mesopores (less than several tens of nanometers) that are inherent to the sol–gel process [89–92]. Fig. 3.1 illustrates the porous structure of the scaffolds made of bioactive glasses produced by means of sol–gel processes. This hierarchical pore structure of the scaffold is beneficial for stimulating interaction with cells as it mimics the hierarchical structure of many natural tissues (e.g., bone) and more closely simulates the physiological environment of mineralized tissues (?). Thanks to the nanopores in the glass, sol–gel derived scaffolds have a very high surface area (100–200 m² g⁻¹). As a result, these scaffolds degrade and convert faster to HA than those of melt-derived glass with the same composition. However, these sol–gel-derived scaffolds have a relatively low compressive strength (2–3 MPa) [93], and consequently, they are primarily suitable for applications focused on low load-bearing orthopaedic sites.

The discovery of mesoporous silica nanoparticles (MSNs) in 1992 was quickly recognized as a breakthrough that could lead to a variety of important applications [94]. These materials have uniform cylindrical pores with a diameter size range of 2–30 nm, along with a large surface area between 700 and 1500 m² g⁻¹ making them ideal for chemical separations, catalysis and biomedical applications [95]. In general, solution synthesis is carried out under a basic condition, which is similar to the traditional Stöber method for preparation of silica particles. Under basic conditions, a part of the silanol group (Si–OH) is deprotonated to form silanolate (Si–O⁻). In order to match the negatively charged silica surface, cationic surfactants are normally used. Thus, the hydrolysis and condensation rates of the silica sources (e.g., TEOS) are highly associated with several factors such as pH, silica source, additives, and temperature. The effect of pH is critical as it affects nucleation and growth. For example, Lu et al. [96] demonstrated that the particle sizes of MSNs were controlled over a wide range, from 30 to 280 nm, by simply adjusting the pH value of the precursor solutions. As the pH decreased from 11.5 to 10.9, the particle sizes decreased gradually. On the other hand, Qiao et al. found that the particle sizes tended to increase from 30 to 85 nm, as the pH values decreased from 10 to 6.28 [97]. These previous reports hint that the minimum particle size can be obtained at around pH 9–10, indicating that the condensation rates, rather than the hydrolysis rate, of silica precursors highly affect the final particle sizes. In fact, Chiang et al. [98] demonstrated that pH had the largest influence on particle sizes of MSNs, rather than the amount of the silica source or reaction time.

There have been very few trials to prepare MSNs under acidic conditions [99,100]. One of the advantages of acidic conditions is the capability of using block copolymers, which have a templating
effect and allow larger mesopores (>5 nm). The condensation rate for the synthesis at pH 5–8 should be faster than at pH < 5. Since the condensation reaction solidifies the silica network, one can expect that a too rapid condensation reaction will create a cross-linked network faster than the mesostructure organizes. This explanation is based on a formation mechanism where the particle formation, silica condensation, and ordering of the pore system are considered as separate processes. Thus, in order to obtain an ordered material the rates of the different processes must be properly adjusted relative to each other [99]. However, compared with basic synthetic systems, it is difficult to obtain MSNs with uniform particle size less than 100 nm and with spherical morphologies, but the large surface area of the pores allows the MSNs to be filled with different biomolecules for biomedical applications [101].

3.2. Phosphate-based materials

Phosphate-based materials are inorganic polymers, based upon the tetrahedral phosphate anion which is linked to form a 3D network [102]. Despite many structural similarities between silica and phosphate-based materials, they exhibit very different chemical behaviors in solution [103]. As explained, the sol–gel chemistry of silica-based materials has been extensively studied, but very little research has been carried out on the sol–gel synthesis of phosphate-based materials and much fundamental work remains to be done.

The novelty of phosphate-based materials in biomedical applications is related to their solubility and controllable dissolution rate in aqueous media compared to silica-based sol–gel materials [104]. Researchers have reported that these materials can be applied as a third generation of biomaterials that are bioresorbable [104,105]. This property of resorption allows for a variety of new biomedical applications, such as temporary implants that can be replaced with natural tissue as the body repairs a wound or DDSs that allow local and sustained release of macromolecules at the implant site [104,106]. Moreover, the breakdown components are monovalent and divalent ions and oligophosphates, which can be easily metabolised in the body [107]. Studies on phosphate-based melt-derived glasses have found that the degradation rate can be easily altered via addition of different modifier oxides such as CaO, TiO$_2$, and Na$_2$O, thus allowing one to tailor the persistent of the biomaterial, which is an important parameter in many tissue engineering and drug delivery applications [104].

The idea of using phosphate-based sol–gel derived glasses for biomedical applications was first introduced by Knowles [104], and a few years later Carta et al. [108,109] successfully synthesized ternary P$_2$O$_5$–CaO–Na$_2$O and quaternary P$_2$O$_5$–CaO–Na$_2$O–SiO$_2$ phosphate-based glasses via a sol–gel route. Following studies by the same group confirmed that the structure of sol–gel synthesized glasses is comparable to the melt-derived glasses with the same composition, and similar bioactivity can be expected for a variety of biomedical applications [109]. Pickup et al. [41,46] developed the sol–gel technique to synthesize binary P$_2$O$_5$–TiO$_2$ and ternary P$_2$O$_5$–CaO–Na$_2$O sol–gel derived glasses at lower temperature. Later, these authors showed the potential drug delivery application of these glasses with the subsequent release of drug molecules into an aqueous solution [106].

Biocompatibility and degradation rate of phosphate-based melt-derived glasses has also been extensively studied, but is outside the scope of this review. The interested reader might see the review by Abou Neel et al. [52]. However, pertinent to the sol–gel process, the degradation rate of the ternary P$_2$O$_5$–CaO–Na$_2$O glass system was found to be too high (of the order of hours) for many tissue engineering applications, so oxide elements like titanium oxides are often introduced to reduce the degradation rates [104,110]. However, limits exist with conventional melt–quench techniques in that achieving a TiO$_2$ content above 8 mol% is generally infeasible. Conversely, the sol–gel approach enables TiO$_2$ contents to reach up to 15 mol%, therefore greatly increasing the potential range of applications for glass-based materials [26]. Moreover, the challenges associated with tailoring the morphology of melt–derived glasses for biomedical applications can be overcome through further development of the sol–gel route. One point to note is that mesoporous phosphate-based materials have yet to be synthesized by the sol–gel route, and it is likely that this is due to a very small processing window between the loss of organics from the structure and the softening temperature at which the glass collapses. This remains a significantly challenging area for future efforts.
3.3. Metal-based materials (Fe–O, Ti–O, Zn–O, etc.)

Considerable efforts have been spent in the development of iron oxide nanoparticles, particularly those with magnetic properties, i.e., MNPs, the understanding of their magnetic behaviors, and the improvement of their applicability within biomedical research [111,112]. MNPs possessing the appropriate physico-chemical and tailored surface properties have been extensively investigated for drug delivery, magnetic resonance imaging (MRI), tissue engineering and repair, biosensing, biochemical separations, and bioanalysis [111–113]. The ferrite colloids, magnetite (Fe₃O₄) and maghemite (γ-Fe₂O₃), are the main representatives of the MNPs. Due to their biocompatibility and biodegradability these materials have received considerable attention in the medical and pharmaceutical fields [111,114]. The range of applications speaks to the potential value of efficient synthesis methods and, as with the previous examples given, the sol–gel route is properly applicable.

Sol–gel is a facile and convenient way to synthesize iron oxides from aqueous iron-based salt solutions. Addition of a base under inert atmosphere prevents the formation of undesired oxides or changes in the oxidation state of the metal cation [115]. Further to this, the size, shape, and composition of the MNPs highly depends on the type of salt based precursors used, the Fe²⁺ to Fe³⁺ ratio, the reaction temperature, the pH value and ionic strength of the media [18–21]. However, as alluded to above, MNPs are unstable under ambient conditions, and are easily oxidized to maghemite or dissolved in an acidic medium. Since the iron species within maghemite are present in the 3⁺ oxidation state, oxidation is of little concern and rather controlling the oxide content of the final ceramic network is most important to proper synthesis. However, both pH sensitivity and the propensity for oxidation to maghemite can be exploited through typical sol–gel means. In this instance, transformation is achieved by dispersing magnetite (a mineral possessing iron in both ferric and ferrous oxidation states) in acidic medium, then addition of ferric iron nitrate. The maghemite particles obtained are then chemically stable in alkaline and acidic medium [18–21]. However, even if the magnetite particles are converted into maghemite after their initial formation, further experimental challenges in the synthesis of Fe₃O₄ by sol–gel lies in control of the particle size and thus achieving a narrow or uniform particle size distribution. Since the blocking temperature depends on particle size, a wide particle size distribution will result in a wide range of blocking temperatures and therefore non-ideal magnetic behavior for many applications. At present, particles prepared by sol–gel unfortunately tend to be rather polydisperse [18–21]. However, it is well known that a short burst of nucleation and subsequent slow controlled growth are crucial to produce monodisperse particles [116]. Controlling these processes is therefore the key in the production of monodisperse iron oxide MNPs.

As one of the most common materials used in biomedical research, methods for the preparation and extended uses of titanium dioxide (TiO₂) have been the subject of interest for many years. Specifically biomedical applications of TiO₂ have motivated strong interest owing to its unique photocatalytic properties, excellent biocompatibility, high chemical stability, and low toxicity [117]. Advances in nanoscale science suggest that some of the current problems associated with alternative materials could be resolved or at least improved through applying TiO₂. For example, the TiO₂ content of phosphate-based glasses produced by the sol–gel route can be far higher than is possible by conventional melt-quench techniques [26]. Titanium itself naturally forms in four main phases: rutile, anatase, brookite, and monoclinic TiO₂. The relative stability of the four titania phases depends on particle size, with rutile being the thermodynamically stable form in bulk titanium but anatase being the most stable phase at sizes below 14 nm [118]. Brookite and monoclinic TiO₂ are metastable forms that are not commonly observed in minerals and are difficult to synthesize in pure form. It therefore stands to reason that the sol–gel approach would be able to improve the range of titanium-based materials that can be effectively produced and would thus enable exploitation of their respective beneficial properties [119].

In early examples, particle sizes obtained via the sol–gel method were several hundreds of nanometers. For example, Matijević et al. [120] described the preparation of uniform titania spheres by hydrolysis of TiCl₄ in highly acidic HCl solutions containing sulfate ions. This step was followed by aging for long periods of time (days or weeks). Similar uniform micrometer-size particles were prepared by Barringer and Bowen [121] through the fast hydrolysis of titanium ethoxide or isopropoxide precursors. The most important step in their preparation was the promotion of homogeneous
nucleation during hydrolysis and uniform growth of the nuclei by guaranteeing colloidal stability to the growing particles. However, porous and nonporous monodisperse spheres were reported more recently by a similar procedure but with addition of polymers to promote the pore formation [122]. Yet more recently, titania particle size was controlled to achieve smaller size regimes. Morales et al. [123] studied the hydrolysis of titanium ethoxide in the presence of hydrolyzing agents. These authors investigated the consequences of adding several acids and bases as additives during the synthesis and found some contradictory results. Use of HCl at mild acidic pH resulted in the isolation of crystalline particles containing all three phases of titania. On the other hand, use of oxalic acid or ammonium hydroxide caused the formation of mainly amorphous titania phase. In another report, in the presence of 0.1 M HNO₃ and titanium butoxide, 3.3 nm anatase particles are obtained [124]. From this brief history, a clear development in the sol–gel approach to the production of TiO₂ NPs can be seen.

One of the main problems faced in sol–gel chemistry is proper control of the hydrolysis and condensation rates when using titanium precursors. As described above, the metal chelating method can be an attractive way to overcome this issue by modifying the chemical activity of the precursors with complexing ligands that reduce the rate of or prevent hydrolysis. Scolan and Sanchez [125] used acetylacetone as such a ligand and conducted the hydrolysis in the presence of p-toluenesulfonic acid to obtain crystalline, dispersible anatase NPs (1–5 nm) whereas Khanna et al. [126] similarly used myristic acid to prepare 5 nm anatase nanocrystals with colloidal stability. Jiu et al. [127,128] used a combination of the surfactants F127 and CTAB to drive the hydrolysis of titanium tetraisopropoxide (TTIP) modified by acetylacetone. However, in this case, anatase particles of 3–5 nm in size were obtained only after calcination at 450 °C to promote crystallization.

In another variation of the classical sol–gel method, in the early 1990s Sugimoto and Sakata [129] developed the so-called “gel–sol” method. Originally applied to the preparation of α-Fe₂O₃ particles [129], it is based on the preparation of a metal hydroxide gel that is then aged to obtain a sol in which colloidal particles are dispersed. By this technique, colloidal dispersions of titania particles can also be produced by a combination of the sol–gel methods and an anodic alumina membrane (AAM) template. TiO₂ nanorods have also been successfully synthesized by dipping porous AAMs into a boiled TiO₂ sol followed by drying and heating processes [130]. By electrophoretic deposition of TiO₂ colloidal suspensions into the pores of an AAM, ordered TiO₂ nanowire arrays can be obtained [131]. Porous TiO₂ based films have been obtained by the sol–gel method using tetrabutoxytitanium and polyethylene glycol (PEG) as the precursor and template, respectively [132]. The morphology of porous TiO₂ was shown to be dependent on the amount of water, type of solvent, complexing agent(s), and the concentration and molecular weight of the template.

Metal oxide NPs, including ZnO, are versatile platforms for biomedical applications and therapeutic intervention, especially in the treatment and imaging of cancers. There is an urgent need to develop new classes of anticancer agents, and recent studies demonstrate that ZnO NPs hold considerable promise. The concentration of various chemical groups (–ZnOH₂, –ZnOH, –ZnO⁻) on the surface of ZnO NPs is pH dependent [133] and the availability of these reactive groups lends ZnO NPs to antibody/protein functionalisation via N-hydroxysuccinimide/1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (NHS/EDC) coupling chemistry [134], as well as other standard coupling approaches which can further improve cancer cell targeting.

ZnO NPs have also been shown to exhibit strong protein adsorption properties, which can be exploited to modulate their cytotoxicity, metabolism or other cellular responses [135]. The sol–gel method proposed by Spanhel and Anderson [136] in 1991 is commonly used to obtain ZnO nanometer-sized particles to prepare nanoparticulate films with strong visible luminescent properties, which are useful for optical and imaging methods [137]. Otherwise, the choice of acid or basic catalysis and the temperature was pointed out to play a key role in the rates of the hydrolysis–condensation reaction and in the equilibrium concentration of dissolved Zn²⁺ species, leading to changes in the size and shape of ZnO-based NPs [138].

The favorable optical properties of NPs obtained by the sol–gel method have also become a common topic of research as reflected in numerous scientific publications [136–138]. Benhebal et al. [139] prepared ZnO powder by sol–gel method from zinc acetate dihydrate, oxalic acid, using ethanol as solvent. Yue et al. [140] also obtained ZnO by the sol–gel method. High-filling, uniform, ordered ZnO nanotubes have been successfully prepared by sol–gel method into ultrathin anodised aluminum oxide
membranes. Integrating the ultrathin anodised aluminum oxide membranes with the sol–gel technique may help to fabricate high-quality 1D nanomaterials and to extend its application as a template for nanostructures growth.

Sol–gel routes are regarded as the optimal method for modifying ZnO quantum dots (QDs), because the synthetic reactions proceed near room temperature and do not harm the structure of the nanomaterial [141]. Previously reported ZnO NPs with various capping groups, including polyvinylpyrrolidone [142], oleic acid together with diethanolamine [143], polyethylene glycol methyl ether [144], and polymethylmethacrylate [145] among others. These materials tend to possess a series of surface associated ligands that effectively link drugs within a stabilizing steric shell. For example, the core–shell model based on ZnO NPs enables drug loading, although further modification of the shell itself can also be achieved via the sol–gel route [146]. The addition of folate groups to the NPs allows for preferential localization toward cancerous tissue which, for the majority of cancers, tend to have a relatively higher proportion of folate receptors in comparison to non-cancerous tissues [147]. Thus, the specific design of the NP system can be tailored to localize agents to a given site and deliver payloads with far greater efficacy with reduced systemic effects [148]. Beyond targeting cell surface receptors, these materials may also be engineered to enter cells through intercellular endocytotic pathways and release drugs to specific intracellular target sites [149].

3.4. Calcium phosphate based materials

Calcium phosphate (CaP)-based ceramics are widely used in medicine as bone substitutes, implants, and coatings on dental and orthopaedic prostheses [150]. Because of their chemical and structural similarities to the inorganic phase of human bone, hydroxyapatite (HA) and other calcium phosphates such as α- or β-tricalcium phosphate (α- or β-TCP) show excellent biocompatibility. In the last decade, HA/β-TCP biphasic materials [151] as well as silicon-substituted HA have been incorporated into clinical practice [152,153]. Most of the relevant application properties of these materials, including their biological influence on tissues and especially their biodegradation behavior, are determined by their chemical composition, morphology and surface topology. Therefore, proper material design offers numerous possibilities for the use of CaP materials in hard tissue replacement or regeneration, and, depending on the processing and formulation, drugs can either be incorporated during the ceramic processing or be surface associated at the end of the synthesis process [154,155]. These uses demonstrate further versatility of the sol–gel process. The CaP system represents one of the most complex families of materials due to the existence of a myriad of phosphate compounds. Furthermore, the stability of each of the phosphates is affected by not only small compositional changes but also by the variations in pH and the reaction. Some of the CaP compositions like α-TCP and tetracalcium phosphate (TTCP) are able to harden in aqueous solution, allowing for cement applications, such as injectable biomaterials to treat bone defects. Among the phases possible within a hydrated system composed of calcium and phosphate only, HA is the most similar to natural tooth and bone. This is also the most stable mineral phase in aqueous solution, meaning that aqueous degradation of CaP-based materials including CaP cements ultimately results in the formation of HA crystals. It is this complexity that makes this family of materials unique and one of the most interesting class of inorganic biomaterials. Understandably, sol–gel synthesis of HA ceramics has recently attracted much attention [15,16,156–166] as this method offers a molecular-level mixing of the calcium and phosphorus precursors and is capable of improving chemical homogeneity of the resulting mineral composite in comparison with conventional solid state reactions [167], wet precipitation [168,169], and hydrothermal synthesis [170]. As alkoxide-based routes maintain a largely anhydrous medium, precipitation of undesirable CaP phases can be minimized and the molar concentration of the reactive species can be raised to a point that favors the desired mineral form before initiation of the reaction. Likewise, this same degree of control is afforded through the prevention of precipitation by chelation-based sol–gel methods.

The synthesis of pure HA requires a correct molar ratio of 1.67 between calcium and phosphate in the final product. Besides the difference in chemical activity of sol–gel precursors, it appears that the temperature that is required to form the apatitic structure depends largely on the chemical nature of
the precursors. For instance, Gross et al. [160] used calcium diethoxide (Ca(OEt)2) and triethyl phosphate (PO(OEt)3) to form pure HA phase at temperatures above 600 °C. These authors also found that an ageing time longer than 24 h is critical for the solution system to stabilize its crystal structure and improve the stoichiometry of the resultant mineral [160]. In a similar fashion, Jillavenkatesa and Condrate [164] synthesized a mixture of HA and CaO at 775 °C using calcium acetate (Ca(C2H3O2)2) and triethyl phosphate as precursors although further hydrochloric acid leaching was required in that process to eliminate CaO, and obtain a pure HA phase. Conversely, Brendel et al. [159] obtained HA at temperatures as low as 400 °C using calcium nitrate (Ca(NO3)2·4H2O) and phenyl dichlorophosphate (C6H5PCl2) as precursors. However, the resulting HA phase produced by Brendel et al. [159] exhibited low purity with a poor degree of crystallinity in the hexagonal unit cell. As expected, further increases in the synthesis temperature resulted in a pure, well-crystallized HA phase. Several other examples exist which serve to illustrate this same point; i.e. although plausible avenues exist for the sol–gel synthesis of CaP-based materials this area is yet to reach fruition. As noted above, one of the most promising aspects of sol–gel methods is the lower synthesis temperatures that lead to a greater range of compatible substrate materials and lower economic costs. Whilst sol–gel methods do generally require lower reaction temperatures, further work is needed in order to produce HA coatings as viable option.

Phosphorus alkoxides have frequently been used as the phosphorus precursors for sol–gel HA synthesis in recent years. Triethyl phosphate and triethyl phosphite are major precursors among them [15,16,156–160,164,166]. However, the hydrolysis activity of the triethyl phosphate is relatively poor and a higher solution temperature together with a prolonged time period are needed to form the HA phase [171]. Alternatively, triethyl phosphite offers a much higher set of reaction kinetics [172,173]. Recent studies also revealed a valence transition from P(III) to P(V) upon ageing with calcium precursors to form HA within 24 h [160]. This indicates a nucleophilic addition of negatively charged OH− groups to the positively charged metal P, leading to an increased coordination number of the phosphorus atom, which is essentially an indication of the polymerization reaction [174]. After subsequent protonation of the alkoxide ligands (−OR) and removal of the charged ligand (−OR)−, P−(OR) hydrolyzes to form P−(OH) [167,175] following interaction with Ca precursor to develop the apatitic structure.

In general, the shape, size and specific surface area of the apatite NPs appear to be very sensitive to both the reaction temperature and the reactant addition rate [176]. Both Han et al. [177] and Liu et al. [24] were able to synthesize HA via non-template-mediated and a template-mediated sol–gel techniques, respectively. Liu et al. [178] used triethyl orthophosphate and calcium nitrate as the initial chemicals for nanosized HA synthesis. This process did however require a high temperature and produces a multi-phase powder. However, a relatively simple sol–gel process using ethanol and/or water as a solvent has also been reported to obtain stoichiometric, nanocrystalline single-phase HA [179]. Nanocrystalline HA powder was also synthesized at a low calcination temperature of 750 °C by the citric acid sol–gel combustion method [177]. The attractive features of this method were that it synthesized materials with a high purity, a better homogeneity and a high surface area within only a single step.

At present, for all those clinical applications where load-bearing properties are required, most of the implants used are metallic, with subsequent and serious problems due to: the large differences in mechanical properties between the artificial implant and the natural bone, giving rise to ruptures, the presence of ions that, released from the artificial implant, could be toxic or harmful and provoke pains, and the impossibility to regenerate natural bone. An alternative option is therefore to coat the metallic implant with bioactive ceramics. This technique is currently being used both for dental implants and hip joint prosthesis. The ceramic coating process on a metallic substrate is, however, quite complicated, and several methods are available in this sense. A great deal of the clinical success depends on this coating, since the quality and durability of the interface attachment greatly depend on the purity, particle size, chemical composition of the coating, layer thickness and surface morphology of the substrate.

The versatility of the sol–gel method thus creates great opportunities to form ‘thin film’ coatings. Whilst somewhat high temperatures are often required, the approach provides significantly milder conditions for the synthesis of HA films. This result in a much better structural integrity whereas
the defects that originated from thick coatings via a conventional plasma spraying can be largely avoided [180]. Furthermore, the lower temperature synthesis particularly benefits the metal substrates where the mechanical degradation or phase transition of the underlying Ti or Ti-alloy (i.e. α to β phase transition) can be prevented. However, thermal treatment of HA sol–gel films under vacuum environment is frequently required to avoid metal oxidation. This leads to structural instability of the HA coating as a result of the evolution of water during thermal treatment. Therefore, from both the economic and practical points of view, thermal treatment of the HA coating should be performed in air, and below the transition temperature of the substrate. In order to minimize oxidation of the underlying substrate, a thermal treatment temperature should be selected at a minimum level that still assures sufficient quality of HA film, in terms of crystallinity, film integrity, and adhesion to the substrate. For examples, at temperatures below 500 °C, oxidation of the underlying Ti or Ti-alloy is negligible due to the presence of a natural, dense oxide layer on the metal implants [181,182]. This means that, for the purposes of coating Ti-based metal implants, temperatures below 500 °C are preferable. Optimization of the film deposition has also been carried out [183]; single phase HA coatings were deposited on Ti6Al4V by the sol–gel dipping technique from aqueous solutions containing triethyl phosphate and calcium nitrate. In order to obtain homogeneous and monophasic HA coatings, the ageing time and temperature of the sol were the variables studied. The pH measurement is also a valuable tool to evaluate the best conditions of sol–gel deposited coatings. The higher the sol temperature, the shorter the ageing time needed to obtain pure HA after coating annealing. When the ageing parameters or the annealing temperature are not adequately controlled, additional phases or poor surfaces are obtained. The conditions to obtain the best coatings have been related with the pH decrease on the aqueous sols observed during the ageing, according to the polymerization reaction between calcium and phosphorous. In order to obtain homogeneous, crack-free coatings, the annealing temperature and thickness of the coatings should also be controlled. Films roughness is related with the viscosity of the sol-precursor used to do the deposition, as well as with the number of coating layers. Thus depending on the exact application, various parameters of the coating that is applied can be adequately controlled.

3.5. Organic and inorganic hybrid materials

Organic and inorganic hybrids are the materials composed of intimately distributed organic and inorganic phases. Each phase of the hybrid materials has scale from 1 to 100 nm and the types of hybrid materials range from molecular level donor–acceptor complexes to fiber-reinforced nanocomposites according to the dimension of each phase. In the late 20th century, the current concept of organic–inorganic hybrid materials, so-called functional hybrid materials, have been developed based on soft chemistry. In the biomedical field, the inorganic–organic hybrids have also gained much interest because each component can complement each other’s features including mechanical properties, biological properties and hydrophilicity. Meanwhile, there are also numerous cases of organic–inorganic hybrid structures in nature, including bone and nacre. This also suggests that the organic–inorganic hybrid materials have high potential in biomedical field.

The inorganic–organic hybrid materials might be sorted by means of the predominant phase, i.e. organic–inorganic or inorganic–organic materials. However, this is not generally accepted because there are many intermediate cases. The hybrid material can be distinguishable from general nanocomposites because it is not a physical mixture and there are chemical reactions between each phase. Thus, the classification of the hybrid materials is usually made according to the nature of interaction between organic and inorganic phases [184,185]. Class I indicates hybrids which do not contain covalent or ionic-covalent bond between the organic and inorganic phases. Instead, each component interacts by only weak interactions, such as hydrogen bond, van der Waals bond, π–π interaction or electrostatic forces. Maya blue, mentioned above, belongs to this class. Contrast with Class I, Class II hybrids refers to materials in which a part of organic and inorganic components are linked to each other by strong chemical bondings such as covalent or ionic-covalent bonds.

Class I hybrids can be produced by several approaches including hydrolysis–condensation of alkoxides in organic polymers and mixing alkoxides and organic components. Some hybrids made in this approach include homogeneously dispersed composites including polyphosphazene–metal oxide
(Si, Ti, Al and Zr) [186–188], silica–PDMAAm [189], silica–PVP composites [190], PDMS–SiO$_2$ [191], Poly(n-butylmethacrylate)–titanium oxide [192] and PMMA–silica hybrids [193]. Fig. 3.2A shows the representative scheme of PVP–silica hybrid material. This class of materials can form hydrogen bonds between hydroxyl groups of sol–gel oxides and amide, carboxyl or carbonyl groups that exist in polymer chains, and can show improved mechanical properties and flexibility compared to those of materials composed of a single polymer component. The second approach is to make hybrids with interpenetrating inorganic and organic substance networks [194,195]. Fig. 3.2B shows the schematic diagram of hybrid material, which has interpenetrating SiO$_2$ and organic gel network structure. The interpenetrating 3D networks are formed by simultaneous gelation of organic and inorganic components. Because the polymer phase is often precipitated in water–alcohol based solvents, cyclic alkenyl monomers, which polymerize by ring opening metathesis or unsaturated alcohols which polymerize by a radical mechanism were selected as organic component. Through this approach, elastic hydrogel, flexible rubber and hard glass with mesoporous networks are obtained, which are highly biocompatible, show reduction of shrinkages and good physiochemical stability [196]. From a biomedical perspective, the most useful inorganic component of organic–inorganic hybrid materials is bioactive glass (BG). The BG usually has SiO$_2$–CaO–P$_2$O$_5$, SiO$_2$–CaO or SiO$_2$–CaO–Na$_2$O systems. For example, calcium-containing hybrids like the PDMS–CaO–SiO$_2$ reported by Kokubo et al. [197] revealed HA-forming ability in SBF solution.

For biomedical purposes, well-known degradable synthetic or natural biopolymers, including PVA [198,199], poly(ε-caprolactone) (PCL) [200,201], gelatin [202] chitosan [203–205] and PEG [206] have been used. Typically, these types of hybrid materials were prepared by mixing of polymer solution with silica–based sol and then gelation. Very recent work has also investigated TiO$_2$–PCL hybrids [207]. Very recently, ZrO$_2$/PCL hybrids have also been investigated as an implant coating [208]. Although these materials are classified as Class I, the organic and inorganic components are sometimes connected by covalent bonds. Pereira et al. [198] reported that C–OH bonds in PVA were converted into C–O–Si bonds by esterification reactions, which were observed by infrared spectroscopy. Because of the good electrospinnability, these hybrids have frequently been obtained as electrospun nanofiber membrane forms which are known to promote desirable cellular behaviors [201,202]. Furthermore, since the process is carried out under mild conditions at low temperature, these hybrids materials can also be used for delivering of biomolecules such as drugs and growth factors [204,209] which would otherwise degrade under harsh synthesis conditions.

In Class II hybrid materials, a part of organic and inorganic components are connected by covalent or ionic-covalent bonding. To obtain this type of material, organically modified alkoxysilanes precursors such as R’$n$Si(OR)$_4$–n or (OR)$_4$–nSi–R’–Si(OR)$_4$–n (with $n = 1, 2, 3$) are firstly prepared and then the hybrid materials formed through hydrolysis and condensation process. In the hybrid, the organofunctional group, R’, affects the networks depending on their chemical structure. If R’ is nonhydrolyzable group it will act as a network modifier. On the other hand, if R’ can react with itself or with additional
polymerizable monomers, it will act as a network former. In similar ways, mixing of di- or tri-functional alkoxysilanes will tune the mechanical and functional properties of produced hybrid materials. Along with Class I hybrid materials, the organic components of Class II hybrid materials should be biocompatible for biomedical use. The representative organic components used in the fabrication of these hybrids are synthetic polymers (PEG, PCL) and natural polymers including proteins (gelatin) and polysaccharides (chitosan). Yamamoto et al. [210] developed PEG–SiO$_2$ ormosil using triethoxysilyl-terminated PEG and TEOS. To obtain triethoxysilyl-terminated PEG, allyl-group terminated PEG was reacted with triethoxysilane with hexachloroplatinic acid as a catalyst. Due to the presence of silanol groups [211], the fabricated hybrid material showed excellent bone-like apatite forming ability in SBF. The PCL–silica ormosil was also prepared in similar way by Rhee et al. [212]. For the sol–gel reaction the PCL molecules were end-capped with TEOS. This functionalised PCL was then reacted with TEOS containing calcium nitrate tetrahydrate as Ca source. As expected, this hybrid sample showed remarkably improved apatite forming ability in SBF. The natural-origin organic molecules also can be modified by TMOS or TEOS for enhancing their reactivity. Gelatin–SiO$_2$ systems showed enhanced apatite-forming ability [213] and osteoblast biocompatibility [214]. Meanwhile, chitosan–siloxane hybrid revealed enhanced apatite-forming ability with interesting photoluminiscent properties [215].

Most recently, new materials called cerasomes were reported by Ariga et al. [216] which served to mimic natural liposomes. These nanostructures were synthesized by a dual process of sol–gel and self-assembly of organic chain containing organoalkoxysilanes [217]. Two structural types of supramolecular materials, of either a lamellar or hexagonal long-range periodicity, could be obtained in a controlled way depending on the amount of silica involved during the synthesis. The intratubular swelling of these materials is related to the flexible nature of the silica walls. The procedure of self-templating synthesis works for cationic surfactants bonded to silica, but it is also expected to work with both neutral (polyoxyethylene-based) or anionic (long-chain carboxylate-, phosphate-, or sulfonate-containing alkyl-based) surfactants. The new compounds could serve to prepare new mesoporous silica materials and also as models for biological membranes and, in particular, for investigations of the diffusion of ions and lipophilic substances through them [217]. These new hybrids are able to incorporate different organophyllic substances, having a high potential for application in DDS [218,219].

4. Physical, chemical and biological properties

4.1. Mesoporous structure

4.1.1. Mesopore generation

The porous structure of sol–gel materials is usually formed during synthesis or by subsequent treatment. Depending on the predominant pore size, the porous materials are classified following the IUPAC system: microporous materials, having pore diameters up to 2.0 nm; mesoporous, having pore sizes intermediate between 2.0 and 50.0 nm; and macroporous materials, having pore sizes exceeding 50.0 nm [220]. The pores are either isolated or interconnected that may have similar (homogenous) or dissimilar (heterogeneous) shape and size distributions. Furthermore, the pore shape can be roughly approximated by any of the following three basic pore models: cylindrical, ink-bottled, and slit-shaped pores [221–223].

These mesopores presenting ordered structures of subnanometers to tens of nanometers in scale could be built from the sol–gel processes, and the templating through a supramolecular chemistry. Overall, a meso-scaled hybrid phase is produced in the first stage composed of inorganic building blocks and an entrapped organized supramolecular template. This step is followed by a template removal, which leads to the actual mesoporous structure. The formation of the first meso-scaled hybrid phase is critical in obtaining a reproducible mesoporous material with tailored features [224]. A complex co-assembly of inorganic and organic building blocks that gives rise to well-defined framework walls and pore template regions takes place during the precipitation or gelation of the system. Control of this first step is essential to define the characteristic interaction lengths that control the nascent meso-phase. The main driving forces toward obtaining organized templated
meso-phases have been presented in the literature and the relevant thermodynamic and kinetic factors have also been analyzed in detail [225,226]. However, the interactions between the inorganic components and the organic template are among the most important thermodynamic drivers, and usually determine the feasibility of meso-structure formation as well as its resultant topology.

Organic–inorganic interactions are in turn determined by the composition of the initial systems, and the adequate size and hydrophilicity of the inorganic building blocks, in order to properly locate both kinds of building blocks in space. Regarding the kinetics, the cooperative formation of an organized hybrid meso-structure is a result of the delicate balance of phase separation and organization of the template and inorganic polymerization. It has been proposed that processes linked to phase separation and organization at the hybrid interface between the inorganic building blocks and the template must be faster than the inorganic condensation that leads to “freezing” of a continuous matrix [227]. Winning this “race toward order” leads to highly organized meso-phases. In the event that the rate of inorganic condensation takes over that of template assembly, or co-assembly at the hybrid interface, poorly ordered meso-phases will result [226,228]. With insight as to processes that govern pore generation, opportunities to exploit are also presented. This has ultimately led to successful manipulation of the synthesis route.

4.1.2. Controlling pore structure

Pore size depends on the chain length of the surfactant employed during synthesis but also on other parameters of the synthesis process. Different methods have been developed to control pore size by adding auxiliary organic molecules, which are solubilised in the hydrophobic micellar region, thus increasing the micellar, and in turn pore size [229,230]. These particular methods not only create the possibility of incorporating larger molecules, but also influence the release rate of molecules as pore size also affects the diffusion of loaded drugs to the delivery medium [231]. However, other factors such as pore ordering [232], particle morphology [233] and the macroscopic form [232] can also determine adsorption and drug release kinetics. In a natural extension from this, the use of binary surfactants of different molecular weights in the synthesis of surfactant-templated meso-structure can also lead to the formation of dual-mesoporous materials. For example, Niu et al. [234] synthesized core–shell structured MSNs with bimodal mesopores consisting of smaller pores (2.0 nm) in the shell and larger tunable pores (12.8–18.5 nm) in the core. This was achieved by utilizing an amphiphilic block copolymer (polystyrene-b-poly(acrylic acid), PS-b-PAA) and CTAB as co-templates. In an aqueous ammonia solution, the hydrophilic PAA blocks of rod-like aggregates could couple with the CTAB micelles via electrostatic interaction between CTA+ and PAA− to form composite rod-like micelles. After the subsequent silicate deposition on the rod-like micelles, and cooperative self-assembly between the rich CTAB and additional TEOS, core–shell dual-mesoporous MSNs were obtained. Wiesner and co-workers also synthesized highly aminated MSNs with cubic Pm3n symmetry and successfully controlled the sizes of particles. In contrast to previous studies, it is worth noting that using ethyl acetate in the aqueous ammonia solution could effectively elevate the amount of aminosilane incorporated in the MSNs, while still maintaining the meso-structural ordering [235].

Although the pore size of sol–gel based materials could be controlled somewhat by varying the chain length of the surfactants used, the range is rather limited (1.8–2.3 nm). It would be desirable to further expand the pore size of MSNs to accommodate larger biopolymers. However, this is often difficult as most synthesis conditions for MSNs are tuned for surfactants such as CTAB. Recently, Fuertes et al. [236] used DMHA and TMB as swelling agents to synthesize pore-expanded MSNs that were able to adsorb a large amount of protein. Likewise, Kim et al. [237] synthesized MSNs using TMB and a sol–gel method to obtain large pore sizes of MSNs for gene delivery whereas Zhang et al. [238] used TMB and decane to synthesize pore-enlarged magnetic MSNs as DNA carriers. An alternative way to make MSNs of larger pore size is to use block-polymers as templates. Whichever the specific way these feats are achieved, their purposes are similar; that is, to increase the loading capacity of the MSNs and to expand the range of agents that can be loaded.

In many applications, pore size can be controlled to meet the given requirements (Fig. 4.1). Moreover, organic functional groups should be kept unchanged throughout the preparation process. MSNs with uniform pore size and a long-range ordered pore structure were first reported in the early 1990s [94,239]. With the abundant availability of various types of surfactants, MSNs with different...
structures have since been developed. However, the defining properties of any mesoporous materials are typically the pore structure and size.

Pore structure can be measured by low angle powder X-ray diffraction and by transmission electron microscopy whereas pore volume is usually measured by nitrogen gas sorption. Wall thickness, on the other hand, is usually determined by a combination of the X-ray diffraction data and the pore size distribution obtained from nitrogen gas sorption analysis. The most common meso-phases in silica with pore sizes between 2 and 5 nm are the 2D hexagonal \( p6m \), the 3D cubic \( Ia3d \) and the lamellar
corresponding to MCM-41, MCM-48 and MCM-50 materials, respectively [240]. For silica materials with larger pore sizes SBA-15, with 2D hexagonal \( p6mm \) structure, was firstly synthesized in highly acidic media using amphiphilic triblock copolymer of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (EO\(_{20}\)PO\(_{70}\)E\(_{20} \)) as a template [241].

### 4.2. Morphology and shape

Materials in a variety of different shapes and morphologies can be produced at the micro- and macro-scale with the use of sol–gel methods. Thin films, bulk glasses, porous foams, fibers, microspheres and nanoparticles are but some examples. However, there is also an obvious prolonged interest in research on coatings for biomedical devices. Such coatings are thought to provide a high degree of biocompatibility and promote the healing response without causing severe reactions [242]. Thin or thick films can be made by spray, spin, and dip coating techniques and used on medical implants. For example, silica nanosols doped with silver are well suited as a simple antimicrobial coating [243]. As with mesoporous NPs, sol–gel derived films can also be used as drug delivery carriers by controlling the thickness and the pore-size distribution of the coating [244]. These materials can have a huge impact as a dressing for wound healing or by preventing wounds from further injury, bacterial invasion, and promoting natural healing process. Biodegradable silica fibers have been shown promise to act as a scaffold for cells with controlled release of drugs and antibacterial properties to promote wound healing [245]. Mesoporous materials are also able to encapsulate relatively large amounts of bioactive molecules. These materials with their high surface area, large pore volume and tunable pore size with a narrow distribution are suitable for various controlled drug release applications. The drug release rate can be designed to range from very slow, by materials with lower total porosity and smaller average pore size, to very fast with higher total porosity and larger average pore sizes [246].

The 3D foam scaffolds based on sol–gel route are considered an important class of biomaterials for tissue engineering that can be processed to achieve hierarchically structured macro- and meso-pores found in many tissues. Recently, some attempts at fabricating multi-scale sol–gel based scaffolds have been carried out by using proper sol–gel precursors and processing techniques in which the pore size and arrangement were carefully controlled and designed. The purpose of such scaffolds is twofold, as they combine the properties of traditional glass-derived scaffolds with the unique features supplied by mesoporous materials. Therefore, the development of highly porous 3D scaffolds with the BG compositions proven in repairing bone tissue can take the benefits of bioactive composition and porous morphology. In particular, the well-defined multi-scale porous 3D scaffolds, compared to the conventional porous scaffolds, can provide advantages for bone regeneration, with their micro- or meso-pores having the capacity to incorporate therapeutic molecules while the macro-pore channels (mostly above 100 \( \mu \)m) allow for cell migration, nutrient delivery, bone ingrowth, and eventually vascularisation [247–250].

The mesopores in the BG compositions are generated in a similar way to those in MSNs, through the supramolecular chemistry in sol–gel process. In fact, the mesoporous BG (MBG) materials can be produced either by melt-quenching or via a sol–gel route [91,247,250–253]. Compared to melt-quenching, sol–gel methods enable a broader range of compositions and mesopore structures. Furthermore, the macro-pore channels can be given to the sol–gel MGB materials by introducing foaming agents (e.g., hydrogen peroxide, surfactants) or porogen materials (e.g., polystyrene spheres, polyvinyl alcohol, polyethylene glycol, etc.) [254–257].

Although the MBGs have the advantages of tunable chemical composition, higher specific surface area and pore volume, and uniform mesopore size, studies on 3D porous scaffolds based on MBGs have just begun [258,259]. Yun et al. [260] prepared hierarchical mesoporous–macroporous MBG spheres with a size of several hundred micrometers in a hydrophobic solvent (chloroform) by the triblock copolymer templating and sol–gel technique. The spheres had interconnected pore structures and excellent \( \text{in vitro} \) bioactivity. Mesoporous hollow BG microspheres with a uniform diameter range of 2–5 \( \mu \)m and a mesoporous shell (500 nm) can be prepared by a sol–gel method using polyethylene glycol as a template [261]. In this strategy, the incorporation of structure-controlling agents (e.g. CTAB, P123 and F127) is essential for obtaining well-ordered structures. Currently, the structure-controlling agents for preparing MBG mainly include CTAB, Pluronic F127 (EO106–PO70–EO106) and P123.
It has been found that the structure-directing agents play an important role in influencing the mesopore shape, mesopore size, surface area and pore volume of MBG. Generally, CTAB-induced MBG have a smaller mesopore size (2–3 nm) than P123- or F127-derived MBG (4–10 nm). P123 induced a two-dimensional hexagonal (p6mm) mesopore structure whilst F127 induced a wormlike mesopore structure [262]. Therefore, it is still a challenge to find much more convenient approaches to prepare MBGs scaffolds and related materials, and to investigate their in vitro and in vivo potential for drug delivery and tissue regeneration.

Fibers are another interesting form of scaffolds that have been widely used for cell culture and tissue repair. A growing interest in scaffold-based tissue engineering is the use of electrospinning techniques to create nanofibrous scaffolds of biodegradable polymers, which mimic the fibrous structure of the extracellular matrix [263,264]. The technique is also being applied to the formation of fibers with sub-micron or nanoscale diameters, and to nanofibrous scaffolds of bioactive glass [265,266]. Because of their high surface area, bioactive glass nanofibers degrade rapidly and can convert to HA. As in the case of sol–gel derived bioactive glasses, the bioactivity of these glass nanofibers is maintained over a larger SiO₂ compositional range when compared to melt-derived glasses. Nanofibrous scaffolds of bioactive glass have been prepared by electrospinning organic–inorganic solutions. A mixture of tetraethyl orthosilicate and calcium nitrate, for example, is typically used as the starting solution for the preparation of 70S30C glass (70 mol% SiO₂, 30 mol% CaO) by the sol–gel process [265,266]. After the electrospinning step, the as-prepared nanofibrous scaffolds are heated to 600–700 °C to decompose residual organic or inorganic groups (e.g. ethyl, nitrate). Bioactive glass fibers with diameters in the micron to sub-micron range, prepared from a melt-derived glass, are available commercially. This material, which is very pliable and has a rapid degradation rate because of its fine fiber diameter, has potential applications in the regeneration of non-loaded bone defects and the healing of soft tissue.

While the individual sol–gel derived mesoporous nanomaterial can be utilized for the loading and release of biomolecules, external modification like the combination of other nanomaterials can provide the ability of stimuli-responsiveness thanks to the so-called gatekeeping concept. Basically, diverse chemical entities are employed to reversibly close the pore entrances to regulate the encapsulation and subsequent release of drug molecules [267]. Thus, these systems acquire the ability to control the biomolecule release on demand in response to different stimuli, such as temperature, pH, ultrasound, light, magnetic field, redox systems, peptides, enzymes, or antibodies [268]. The great advantage of these capped drug delivery NPs is the protection of the drug against any possible degradation and/or inhibition of premature release; the latter of which is of vital importance when the cargo to be delivered can give rise to side effects. Thus, a so-called “stair-case” release profile can be obtained, which is one of the most important characteristics of stimuli-responsive systems: the drug release can be tailored to be provided on demand. Advances made in this respect are detailed in Section 4.5 of this review.

4.3. Mechanical properties

In general, inorganic materials, including ceramics, have relatively higher hardness, tensile strength and elastic modulus when compared to other materials available [269]. These properties are due to their molecular structure composed of ionic and covalent bonding, which is much stronger than metallic bonding. Since dislocation motion is difficult in these bonding structures, the strength of the materials increases. However, the materials also become brittle because the stress concentrated at flaws in the structure cannot be released [270]. In this kind of brittle fracture, called ‘catastrophic fracture’, the cracks propagate rapidly without any predictable sign or appreciable amount of plastic deformation. Most sol–gel derived inorganic materials also show this same degree of brittleness. Moreover, because they are, for the most part, highly porous structures, particularly for aerogels (80–98%) [271], the mechanical properties of the sol–gel derived materials can often be much lower than that of dense materials. For instance, the flexural strength of the TMOS-derived silica aerogel with 95% porosity is 0.02 MPa [272], which is 0.0002% of that of the dense silica material [273]. Therefore, in order to use the sol–gel based materials in bulk or coating state, not only analyses of the mechanical properties but also developments of methodologies for improving the mechanical properties are important.
The mechanical properties of sol–gel based materials are significantly influenced by network properties. These in turn can be altered by adjusting the process parameters including the water to precursor molar ratio, pH, type of precursor as well as doping agents. When water content increases, hydrolysis reactions are accelerated; however, the density of the material also decreases because the concentration of reactants decreases. Murtagh et al. [274] reported the effect of water content on the elastic modulus of TEOS-based silica gel. They prepared the silica aerogel with various water contents and evaluated the elastic modulus by ultrasonic analysis. Their results showed that the elastic, shear and bulk moduli of the silica gel decreased from 9.95, 3.95 and 6.53 GPa to 6.55, 2.83 and 3.51 GPa, respectively, when the water content increased from 4:1 to 24:1. In instances where such elastic or tensile moduli closer to that of natural tissues are required to maintain adequate axial forces and, hence, prevent loss of bone mineral density, the parameters of a material can thus be manipulated in whichever direction is necessary though simple control of water content during the gel formation stages of the sol–gel process.

As stated above, the pH of the sol directly affects hydrolysis, however indirect effect on the mechanical properties of the resultant materials also occurs. Sol–gel based materials can fail to meet specification due to internal stresses resulting from shrinkage during the drying process, and this can be related to the pH of the synthesis solution. For example, Woignier et al. [275] observed shrinkage of TEOS-based gel decreased from 15% to 1.5% when the pH of the sol increased from pH 1.5 to pH 9. The authors concluded that the network of silica gel is built by larger particles when the pH of the sol is high and these large particles make the silica branches stiffer and reduce the overall shrinkage during the drying process. Understandably, internal stress can cause a decrease in the initial and long-term mechanical stability of these materials [276].

The type of precursors used during synthesis influences not only the reaction, but also the final material mechanical properties. For example, TMOS- and TEOS-based silica xerogels that were prepared from a solution with a 16:1 M ratio of water to Si had a porosity of 50% and showed strengths of 20 MPa and 10 MPa, respectively [277]. These issues with brittleness, limit the wider applications regardless of the relatively high strength or moduli. However, flexible silica aerogels derived from hydrophobic precursors have recently been reported. Rao et al. [278,279] developed a superhydrophobic and flexible silica aerogel using alkylalkoxysilane precursors such as MTMS and MTES by a two-step process. These types of coatings are covered in a recent review by Yu et al. [280]. Since the alkyl group of the alkylalkoxysilane did not participate in the network formation and the inter-chain cohesion in the silica is minimized, the result was networks with increased flexibility.

Substitution of the Si or O in the silica network with other elements also improves the mechanical properties of the sol–gel derived silica. Li et al. [281] prepared silica, which has a composition of 45S5 bioactive glass by sol–gel method, and observed the effect of the additives on the mechanical properties by adding small amounts of MgO, TiO₂ or CaF₂ to substitute Mg²⁺, Ti⁴⁺ for Ca²⁺ or F⁻ for O²⁻. The mechanical properties of the glasses as evaluated by 3-point bending test were improved for all cases. The Dietzel's ionic strength of Mg²⁺ ion is stronger than that of Ca²⁺ ion. Therefore the substitution of Mg²⁺ for Ca²⁺ makes the glass network stronger due to decreased bond lengths [282], although the biocompatibility decreases slightly. Like the Si⁴⁺ ion, the Ti⁴⁺ ion is able to connect with four O²⁻ ions. Ti⁴⁺ ions substituted for Ca²⁺ ions thus make the glass network stronger by connecting with more O²⁻ ions [283]. As a result, Mg and Ti substituted glass showed improved bending strength, fracture toughness and hardness. In contrast, the fluorine ions due to the substitution of CaO by CaF₂ disrupted the glass network, with an increased tendency to crystallization. Therefore, the CaF₂-doped 45S5 lacks favorable mechanical properties.

Mechanical properties of sol–gel derived gels can be enhanced by reducing the pore size during the sintering process [273]. One of the advantages of sol–gel derived materials is the ability to release therapeutic agents including drugs and growth factors in controlled manner by loading during sol preparation process [284]. For most DDSs, however, the final sintering processes cannot be used as heat can damage many therapeutic agents and so the materials remain in a weaker form. Therefore, hybridisation with organic materials is considered a more promising technique for toughening sol–gel based inorganic materials applied to DDSs. For biomedical applications, biocompatible and biodegradable polymers, including collagen [285,286], gelatin [286], chitosan [204], PCL [287], PLLA [288], have been hybridised with silica. For example, Lee et al. [204] reported a hybrid system of silica xerogel and
chitosan. In this instance, the authors prepared TMOS-based silica xerogel with differing chitosan contents. After hybridisation with 50 vol% of chitosan, the final compressive strength and elongation of silica xerogel increased from 10 MPa to 70 MPa and from almost zero to 10%, respectively. This hybrid material also exhibited sustained release of a pre-loaded drug, the antibiotic Vancomycin. Tailored release of the drug was also achieved by altering the chitosan content. These same authors also developed a silica–chitosan hybrid membrane for guided bone regeneration [203] and a coating system [289] with the same improved mechanical properties, therefore demonstrating the aforementioned flexibility of inorganic–organic hybrid systems for the production of sol–gel based biomaterials.

4.4. Chemical properties

4.4.1. Surface chemistry

4.4.1.1. Surface functional groups. As mentioned above, sol–gel materials can be synthesized with different structural features. It was also indicated that by altering their structures or surface functional groups, the physical and chemical properties of sol–gel based materials can easily be tuned. The ease with which tuning their surface properties can be achieved has also extended previously assumed limits of biocompatibility and drug carrying capacity. Surface-functionalisation of sol–gel based materials are widely used in biological applications because many such materials, especially those containing judiciously chosen functional groups, have improved adsorption capacities for loading of bioactive molecules or drugs, and increased binding affinity to deliver these molecules to specific cell and tissue targets, along with enhanced overall biocompatibility [3]. The surface functionalisation of mesoporous silica based materials, for example, is typically achieved by introducing functional groups either on the external surface of the material or the inner surface of the pore or, indeed, both. This can be accomplished through one of two basic synthetic strategies: stepwise synthesis (post-synthetic grafting) or one-pot synthesis (co-condensation method). The presence of a high density of surface silanol groups capable of being functionalised using a wide variety of entities represents one of the great advantages of MSNs [290–292]. When nanomedicine applications are required, the surface functional groups of MSNs can play diverse roles, such as tailoring the surface charge of NPs, allowing the grafting of functional molecules inside and outside the pores, or facilitating the capping of the nanopore openings to prevent premature release of entrapped drugs [290].

Adjustment of the polarity of the pore surfaces by the addition of organic building blocks considerably extends the range of materials that can be used in biomaterial applications [293]. Equally useful is modification with organic functionalities such as C–C multiple bonds, alcohols, thiols, sulfonic and carboxylic acids, and amines. These allow for localized organic or biochemical reactions to be carried out on a stable, solid inorganic matrix. As a consequence of the functionalisation, organic–inorganic hybrid materials are obtained which can adsorb drug molecules through weak electrostatic interactions. Functionalisation with hydrophobic functional groups is a good alternative to increase the loading capacity of most non-polar drugs. This strategy is also employed to delay the release of certain drugs from mesoporous channels in aqueous medium by manipulating the wettability of the material surface [294]. On the other hand, there are other situations in which pharmaceutical molecules can be confined into the mesoporous channels. However, higher loads and slower release kinetics can be achieved if the mesoporous silica wall is functionalised with different functional groups [295].

Thus, the capability to cross cell membranes, avoid endosome capture and similar undesired intracellular binding processes, can make the MSN’s more effective. These properties are often necessary so that they can reach their desired target with the maximum drug payload intact. Among these processes, the endosomal escape of MSNs constitutes a crucial step or checkpoint, which can be significantly improved by proper functionalisation of the external MSN surface [296–298]. For example, Serda et al. [298] used polycations (such as poly-L-lysine, PEI, polyamidoamine dendrimers, and natural chitosan) along with endosome-disrupting peptides to coat the outer surfaces of silica based NPs and thus dictate cellular uptake and trafficking responses. Furthermore, as alternative molecules such as branched chain PEI contain a high density of amine groups, the positive charge imparts a so-called “proton sponge effect” and can serve as an excellent buffering agent during endosomal encapsulation [296]. Due to this high endosomolytic activity, PEI-coated MSNs exhibit an improved ability to deliver
nucleotides and drugs to intracellular or even nuclear sites [297]. It is the precise manufacture of these materials that is made possible by the sol–gel process.

4.4.1.2. Sol–gel processed surface chemistry. Given the interests in the potential biological applications of silica based materials, sol–gel methods that make silica based materials as biocompatible as possible are necessary. In this regard, surface modification of silica based materials is very important as it may result in enhanced cellular uptake and endosomal escape, and reduced collisions with some undesired organelles during cellular trafficking of the NPs themselves. This can be best exemplified by the work performed by Lin and co-workers, in which a variety of surface-functionalised MSNs were found to be internalised differently into human cancerous HeLa cells expressing folate receptors [299]. The different surface-modified MSNs were synthesized by functionalising MCM-41 with 3-aminopropyl (AP), guanidinopropyl (GP), 3-[N-(2-guanidinoethyl)guanidine] propyl (GEGP), and N-folate-3-aminopropyl (FAP) groups. All these organic-functionalised MSNs exhibited lower surface areas and pore volumes compared with their parent MCM-41 [91]. Interestingly, the degree of particle uptake by the HeLa cells increased in the same trend as their surface charges, (i.e., in the order of parent MCM-41 < AP-MCM-41 < GP-MCM-41 < 0 mv < GEGP-MCM-41 < FAP-MCM-41) [91]. Furthermore, among these materials, the FAP-modified MSNs were internalised to a greater extent by the folate receptor-expressing cells, indicating that the chemical affinity of functionalised particles to cells could play more significant roles than physical properties in determining the degree of endocytosis of MSNs. The mechanisms for endocytosis of particles, except for GEGP-functionalised MSNs, were also included in the study, and they were reported to vary from material to material [299]. The authors concluded that the parent MCM-41 MSNs were ingested through a clathrin-mediated pathway, whereas the AP- and GP-grafted MCM-41 MSNs were internalised into cells via a caveolae-dependent mechanism, and the FAP-MCM-41 MSNs were engulfed by both clathrin and folate receptor-mediated endocytosis followed by greater confinement of positively charged particles inside endosomes [299]. These results clearly showed the effects of surface properties on the cellular uptake of MSNs and their subsequent endosomal escape. Notably, a particular surface chemistry, surface charge determined by surface functional groups, may not only help one of the steps in the given biological process, but may also inhibit the others in the process. Therefore, all the steps in a given biological process need to be taken into account when rationally designing and synthesizing sol–gel based nanomaterials for biological applications.

By using HA NPs with similar particle size, shape, and crystallinity but different surface charges, Liang et al. [289] recently showed that differently charged NPs underwent different cellular uptake in murine osteoblasts in vitro. The particles with positively charged surfaces accumulated inside cells more so than their negatively charged or neutral counterparts. This result is consistent with a previous report on the uptake of differently charged MSNs by HeLa cells [300]. Interestingly, the cells even with highest particle uptake (i.e., those containing positively charged HA NPs) maintained their membrane integrity, viability, and normal replication, with no difference compared to those containing fewer intracellular particles [289]. This result was unprecedented, considering the fact that substantial residues of nanomaterials remained inside the cells or tissues and that such materials are traditionally expected to result in severe injury or toxicity based on previous findings on the internalisation of HA NPs in other cells (e.g. fibroblasts or hepatic cancer cells) and their cytotoxicity and cell growth inhibitory effect [289]. Nonetheless, this again corroborated the results that surface charge on MSNs could play a major role on cellular uptake of materials and subsequent cell viability. Moreover, the results showed that the degree of uptake of the functionalised MSNs and their subsequent biological effects and biocompatibility could depend on the cell type.

The most often used method for the loading of small drugs into the pores of MSNs is physical adsorption from solution. In this instance, the presence of surface silanol groups on the silica surface means that, in the absence of specific ion adsorption, the silica surface is negatively charged under biologically relevant conditions. Therefore, electrostatic adsorption of positively charged adsorbates is an attractive method for incorporating water-soluble cargos onto siliceous MSNs. The extent of adsorption can be further increased by introducing functional groups, very often weak acids and bases like carboxylic acids or amines, which can then be used to tune the effective surface charge under given pH conditions and also provide the possibility for additional specific adsorbate–adsorbent
interactions. Hydrophobic drugs are typically adsorbed from organic solvents, followed by vacuum drying in order to remove the solvent. Naturally, pore size effects do play a role for the extent of adsorption if the molecular size of the drug is in the range of the pore diameter.

Balas et al. [301] reported amino-functionalised MCM-41 and SBA-15 mesoporous silica-based materials containing alendronate for bone repair or regeneration. Alendronate belongs to the bisphosphonate family, which inhibit bone resorption by osteoclasts. In both surfaces, the systems of the pore walls were organically modified with amino groups. After 24 h in an aqueous alendronate solution, the amino-modified materials showed a drug loading almost 3 times higher than that of the unmodified materials. This different behavior could be explained by the different chemical interaction between the phosphonate groups in alendronate with the silanol groups in the case of the unmodified materials and with the amino groups covering the surface of the mesopore walls of the modified materials. Song et al. [302] also reported the functionalisation of MCM-41 and SBA-15, respectively, with amino groups as an effective method to control drug release. The ionic interaction between the carboxy groups in drug molecules and the amino groups on the matrix surface allows the release rate of the drug from amino-functionalised MSN to be effectively controlled.

4.4.2. Bulk chemistry

The microstructure of sol–gel derived silica is closely related with the chemical properties of itself. For example, many acid-catalyzed sol–gel derived silica materials have fractal structures as shown in Fig. 4.3A. This fractal structure is formed by agglomeration of particles with a diameter of 4–6 nm (secondary particle), which are also composed of small particles with a diameter of 1–2 nm (primary particle) [303]. West et al. [304] estimated the size of primary particles using an intermediate neglect of differential overlap (INDO) molecular orbital model. This approach led them to first propose two silica structures: one is a ring structure (Fig. 4.3B), and the other is chain structure (Fig. 4.3C). In each structure, each silicon, except those at the end of the chain, is bonded to two bridging oxygens and two non-bridging oxygens. Each non-bridging oxygen is bonded to hydrogen for terminating the structure and balancing the charge. The INDO energy per silica tetrahedron of ring structured silica was about −55.8 AU regardless of the number of silica tetrahedra. On the other hand, the INDO energy per silica tetrahedron of chain structure silica was initially lower than that of ring structured silica, but increased with the number of silica tetrahedra [48]. Based on their data, the difference in the INDO energy per silica tetrahedron between the two structures becomes zero when the number of tetrahedra is between 10 and 12. This means that the silica initially grows in a chain structure and converts to

![Fig. 4.3. Schematic diagrams of (A) fractal structure acid-catalyzed sol–gel derived silica gel network, (B) ring structure of 4 silica tetrahedra and (C) chain structure of 4 silica tetrahedra.](image-url)
a ring structure when the number of silica reaches 10 or 12, because the driving force to produce ring structure becomes more favorable, and this conversion to a ring structure of the primary particles results in the development of fractal structure. The size of primary particles estimated by this model is similar to the radius of gyration of the primary particle calculated by SAXS analysis [303]. Meanwhile, the molecular energy of silica with ring or chain structures was recalculated by Davis et al. [48] using AM1. In their calculation, extra water molecules in the chain, as compared to the rings, were considered, and the energies were corrected for zero-point energy with subsequent conversion to enthalpies at 25 °C. Due to these corrections, the number of silica tetrahedral where the chain-to-ring conversion occurs moved from 10–12 to 3–4. This calculation suggested that the primary particle might be built with a 3- or 4-membered ring structure. Moreover, the size of 3- or 4-membered ring corresponds very closely to the 1–2 nm primary particles.

Because many properties of sol–gel derived materials are affected by their chemistry, the investigation of chemical reaction and chemical structure of the sol–gel materials is central to their development and optimization. Generally, the chemical properties of the sol–gel networks are analyzed by infrared [305–308], Raman [305,306] and/or nuclear magnetic resonance spectroscopy [306,309–311]. In infrared spectroscopy, silica exhibits three major absorption bands at ~460 cm⁻¹, ~800 cm⁻¹ and ~1100 cm⁻¹, which respectively correspond to the rocking motion of oxygen perpendicular to the Si–O–Si plane, symmetric stretching motion of oxygen along the bisector of the Si–O–Si bridging group and asymmetric stretching motion of oxygen parallel to the Si–O–Si line [307]. Because these main peaks resulted from the Si–O–Si backbone of sol–gel derived silica, when the network is disrupted by network modifiers the spectra would be changed significantly.

For example, Ca²⁺, an abundant element in human body, has been used for making the bioactive glasses because it promotes bone regeneration. In the silica network, the Ca²⁺ ion acts as a network modifier, which disrupts the glass network by breaking Si–O–Si bond and generating non-bridging oxygen species. As shown in Fig. 4.4A, as increasing Ca content in the glass, the intensity of absorption bands corresponding to symmetric and antisymmetric motions decreased and the peak corresponding to Ca–O formed at ~1220 cm⁻¹. Moreover, the bands of Q⁰, Q¹, Q² and Q³ which respectively indicate symmetric stretching motion of silicon with 4, 3, 2 and 1 non-bridging oxygen atoms [312] were detected, suggesting that the addition of Ca ions disrupted the polymeric structure of silica [305]. This kind of information related to molecular motions can also be obtained by Raman spectroscopy. Raman spectroscopy is a sensitive analysis method in which the scattering of the visible near infrared or near ultraviolet light is measured, while the absorbance or transmittance of the light is measured in IR spectroscopy. In the Raman spectrum of sol–gel derived silica, the main peaks are detected at ~980 cm⁻¹ (Q⁰), ~493 cm⁻¹ (D¹) and ~606 cm⁻¹ (D²), which respectively correspond to Si–O stretching in the silanol group, vibration motions of oxygen atom in 4- and 3-membered rings [313]. It is noteworthy to point-out that these kinds of peaks are not detected in crystallized silica such as quartz [305].

The addition of network modifier cations, such as Ca²⁺, also affects the Raman spectra of silica. Fig. 4.4B shows the Raman spectra of sol–gel derived silica with Ca contents ranging from 0 to 40 mol%. The D¹ and D² peaks were not influenced by Ca addition, but shifted when the Ca content reached 40 mol%. In addition, new bands corresponding to Q² and Q³ appeared at ~980 cm⁻¹ and ~1050 cm⁻¹, respectively [305]. Meanwhile, NMR is one of the most useful tools for investigating the chemical reaction during the sol–gel process. The terms Qⁿ (n = 0–4) were originally used in ²⁹Si NMR spectroscopy [305,311] where solid-state ²⁹Si NMR allows one to observe the connectivity and degree of condensation of sol–gel derived materials by comparing the intensities of Qⁿ signals [311]. Homogeneity of the element is one of the main advantages of the sol–gel technique when making the composite or compound. However, if the process is not sufficiently optimized, so that the self-condensation between the same precursors is more dominant than co-condensation between the different precursors, phase separation will occur and desirable properties cannot be obtained.

Energy dispersive spectroscopy or X-ray photoelectron spectroscopy is a more accessible way to investigate the chemical composition of surface layers, although it is difficult to investigate chemical reactions using these methods. Although ²⁹Si NMR gives much information about silica network, such as D¹, D², Q²–Q⁴, it does not give information about the reaction between different precursors, while ¹⁷O NMR is more valuable in this respect [310]. Fig. 4.5 shows (A) ¹⁷O NMR spectra of (A) pre-hydrolyzed TEOS sol and (B–D) mixture sol of TEOS and Ti(OBu⁴)₄ with 1:1 M ratio at (B) 15 min,
The bands indicating Si–O–Si, Si–O–Ti and Ti–O–Ti are clearly detected without any overlap [310] suggest that $^{17}$O NMR may help to optimize the conditions of sol–gel processing.

The sol–gel derived silica materials have chemical structures based on the Si–O–Si backbone; thus, it would stand to reason that the many properties of sol–gel derived silica might be significantly affected by the elements which influence the network. The number of alkoxy groups in the precursor molecules is one example. TEOS/trimethylethoxysilane (TMES) system was prepared to functionalize the silica by using a hydrophobic precursor TMES. Since the TMES molecule has only 1 hydrolysable group, this mixture did not set when the molar ratio of TMES was higher than 0.6 [314]. For methyltrimethoxysiloxane (MTMS) or methyltriethoxysiloxane (MTES), these can set alone, because...

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**Fig. 4.4.** (A) IR [312] and (B) Raman spectra of $x$CaO–(1–$x$)SiO$_2$ glasses ($x$ = 0–0.4) [305].

**Fig. 4.5.** $^{17}$O NMR spectra of a prehydrolyzed solution of TEOS aged during 4 h, (a) before and (b–d) after addition of Ti(OBu$^n$)$_4$: (b) 15 min, (c) 1 h, and (d) 2.75 h (TEOS/H$_2$O/EtOH 1/1/4 (pH = 1.6); Ti/Si = 1) [310].

(C) 1 h and (D) 2.75 h. The bands indicating Si–O–Si, Si–O–Ti and Ti–O–Ti are clearly detected without any overlap [310] suggest that $^{17}$O NMR may help to optimize the conditions of sol–gel processing. The sol–gel derived silica materials have chemical structures based on the Si–O–Si backbone; thus, it would stand to reason that the many properties of sol–gel derived silica might be significantly affected by the elements which influence the network. The number of alkoxy groups in the precursor molecules is one example. TEOS/trimethylethoxysilane (TMES) system was prepared to functionalize the silica by using a hydrophobic precursor TMES. Since the TMES molecule has only 1 hydrolysable group, this mixture did not set when the molar ratio of TMES was higher than 0.6 [314]. For methyltrimethoxysiloxane (MTMS) or methyltriethoxysiloxane (MTES), these can set alone, because...
they have three hydrolysable groups \[279,315,316\]. Due to the hydrophobic methyl group, the properties of silica aerogels derived from these precursors differ from silica aerogels derived from TMOS or TEOS. For example, MTES-derived silica aerogels showed flexible properties due to the flexibility of the final glass network \[279,316\] and also exhibited buoyancy in water because of the hydrophobic property imparted by the methyl group \[316\]. The incorporation of ions in the glass network also significantly affects the properties of sol–gel derived silica as described previously. The elastic modulus and strength of the glass decreased with increasing polymer content, because the stiffness of the Si–O network is reduced by incorporation of soft polymer molecules. However, the ductility and toughness of the glass increased \[317,318\].

Metallic ions also influence physicochemical properties, such as degradability. When Na\(_2\)O is substituted for CaO, the glass network became more disrupted because two Na\(^+\) cations should exist between oxygen for charge balancing \[319\]. Furthermore, Na\(^+\) ion can be released from the network more easily because the bond energy between Na\(^+\) and O\(^2-\) is lower than that between Ca\(^{2+}\) and O\(^2-\) \[320\]. Thus, the chemical stability of Na-substituted 58S decreased compared to 58S glass. In the 58S glass system, Ti\(^{4+}\) substituted for Ca\(^{2+}\) results in covalent O–Ti–O bonding because the electrostatic field strength \((z/a^2, z:\) cation charge, \(a:\) internuclear distance between cation and oxygen) of Ti\(^{4+}\) \((1.05 \times 10^{-20} \text{ m}^{-2})\) is higher than Ca\(^{2+}\) \((0.36 \times 10^{-20} \text{ m}^{-2})\) \[321\]. Put more simply, the lower difference in electronegativity between Ti and O \((\text{in comparison to Ca and O})\) favors covalent character between these elements. Since Ti has the same valence as Si, Ti can assist Si in forming the glass network. The Ti\(^{4+}\) cation is also known to strengthen phosphate glasses by cross-linking the chains effectively \[322\]. Therefore, Ti-substituted 58S glasses exhibit decreased degradability. As the composition of the glass becomes complex, it is difficult to predict the effect of each component on the degradability. Hill et al. \[323,324\] have suggested a systematic method to estimate the relationship between the dissolution rate of the glass and composition using split network or network connectivity (NC) models. NC is defined as the average number of bridging oxygen atom per network forming element and is a measure of glass structure polymerization. The network connectivity also affects bioactivity by modulating the rate of HCA layer formation \[40\]. When the NC value is too high (>2.4), the glass is insoluble and has decreased bioactivity. On the other hand, when the NC is too low, the glass degrades very quickly. Hill et al. \[323,324\] recommended an optimum range of NC from 2.0 to 2.4 for biomedical materials based on the percolation theory by Philips and separately by Thorpe \[325,326\].

Since many trace elements (such as Sr\(^{2+}\) \[327,328\], Mg\(^{2+}\) \[329,330\], Zn\(^{2+}\) \[331,332\], Fe\(^{3+}\) \[333,334\], B\(^{3+}\) \[335,336\], and Co\(^{2+}\) \[337,338\]) present in the human body are known for their anabolic effects on physiological processes such as bone metabolism \[339,340\], new approaches for enhancing bioactivity of the sol–gel BG-based materials are continually investigated by introducing such therapeutic ions into the structure. As indicated above, the subsequent release of these ions after exposure to a physiological environment is believed to favorably affect the behavior of human cells and to enhance the bioactivity of scaffold materials. Thus, research efforts are devoted to incorporate these ions in different implant materials. Furthermore, these substitutions often result in altered dissolution behavior and modified biological performance. In addition, single inorganic ions such as calcium \[341,342\], phosphorous \[343\], and silicon \[344,345\] are known to be involved in bone metabolism and to play a physiological role in angiogenesis, growth and mineralization of bone tissue. However, the variations in dissolution behavior are testament to effects on the chemical structure of the materials produced. This can, in some cases, mean that synthesis conditions are also altered. For example, borosilicate glasses dissolve much slower than unsubstituted silicate glasses \[335\]; however, significantly higher synthesis temperatures are also required to produce these glasses. For the purposes of coating implant materials, sol–gel approaches may be far more efficacious although, at present, sol–gel approaches have yet to be fully developed for this particular synthesis pathway.

4.5. Biological properties

4.5.1. Biodegradation

One of the most important properties that can be exploited in some sol–gel derived materials is their biodegradability. Compared to non-degradable materials, biodegradable materials show higher reactivity, which can have a close relationship with formation of an HCA layer \[323\]. However, as
noted above degradation is intrinsically linked to NC and this relationship does appear to show some optimum range with upper and lower limits [40]. Nevertheless in many applications, degradable materials are more ideal than permanent implant materials because they can be resorbed into the body and replaced with regenerated tissues [346] or one a drug payload has been delivered [284,347]. However, the controlling the rate of degradation is complex, excessive degradation rates must be avoided because the materials have to maintain space during the healing process with appropriate mechanical properties and Bioglass® can degrade at an excessively fast rate which can detrimental affect cellular local conditions [348]. Therefore, the understanding of the degradation mechanism and the factors which influence the degradation rate of sol–gel based materials is required. In this section, the ionic degradation by reaction with aqueous solution will be covered for biological applications.

In general, the dissolution tests are carried out by immersing the materials into solutions such as simulated body fluid (SBF) [67,349,350], tris buffer solution [67,350,351], phosphate buffered saline [347,350], and cell culture media [349], and measuring changes at pre-determined periods. Among the various ways to investigate the dissolution rate, measurement of weight loss is the simplest method. Other ways to examine the dissolution of the materials is measuring the concentration of released ions. The pH of the solution is another index of dissolution of the materials. For example, when the glass with network modifier such as Na+ or Ca2+ is dissolved, ion exchange occurs between protons from the solution and the modifier ion from the glass and the pH of the solution increases [349,352]. The change in pH is closely dependent on the buffer capacity of the solution (or local environment in vivo) [349,350]. In most cases, dissolution tests are performed in static condition, where the fixed volume of solution is refreshed periodically, but the dissolution is significantly inhibited if the released ions are saturated in the solution. This complication means that, in some cases, that perfusion systems are highly valuable to investigate dissolution behavior of sol–gel materials in dynamic conditions [352,353].

When silica-based glass is immersed into aqueous solution, the following reactions occur simultaneously: (i) the penetration of water into the glass, (ii) hydrolysis of M–O bonds to form hydroxyl groups and (iii) ion-exchange reactions between protons and network modifier ions. The water molecules can penetrate into the glass matrix by diffusion through voids between oxygen atoms or by hydrolysis and condensation reactions. Usually the latter case is dominant when the water molecules are incorporated into the glass matrix, because most glass structures do not contain the voids which are large enough for water diffusion [354]. During the hydrolysis reaction, the SiO4 4− sites in the glass structure are attacked by a nucleophile such as OH− and five-coordinated intermediates are formed. These intermediates are highly reactive and easily decompose to facilitate the breakage of Si–O–Si bonds [355].

Since the breaking of the Si–O–Si network is a major part of the reaction of dissolution of silica glass, the dissolution rate is closely related to variables that influence the glass network, and the chemical composition of the materials is one of the main factors, which can affect the network structure. For pure silica, the hydrolysis reaction is inhibited because high connectivity of the glass structure makes the formation of five-coordinated intermediate difficult. The addition of network modifiers disrupts the network connectivity and forms the non-bonding sites, which decrease network stability. In general, the reactivity of silica decreases as network connectivity goes from Q1 to Q4 [354]. In the NC model, the role of the network modifier on the dissolution rate is systematically summarized [323,324]. As noted above, materials which have an NC value between 2.0 and 2.4 [324] or 2.6 [356] are recommended for biodegradable materials due to their appropriate dissolution rate and bioactivity. Furthermore, the network modifiers can be released from the network faster than Si ions that are bonded, leading to void development, which helps the diffusion of water molecules into the matrix. Therefore, the dissolution rate increases with increasing ionic radii of network modifier ions. For instance, in the case of alkali metal network modifiers, the effect of ions on the reactivity of the glass follows the trend K > Na > Li [357].

The dissolution reaction occurs at the interface between the material surface and solution. Thus, the specific surface area can be an important parameter which influences the dissolution rate [358]. Due to the larger surface area, the dissolution rate of mesoporous sol–gel derived glasses is higher than that of melt-derived glasses, which are relatively dense [349]. Moreover, when the pore size
increases the circulation of solution in the pore becomes easier, resulting in increased reactivity [358]. Meanwhile, sol–gel derived materials are often heat-treated to remove the organic phase [359] or increase the stability [360]. The heat-treatment process affects not only the materials porosity but also the crystallinity of the materials. Li et al. [361] compared the reactivity of silica with the same composition but different crystallinity using SBF solution. Results revealed that sol–gel derived silica had the highest reactivity among all the samples tested. The silanol groups (Si–OH) which are more abundant on the surface of amorphous silica gel may also affect the reactivity of the silica in providing a site for ion exchange or HCA nucleation [211]. In another example, the dissolution rate of sol–gel derived 45S5 subjected to different heat-treatment temperatures was evaluated. These investigations found that crystalline phases were detected when the heat-treatment temperature was 1100 °C, and this bioglass (BG) showed much slower dissolution behavior than glass which was treated at 700 °C [362]. As expected, these findings can be traced back to a lower degree of crystallinity within glasses when subjected to lower heat treatment temperatures.

The dissolution behavior of sol–gel derived materials is affected not only by the intrinsic conditions described above but also by environmental conditions such as solution temperature, pH and composition. First, the probability of activation of the hydrolysis reaction, as well as the solubility of Si(OH)₄ phase in the aqueous solution, increases with increasing temperature [354]. Thus, the solution temperature significantly influences the dissolution behavior of sol–gel derived silica materials. This trend is also shown in phosphate-based glass system [363]. The pH of the solution also affects the dissolution behavior of sol–gel based materials. For silica glass, the dissolution rate increases as pH increases because Si–O–Si bonds are broken by nucleophilic attack of OH⁻ in aqueous solution [364]. From a thermodynamic point of view, the solubility increases with pH when the pH is above ~8, while the solubility shows a small dependencies on pH within a range of 2–8 [364]. Alternatively, a kinetic view of dissolution shows that the rate increases with increasing pH from 2 to 8.5 and further increases above pH 8.5 because more soluble ions (SiO(OH)₃⁻) are formed above pH 8.5 [39,365]. The composition of the solutions is also an important factor, which determines the dissolution rate of the materials. There is no Ca or P source in Tris-buffered solution; thus, the apatite layer is formed only by the Ca and P ions released from the materials. On the other hand, in SBF or PBS solution, formation of an HCA layer is accelerated and the further dissolution is inhibited because a number of calcium or phosphate ions are contained in these solutions [350]. Furthermore, the biomolecules in the solutions also affect the dissolution behavior. Sepulveda et al. [349] compared the dissolution behavior of sol–gel derived 58S glass in SBF solution and culture medium. ICP results showed the dissolution rate of 58S glass is slower in culture medium than in SBF due to serum proteins. These positive-charged proteins can adsorbed on the materials surface and suppress the dissolution of glass. Thus, it is important to bear in mind the limitations of non-biological in vitro model systems when considering materials that will ultimately be applied in vivo.

From the above, a consensus view that bioactive materials should be tested for their biocompatibility with the physiological environment upon their implantation is without question [366,367], although factors pertaining to degradation of the material itself are but one facet. The bioactivity, or influence, of the material on its in vivo environment is equally important to consider. Critical levels of the ions released by bioactive glasses regulate genes in osteogenic cells, which can initiate a self-repair mechanism for tissue regeneration [368,369]. In other words, dissolution products of bioactive glasses should not be detrimental to the tissues of the host. As yet, bioactive glasses have been implanted in many mammalian species and no evidence of toxic effects have been found [370]. However, just as the release of ionic species can prove beneficial, so too can the release of potentially toxic agents be detrimental. An illustration of this process lies in the 45S5 Bioglass® composition. These granules have been implanted in rabbits to determine the pathway of silicon released during the degradation of glass in vivo [371]. After 7 months of implantation, histopathological analyses of tissues indicated the excretion of silica through urine in soluble form. Although 45S5 is the standard bioactive material, it has certain limitations such as its slow degradation rate and conversion to HCA-like material [372,373]. Hence, the rate at which new tissue formation takes place is not in equilibrium with the rate at which scaffold degrades, thus providing a distinct example of the pervasive nature inherent in the use of biomaterials and the care that should be taken in ensuring that secondary complications are avoided.
4.5.2. Possible interactions with molecules

With the versatile and tunable structures, mesoporous silica-based materials have been proven to be capable of loading a variety of guest molecules including pharmaceutical drugs, therapeutic peptides, proteins, and genes. MSNs have been used as DDSs for various kinds of pharmaceutical drugs with different hydrophobic or hydrophilic properties, molecular weights, and biomedical effects. This includes commonly used agents such as ibuprofen [374], doxorubicin [375], camptothecin [376], cisplatin [377], and alendronate [295]. Peptide and protein drugs have been developed as potent therapeutic agents in many medical applications including cancer therapy, vaccination, and regenerative medicine. However, protein delivery is difficult, in part related to their intrinsic properties of large molecular weight and a fragile structure that must be maintained for activity [378,379]. Attributed to the porous and stable nature, MSNs as DDSs can protect biomolecules against such premature degradation.

The release of the drug from the mesoporous matrix would take place through diffusion of the pharmaceutical molecule into pore channels. However, when targeting a truly controlled release, it is necessary to design stimuli-responsive systems. The use of different external or internal stimuli such as pH, temperature, ultrasounds, and light among others has been employed in order to achieve a controlled release of drugs trapped inside mesoporous silica NPs [268]. Different aspects should be considered for the development of an effective stimuli-responsive system to retain the drug until reaching the target zone and also releasing only the required dose. The combination of supramolecular chemistry with material science may open up excellent opportunities to achieve this goal. In 2001, the Vallet-Regi research group described for the first time that it was possible to load and release different drugs from a mesoporous silica matrix [374] and identified that these materials could host many different molecules. The performance of the silica nanocarriers was monitored employing a reporter molecule. Reporter molecules should present a convenient and relevant size that allows it to enter and exit through the pore, an adequate electrostatic charge and solubility in the environment (usually aqueous solutions) and finally, the molecule should be easily detected (e.g., fluorescent molecules).

The next step is to achieve a controlled release of the trapped drugs in such a way that it only happens in the presence of certain stimuli. The system has to be able to transport a cargo to specific places in the body without premature release during transport and release the payload by the application of the trigger stimuli once reaching the target zone. Other potentially useful properties include switchability [380], which means that a capacity to be repeatedly activated by the trigger stimuli after reaching the targeted cells or tissues can be integrated into the delivery system.

Ibuprofen, a common anti-inflammatory drug and also a useful marker as it is readily measured, was the first to be confined inside of MCM-41 type mesoporous materials by using MNPs with different pore sizes and with subsequent release in SBF [374]. Since then, the interest in this field has grown exponentially as reflected by the number of research papers concerning mesoporous materials as DDSs [381,382]. Factors such as textural parameters and the functionalisation of the pore surface by organic molecules have been shown to influence the loading and release rate of biologically active molecules (Fig. 4.2). Commonly, pore diameters larger than the drug molecule dimensions (pore/drug size ratio >1) are enough to allow the adsorption of drug inside the pores. As can be expected, one of the most important characteristics of mesoporous materials is that the pore diameters can be tuned from a few nanometers to several tens of nanometers by changing the chain length of the surfactant, employing polymeric structure-controlling agents, or solubilising auxiliary substances into micelles [383,384]. Therefore, the loading capability of mesoporous materials can be large, ranging from small molecules up to macromolecules such as proteins [302,385,386]. Moreover, pore size has also been demonstrated to influence the release rate of molecules since this parameter affects drug diffusion to the delivery medium [231,387]. Finally, it must be highlighted that the cornerstone in the development of silica mesoporous materials as DDSs has been organic modification of the surface or functionalisation. As described above, functionalisation of the surface through organic groups provides numerous possibilities to control drug adsorption and release [294,388]. For this purpose the surface is functionalised with chemical groups that are able to link to the drug molecules through ionic bonds or through hydrolytically degradable ester groups and thus, to enhance the loading and release properties via increasing the guest-matrix affinity.
Surface area and pore diameter are critical factors for drug adsorption and release in implantable drug-delivery systems. In this case, the outermost mesopore surface of virtually insoluble porous frameworks is responsible for the interaction with the loaded drug, and the first approach in achieving this involves manipulating the amount of surface available for the drug molecules. So long as the pore size allows the drug to enter the matrix, the higher the surface area the higher the amount of drug adsorbed. However, weak drug–drug interactions can result in under loading conditions and can lead to the pores filling before reaching maximum capacity. In this case, the pore volume is a key factor in determining the amount of drug adsorbed. It was recently reported that several consecutive loadings of drug in ordered mesoporous materials lead to larger filling of the mesopores, which is attributed to the increased drug intermolecular interactions within the pore voids whereby larger pore volumes may result in greater drug loading [389].

In many clinical situations however, the administration of therapeutic compounds is hampered by impaired drug absorption or tissue-unspecific delivery [390, 391]. Further to this, in clinical fields such as oncology, the high toxicity associated with currently employed cytotoxic drugs can create an unmanageable situation with respect to risk. In this particular field, the development of nano- or micro-particles able to deliver different therapeutic agents and release them in a controlled manner in the affected tissues has emerged as one of the most groundbreaking applications in nanomedicine today [392–395].

4.5.3. Possible interactions with cells and intracellular delivery

Advances in nanotechnology research have generated a growing list of contrast agents, therapeutics, scaffolds, and delivery vehicles. Many colloidal suspensions of NPs produced by sol–gel methods, which typically have at least one dimension in the 1–100 nm size range, are engineered as DDSs. Beginning with this aspect, the consequent cellular events can depend on the various physical and chemical factors of the nanomaterials (Fig. 4.6). Hence, evaluation of the combined effects of all the material variables in a series of biological processes, which consist of membrane trespassing, vesicular coating, endosome development, and lysosome degradation, are necessary for one to determine how exactly the cellular trafficking of MSNs occurs.

Considering cellular transport in more detail, the intracellular milieu is physically segregated from the environment by the plasma membrane, an elastic lipid bilayer embedded with domains of lipids, carbohydrates, and membrane proteins. In order to deliver NPs into cells and to their subcellular targets, NPs must first be able to traverse this plasma membrane, which is not always feasible. For example, it was previously found that quaternary amine-functionalised MSNs tended to adhere to negatively charged components of cell membranes rather than penetrating the cells and entered the cytoplasm [396]. This effect was most likely due to the non-polar tail region within the cell membrane lipid bilayer preventing further transport of the MSN. This, in turn, would also suggest that functionalisation of the MSNs surface to the point of introducing larger non-polar domains may prove advantageous.

Aside from passive diffusion, NPs may also be internalised either directly by interacting with membrane-embedded receptors or indirectly by associating with the lipid bilayer. In the first approach, NPs are functionalised with ligands that bind to receptors on the cell with high affinity and specificity. Ligands can be selected or engineered to target over-expressed receptors on healthy and diseased cells [397]. Internalisation of the resulting receptor–ligand complexes then leads to receptor-mediated endocytosis of the NPs. Alternatively, NPs can interact with the membrane via hydrophobic and electrostatic interactions and be taken into the cell through pinocytosis, a form of fluid-phase uptake where cells take in the local extracellular environment through invagination [398]. The variety of pathways available adds a further level of complexity in predicting the activity of such DDSs however with this complexity come further avenues for research.

Controlling the route of NP uptake is important for mediating their intracellular fate and biological response. An increasing number of mechanistically distinct and highly regulated endocytic pathways are used by cells to traffic the extracellular cargoes to different intracellular locations and interactions [399–401]. However, recent studies show that the endocytic processes not only function to internalize nutrients and membrane-associated molecules but orchestrate the spatio-temporal dynamics of cell signaling circuitry [402], meaning that a deep understanding of the cellular uptake mechanisms of
NPs is necessary to determine what and how they would modulate signaling and the subsequent molecular response of the cell [403–405]. In order for a material to be taken up efficiently by non-phagocytic cells, for example, the particle size of the material needs to be on the sub-micron scale [400,406]. For intracellular drug and gene delivery applications, this limitation has led to extensive research efforts on different designs of materials with precise control of the particle size (Fig. 4.7). Furthermore, it is important for the drug carrier to have proper surface properties and favorable interactions with the drug molecules to achieve high loading. This would allow the release of the drug with high local concentrations at the site of interest intracellularly.

Cellular uptake of molecules is often facilitated by the specific binding between the molecule in question and membrane-bound receptors (e.g., LDL or transferrin receptors). In contrast, delivery systems aimed at cells lacking receptors can still be taken up by constitutive “adsorptive” endocytosis or by fluid phase pinocytosis [400,407]. Silica particles are known to have a great affinity for the head-groups of a variety of phospholipids [408]. Therefore, the high affinity for adsorbing on cell surfaces that eventually leads to endocytosis is not surprising. Similarly, chitosans, poly(alkyl acrylic acids), and anionic amphiphilic peptides (derived from viral fusion peptides), which are protonated in response to pH decrease, can also be tethered on MSNs’ surfaces via covalent bonding or electrostatic interaction. This functionalisation can help MSNs accumulate with high charge density when acidity increases during endosome development, destabilising the endosomes’ membranes [298]. In addition, surface modification of MSNs with functional groups, such as photosensitive porphyrins, have been used to develop a novel MSN-based delivery system for photo-induced endosomal escape in living cells [409]. After entering cells via endocytosis and binding to endosomal membranes, photosensitised MSNs can be excited by light and further quenched by a triplet oxygen moiety to produce a singlet oxygen species [409]. The singlet oxygen species then damaged the endosomal membranes, thus releasing the cargo-loaded MSNs.
Although few studies have been conducted on exocytosis, there have been some reports on exocytosis of MSNs in a number of cell lines. PEG has been widely utilized for these purposes as it has the ability to improve the surface hydrophilicity of MSNs, reduce their protein adsorption, and shield them from nonspecific binding [411]. Compared to their naked counterpart, PEGylated NPs (including PEGylated MSNs) have been shown to have much higher dispersion and stability in biological media and much lower adsorption of serum proteins with less uptake by macrophages [411–413]. PEGylated MSNs with enhanced biocompatibility could be produced more easily by applying PEGs with molecular weights >10 kDa on the NPs and by ensuring homogeneous surface coverage and optimal PEG density on the NPs’ surfaces rather than by increasing their chain lengths [411–413]. Properly performed PEGylation could also help the mesoporous structures of MSNs remain intact [413,414]. Furthermore, PEGylated MSNs were shown to exhibit significantly lower effects toward hemolysis of human RBCs, possibly because the PEG groups shielded the silanols on the surfaces of MSNs and prevented the latter from possibly generating ROS that could damage cell membranes. This was confirmed both with in vivo and ex vivo mouse model studies in which the distributions of PEGylated MSNs in various tissues following intravenous injection were probed. PEGylated NPs exhibited longer retention times in the bloodstream and lower accumulation in the spleen, liver, kidney, and urinary bladder, compared to their parent MSNs or their carboxylated or hydroxylated counterparts with similar particle sizes [415,413,416]. In addition, PEGylation prolongs the blood circulation time of MSNs by reducing RES organ uptake, bypassing renal and hepatobiliary clearance, and thus enhancing permeability and retention of the NPs. Hence, the surface modification of MSNs, using PEG or other similar functional groups, not only improves the biocompatibility of MSNs but also promotes the ability of the MSNs to deliver drugs or biomolecules to desired sites in a sustained manner.

4.5.4. Possible interactions with cells and morphological traits

One of the main advantages of sol–gel synthesized materials over other materials is the tunability of the process to shape different morphologies, including films, fibers, foams and particles. Among these, the nanoparticulate form of sol–gel materials hold unique properties derived from the
morphological trait. In this section, we focus on this aspect of sol–gel materials and discuss briefly the possible effects of size and shape of NPs on pertinent biological responses.

4.5.4.1. Particle size. As discussed above, NPs are promising candidates for DDSs, contrast agents for imaging, and other biomedical applications [417–419]. The high surface area of NPs can also be modified by various functional groups for targeting a variety of biomedical applications. Particle size is also considered to be one of the most important properties of nanomaterials including interactions with cells and tissues. Although it is still uncertain as to what the exact relationship is between particle size and potential toxicological effects of nanomaterials [420], size is certainly a property that can be exploited.

For the biotranslocation of MSNs, Lee and co-workers investigated the influence of particle size on the cellular uptake of MSNs by HeLa cells [421]. The cellular uptake amount is size-dependent in the order 50 nm > 30 nm > 110 nm > 280 nm > 170 nm and this may be due to statistical variation. The cellular uptake amount of 50-nm NPs was about 2.5 times higher than that of 30-nm particles. This result is consistent with the trend of other NPs systems that showed 50-nm particles led to maximum cellular uptake [422,423]. It suggests that 50-nm MSN may be the most effective in drug delivery from the perspective of cellular uptake. The distribution in the liver and spleen increased with the increase of particle sizes from 80, 120, to 200 nm at 30 min post-injection, but the particles with a diameter of 360 nm exhibited a different effect, being localized in the spleen [415]. Importantly, particles of smaller size had longer blood-circulation lifetimes. The excretion from urine also increased with increasing particle size, which may reflect the in vivo degradation rates and the excretion amount of degradation products. Research on the cytotoxicity of spherical MSNs with particle sizes of 190, 420, and 1220 nm found that cytotoxicity is strongly correlated with particle size [424]. 190 nm and 420 nm MSNs showed significant cytotoxicity at concentrations above 25 mg ml−1, while microscale particles of 1220 nm showed slight cytotoxicity due to decreased endocytosis. Others have reported that MSNs with sizes of 150 nm, 800 nm, and 4 µm showed a size-independent toxicity in vivo, and instead there was a significant dependence on route of administration [300]. Clearly, further work is needed to decipher role of particle size on both cellular and systemic levels, although what is certain is that sol–gel approaches to the production of such NPs will provide the degree of control and homogeneity needed to produce safe and effective treatments with the efficiency and the flexibility required.

4.5.4.2. Particle shape. In the past few years, particle shape has gained increasing attention [425,426] due to the possibilities that arise with being able to fabricate sol–gel based materials with different shapes and similar composition, structure, diameter, or dispersity. This control enables research into shape effect on nanotoxicity, biodistribution, and performance for drug delivery. Until now, research on the shape effect of sol–gel based materials has mainly focused on in vitro cellular uptake. As reported, cellular uptake of spherical (size from 80 to 150 nm) and tube-shaped (600 nm in length and 100 nm in width) MSNs by Chinese hamster ovarian (CHO) cells and human fibroblast cells was both morphology- and cell line-dependent [427]. For CHO cells, the endocytosis rates for both MSNs were similar and rapid, whereas endocytotic rate of sphere MSNs was significantly faster than that of rod-like MSNs by fibroblast cells. Another study by Yu et al. [428] reported on the cytotoxicity of MSNs with diameter of 80–150 nm and aspect ratio of 1, 2, 4, and 8 showed that aspect ratio had no significant effect on the particles’ acute cytotoxicity, proliferation inhibition, plasma membrane integrity, and cellular uptake to murine macrophage, human lung carcinoma cells, and human erythrocytes. However, the hemolytic activity was shape-dependent: MSNs with higher aspect ratio showed lower hemolytic toxicity. The different cellular uptake behavior of MSNs with similar aspect ratios in this respective study is likely due to the difference in other parameters including diameter of MSNs, selection of cell line, and existence of serum protein in the research systems. With controllable synthesis of MSNs and fluorescent counterparts with aspect ratios from 1 to 10, the interaction between A375 human melanoma cells and MSNs with diameter of about 100 nm and aspect ratio of 1, 2, and 4 [429] also were examined. Overall, particles had a greater impact on different aspects of cellular functions including cell proliferation, apoptosis, cytoskeleton formation, adhesion and migration with the increase of the aspect ratio (Fig. 4.8B) [429], which may result from the accelerated cellular internalisation rate and increased uptake amount (Fig. 4.8C) [429]. In addition to cellular behavior, the protein...
Fig. 4.8. Controlled fabrication of MSNs with different aspect ratios and their effect on the in vitro and in vivo behaviors.
(A) Illustration of the fabrication of MSN–FITC with aspect ratio from 1 to 5 and the TEM images of NPs [416]. (B) A375 cellular adhesion was influenced by nanoparticle with different aspect ratios. (a) Negative control, (b) sphere MSN, (c) short-rod MSN (aspect ratio = 2), and (d) long-rod MSN (aspect ratio = 4) [429] Copyright 2010, Elsevier. (C) A375 cellular uptake simultaneously incubated with two kinds of MSN labeled with different fluorescence. (a) Sphere MSN (RITC-labeled, red), short-rod MSN (aspect ratio = 2; FITC-labeled, green), and merge fluorescent image; (b) sphere MSN (FITC, green), long-rod MSN (aspect ratio = 4; RITC-labeled, red) and merge fluorescent image; (c) long-rod MSN (aspect ratio = 4; FITC-labeled, green), short-rod MSN (aspect ratio = 2; RITC-labeled, red) and merge fluorescent image. FITC: fluorescein isothiocyanate; RITC: rhodamine B isothiocyanate. Reproduced with permission from Ref. [429]. Copyright 2010, Elsevier. (D) Relative silica contents in liver, spleen and kidney at 24 h post intravenous injection of short-rod MSN (aspect ratio = 1.5) and long-rod MSN (aspect ratio = 5). Reproduced with permission from Ref. [416]. Copyright 2011, American Chemical Society. (E) Si content in urine at 24 h post intravenous injection of short-rod MSN (aspect ratio = 1.5) and long-rod MSN (aspect ratio = 5). Reproduced with permission from Ref. [416].
expression related to cell adhesion (e.g., cell adhesion molecule-1 (ICAM-1) and melanoma cell adhe-
sion molecule (MCAM) at the protein and mRNA expression level) were investigated. The influence of
particle aspect ratio on protein expression followed the same trend as the cellular behavior. The dif-
ferent cellular uptake pathways would finally determine the intracellular fate of the NPs as DDSs.

Clearance, in vivo biodistribution, and biocompatibility of MSNs and PEGylated counterparts with
aspect ratios of 1.5 and 5 and diameters of 110–150 nm has also been shown to be dependent on both
shape and the degree of surface modification [430]. At 2 h post-intravenous injection, short-rod MSNs
(NSR) were easily trapped in the liver, while higher amounts of the long-rod MSN (NLR) were distrib-
uted in the spleen (Fig. 4.8D) [416]. PEGylation can decrease the RES sequestration by the liver
and spleen for both shaped MSNs. In terms of the circulation time of particles in blood, the long-
rod MSN (NLR) and short-rod MSNs (NSR) did not show detectable concentration differences in blood
at 2 h after administration. At 24 h, the Si content of NSR in blood significantly decreased, whereas
that of NLR maintained at a similar level as that of 2 h, indicating that NLR has a longer blood circu-
tlation time than NSR. These results also showed that short-rod MSNs had a more rapid clearance from
urine and feces than long-rod MSNs in both excretion routes (Fig. 4.8E) [416]. All these findings may
provide useful information for the rational design of efficient drug delivery nanocarriers and therapeu-
tic systems and provide further insights into nanotoxicity.

Understanding the shape effect of sol–gel derived HA microparticles on cellular behavior is impor-
tant for enabling new kinds of biological and biomedical applications. Yang et al. [431] prepared sol–
gel HA microparticles with different shapes and investigated the interaction between the particles and
bone marrow mesenchymal stem cells (BMSCs). The results revealed that the shape of HA has a strong
influence on cellular behavior, and that the sphere-like particles performed better than the rod-like
particles. Moreover, the HA microspheres performed better than the microrod particles for promoting
osteogenic differentiation of BMSCs.

To confirm the influence of shape of MSNs more precisely, more rigorous research is needed. Cur-
rently, evidence clearly shows that the shape does have an important influence on the cell–nanopar-
ticle interaction and in vivo particle biotranslocation. In a recent study [432], it was found that a rod of
15 nm diameter and 54 nm length (CdSe/CdS quantum dot (QD) coated with silica shell) penetrated
tumors 4.1 times faster than 35-nm diameter spheres (CdSe QD). This result serves as a reminder that
MSNs with large aspect ratios and suitable diameters may have longer circulation time and more effi-
cient tumor accumulation than the spherical ones, which may significant enhance the therapeutic effi-
cacy of nanoparticulate DDSs [433]. However, techniques to produce particles with different shapes
using biocompatible materials are limited [434]. Regarding their effect on endocytosis, a theoretical
model was proposed in 2005 to compare cell membranes containing diffusive mobile receptors that
wrap around ligand-coated cylindrical or spherical particles [244]. In this model, the concept of
“wrapping time” was introduced to explain the faster endocytosis of spherical particles than the cylin-
drical counterpart. To some extent, these models provide useful guidance for the consideration of
shape in designing DDSs. However, other approaches have focused on adjusting the concentration
and the molar ratio of specific aspect of the system more directly. For example, by adjusting the CTAB
and NaOH concentrations, Huang et al. [416] were able to precisely control the aspect ratio of MCM-41
from spherical to rod-like structure with constant diameter. With the ability to tailor any one of the
parameters of diameter, aspect ratio, pore size, and geometry while keeping other parameters con-
stant (Fig. 4.3a), the effect of certain chemo-physical properties on biocompatibility and performance
in drug delivery of NPs can be more easily investigated.

4.5.4.3. Structure and surface area. Structure is one of the greatest contributors to the properties of sol–
gel materials; however, this area of research has been given little attention with respect to biocom-
patibility. The high specific surface area may be advantageous for encapsulating guest molecules, but
may pose an increased risk and hazards with higher reactive and oxidative activity [435]. It is now
generally accepted that the specific surface area is positively correlated with the toxicity of NPs.
NPs with large surface areas and abundant silanol groups have the ability to generate reactive oxygen
species (ROS) which in turn play a significant role in nanomaterial-related injury [435]. It has also
been demonstrated that MSNs can inhibit cellular and mitochondrial respiration [436] and cause
oxidative stress [437], which itself is not surprising given the correlation between ROS and available
surface moieties. However, comparison of the toxicity of porous and non-porous silica NPs results in differing opinions. The reason is deduced as follows: the “cell-contactable surface area” of NPs dictates the nanoparticle-organism interaction. Although the surface area is higher for porous silica than the nonporous counterpart, the “cell-contactable surface area” (the area which cell membrane, cell-bound proteins, and cell-associated molecules can interact) is lower for porous silica NPs instead [438].

Two independent groups also showed that MSNs exhibited less hemolytic activity compared with their dense silica counterparts possessing sizes similar to those of the MSNs but no mesoporous structures in them [439,440]. The authors attributed this difference in the hemolytic effect exhibited by these two materials to the differences in their surface silanol density and overall cell-contactable surface areas. MSNs with fewer silanol groups on their cell-contactable surfaces were considered to trigger the hemolysis of RBCs at a lower rate than their nonporous silica counterparts containing higher density of cell-contactable surface silanol groups [413].

As introduced above, MSNs can be synthesized with different mesostructures such as hexagonal, cubic, and worm-hole. Besides sizes and shapes, the type of mesostructures existing in MSNs was also found to affect the MSNs’ biological activities as illustrated with the following examples. The first example deals with MSNs’ catalytic activity toward the oxidation of epinephrine. As mentioned above, both MCM-41 and SBA-15 MSNs were shown to catalyze the oxidation of epinephrine; however, the two materials exhibited different catalytic activities toward this reaction, which was attributed mainly to their differences in mesostructures [441]. In another example, whereas SBA-15 type MSNs impaired cellular respiration and mitochondrial electron transport chain in cells when incubated in vitro, MCM-41 type MSNs at the same dosage barely resulted in any noticeable effect [436]. In addition, the MCM-41 type MSNs generally produced milder toxicity than SBA-15 type MSNs [442]. Although these two types of MSNs have similar hexagonally ordered mesostructures, they have some subtle differences. For instance, in contrast to MCM-41, SBA-15 has a unique porosity as it possesses pore interconnectivity between the ordered cylindrical channel pores; this in turn, contributes to a substantial part of total surface area and leads to a different catalytic activity from MCM-41 [441]. Thus, some different biological effects exhibited by these two types of MSNs can be explained based on these structural differences. The relatively larger surface area and smaller pore diameter of MCM-41 can make these nanoparticles thermodynamically more favorable for cellular ingestion, according to the proposed model regarding nanoparticle endocytosis [414].

Studies on 3D porous scaffolds confirmed that surface area, volume, and size of the pores have a considerable effect on cell adhesion, growth, and proliferation [242]. An ideal scaffold needs to mimic the extracellular matrix of the tissue that is being replaced and act as a template for cells to attach, proliferate, migrate and function [73]. The pores should be interconnected with minimum diameters of 100 µm for cell penetration, tissue ingrowth and vascularisation, and with the passage of nutrients to the center of the regenerating tissue [242]. The shape of the scaffold also has an effect on tissue cells and sharp edges may elicit more of an inflammatory response than spheres [443]. The biocompatible scaffold structure provides a 3D environment which promotes cell attachment and proliferation [444]. Apart from being 3D, the scaffold should be made from a biocompatible degradable nontoxic material and should be highly porous to permit the diffusion of nutrients, oxygen, and waste products [445]. This is where nanotechnology can have a significant role to play, since nanotechnology permits the creation of a specialized scaffold structure that can be specifically designed for the particular cell or tissue type. Furthermore, the scaffold can be enhanced to provide the maximum environmental conditions for optimal cellular growth.

5. Biomedical applications

5.1. Scaffolds and matrices for tissue repair and regeneration

Sol–gel technology is a contemporary advancement in science that requires a multidisciplinary approach for its various applications. As noted previously, it is the process of making crystalline and amorphous materials at a relatively low temperature that allows doping of various inorganic,
organic and biomolecules during the formation of a glassy or more ordered matrices [74]. Among the matrix networks, silica-based systems and its combination with modifiers in the biocompatible ionic source categories have gained the greatest attraction in biomedical fields. This class of materials, known as silica-based sol–gel bioactive glasses (BGs) have unique merits as the BGs produced by conventional melt-quenching methods have been limited by the prerequisite of high temperature processes for many decades.

One area of profound impact of BGs relates to bone grafts. Bone grafts are used for augmenting or stimulating the formation of new bone in cases such as the healing of skeletal fractures or between two bones across a diseased joint, to replace and regenerate lost bone as a result of trauma, infection, disease or improve the bone healing response and regeneration of the tissue around surgically implanted devices. The high incidence of these conditions is evidenced by the 2.2 million bone grafts used in orthopedic procedures worldwide annually. The tissue regeneration capacity of these grafts is measured in terms of their osteogenic, osteoconductive, and osteoinductive potential [446]. The osteogenic potential of a bone graft is given by cells involved in bone formation, such as MSCs, osteoblasts, and osteocytes. The term osteoconductive refers to a scaffold or matrix, which stimulates bone cells to grow on its surface. Osteoinductive capacity of a bone graft is perhaps the most challenging property in bone healing, as it can refer to the stimulation of MSCs to differentiate into preosteoblasts and thus to begin the bone-forming process [446].

The properties required to actively stimulate cells and bone formation can be improved by the design of scaffolds as biomimetic (i.e. with an ability to simulate natural tissues) and often involves the delivery of osteoinductive factors (?). To design biomimetic bone scaffolds involves building a proper representation of bone, which, in turn, requires understanding of bone biology and clinical physiology. Bone can be seen as an open cell composite material composed of osteogenic cells, ECM proteins, growth factors, mineral in the form of HCA, and a complex vascular system. The cells that make up the bone represent about 10% of the total volume and include osteoprogenitor cells of mesenchymal origin. Pre-osteoblasts are bone progenitor cells located in the periosteum, endosteum, and Haversian canals that derive from MSCs and which are stimulated through specific growth factors. An ideal mimic for the bone matrix would be facilitated by the tissue engineering approach, a concept to engineer tissue equivalents through the use of scaffolding matrix and tissue forming cells. Tissue engineering has emerged as a promising approach for the repair and regeneration of tissues and organs lost or damaged as a result of trauma, injury, disease or aging [447]. It has the potential to overcome the problem of a shortage of living tissues and organs available for transplantation. In the most common approach, a biomaterials scaffold with a well-defined architecture serves as a temporary structure for cells to guide their proliferation and differentiation into the desired tissue or organ. Growth factors and other biomolecules can be incorporated into the scaffold, along with the cells, to help and guide the regulation of cellular functions during tissue or organ regeneration [448,449]. The overall purpose of this scaffold-based tissue engineering approach is to provide the temporary support structure for tissue forming cells to synthesize new tissue of the desired size and shape.

Scaffolds for tissue engineering are commonly constructed from biodegradable polymeric materials of both synthetic and natural origin [450,451]. However, for the regeneration of load-bearing bones, the use of biodegradable synthetic polymer scaffolds is challenging because of their low mechanical strength and lack of bioactivity. Attempts have been made to reinforce biodegradable polymers with a biocompatible inorganic phase, commonly HA [452,453]. Although brittle, scaffolds fabricated from inorganic materials such as CaP-based bioceramics and BGs can provide higher mechanical strength than polymeric scaffolds alone. Biodegradable metals are currently under investigation [454], but their corrosion behavior in vivo remains a key concern. In addition, the situations concerning load-bearing bone grafts often become more complicated from a tribological perspective in that mismatch between the compressive strengths or plastic deformation characteristics of the implant and the natural tissue can result in direct failure due to shear stress induced fractures. Likewise, inappropriate materials which absorb too high a degree of the longitudinal force can lead to a reduction in bone mineral density in the adjacent tissues, thus indirectly exacerbating the differential and increasing the likelihood of implant or joint replacement failure. Whilst the bulk properties of implant materials are outside the scope of this review, applied sol–gel coatings have the potential to provide a biocompatible barrier and therefore stand to increase the range of materials that can be used for their mechanical benefits.
alone. The biocompatibility of such materials is therefore less of a concern as the interface between the natural tissue and the implant materials is mediated by the coating that is applied. In this part, we will focus on BGs and their hybrids with organic phases, and other sol–gel compositions for biomedical scaffolds and matrices to stimulate cells and proper bone formation.

5.1.1. Sol–gel bioactive glasses and hybrids

Among the sol–gel compositions, silica-networked glasses have been the most widely studied. In fact, the greatest commercial success of BGs is the silica-based bone graft known as 'Bioglass 45S5' (45% SiO$_2$, 24.5% NaO$_2$, 24.5% CaO and 6% P$_2$O$_5$ in weight). This material is processed using melting temperatures in the range of 1300–1450 °C followed by casting of bulk implants or quenching to allow formation of powders [455]. Since its discovery, a series of sol–gel compositions have been used to demonstrate in vitro bioactivity in SBF with up to approximately 90% SiO$_2$. These glasses have indeed shown the formation of a HCA layer. Moreover, the rate of surface HA formation for the 58S sol–gel derived compositions was higher than that for the conventional melt-derived 45S5 Bioglass most probably due to a greater surface area and surface reactivity achieved by the low-temperature route and the mesoporosity that is generated. These results encourage the pursuit of potential processing methods for molecular and textural tailoring of the biological behavior – a so called third generation of bioactive materials [456].

For the most part, bioactivity results in the formation of a bond between the tissue and that material, of which two kinds of bioactive materials can be classified according to the tissue response: Type (A) or osteoinductive (which have and connective tissue bone bonding properties and enhance bone formation) and type (B) osteoconductive which are able to bond to bone but at slower rate than the previous Type A and they lack the ability to bond to soft tissue [457]. In other words, these sol–gel derived glasses are considered as bioactive glasses because they have the ability to regenerate bone, and to release ionic stimuli that promote bone cell proliferation by gene activation [242]. Specifically, these properties are thought to be related to the formation of an apatitic surface layer; [458] however, the balance of apatite formation, degradation rate and the presence of extraneous ions are currently the subject of active research.

For the case of sol–gel processed BGs, the effects of ions present in the glass compositions have been substantially researched [459,460]. The presence of major ions, like silicon and calcium, has already shown significant influence on the proliferation and differentiation of cells including osteoblasts and MSCs [461]. Furthermore, some recent attempts have been made to alter the composition of glasses, i.e., incorporating different ionic elements such as phosphate, magnesium, strontium and zinc, in partial replacement of calcium [459,461,462].

Kaur et al. [463] demonstrated the substitution of calcium with phosphate in sol–gel derived glasses with four different compositions, and it was found that the increase in phosphate content led to an increase in the formation of an HCA crystal layer. Moreover, it was found that the growth in this layer increases with glass solubility, and the acidity when immersed in SBF has a direct relation with phosphate content and that there is an association between the decline in cell toxicity with the increase in crystallization. Olmo et al. [464] prepared three different types of sol–gel derived glass compositions $[(\text{SiO}_2)_{0.75−x}(\text{CaO})_{0.25}(\text{P}_2\text{O}_5)_x]$ when $x = 0, 2.5, 5$ and found the rate of HCA layer formation coincided with the high phosphate percentage. In addition, osteoblast cell attachment and spreading were the highest at the highest phosphate composition in comparison to the other compositions in the experiment [464]. Substituting magnesium as (MgO) into (SiO$_2$–CaO–P$_2$O$_5$) sol–gel systems in different percentages showed that replacing the magnesium with calcium led to a decrease in the formation rate of HCA layer, and led to the formation of a thicker HCA layer along with a second whitlockite like layer that attached to the apatite layer formed on the surface of bioactive materials when exposed to physiological solutions. This caused an increase in foam surface area and pore volume with little decrease in pore size [465,466]. In addition to that, adding magnesium to sol–gel derived glasses showed them to be biocompatible and had the ability to stimulate human fetal osteoblastic cells (HFOB) growth [467]. Strontium addition led to strontium traces in the HCA layer formed after immersing it in SBF. In addition to this, the MTS viability assay showed that there was no cytotoxic effect of the added strontium to bone cell viability and calvarial bone cells were seen to colonize the strontium-modified BG. Also examination of osteoblast differentiation markers:
Collagen I and ALP both of which are associated with osteoblast differentiation displayed levels significantly increase over the culture experiment time, and the Runx2, Osterix (Osx) and Dlx5 genes, which are three transcription factors strongly involved in skeletal formation and bone repair, were highly expressed [468,469]. On other hand, zinc-modified sol–gel derived glasses were found to form a thick mineral layer after immersion in SBF and little decline in silicon and calcium dissolution rate was noticed with incubation for seven days. Culturing extracted rat bone marrow mesenchymal stem cells (MSCs) in zinc-modified glass granules resulted in clear increases in the ALP level in relation to zinc concentration and bone sialoprotein (BSP) secretion which is an indicator osteogenic differentiation, were evident especially around the glass granules. This enhanced osteogenesis (?) was significantly enhanced by the zinc BG granules in comparison to that in the zinc-free BG granules after examination by immunofluorescent staining. In addition there was some evidence of increased cell mineralization in the zinc containing samples when viewed by using Alizarin Red S (ARS) staining [470].

As explained above, ionic substitutions have been proposed as an effective tool to improve the biological performance of sol–gel based inorganic materials. Several inorganic materials have been shown to be bioactive and resorbable and to exhibit appropriate mechanical properties, which make them suitable for bone tissue engineering applications. However, the exact mechanism of interaction between the ionic dissolution products of such inorganic materials and human cells are not fully understood. What is certain is that for such materials it is necessary they degrade in vivo so that the trace elements can be released. Further to this, release rates should be maintained at a safe level so as to avoid complications that may arise from toxicity. Hoppe et al. [459] describe a schematic overview of biological responses to ionic dissolution products of sol–gel based biomaterials (Fig. 5.1). However, inorganic dissolution products are by no means the only active agents that can be delivered from sol–gel materials. As discussed above (Section 3.5), the production of organic–inorganic hybrid materials is particularly akin to the sol–gel route.

One of the first hybrids reported was based on PMMA–SiO₂ [210]. This material provides a good environment for cell attachment, proliferation, and differentiation for mouse calvarium osteoblast cell cultures when seeded on these silicate BG surfaces. This observation is possible for two reasons: the

![Fig. 5.1. Overview of biological responses to ionic dissolution products of sol–gel based bioactive glasses [459].](image-url)
PMMA–SiO₂ materials retain the ability to nucleate a favorable CaP surface layer in cell culture media whilst they also release silica (as silicic acid), which itself can aide in osteoblast mineralization. In another example, Chen et al. [317] explored the effects of calcium within a PDMS-modified CaO–SiO₂–TiO₂ system. Here, surface calcium was shown to stimulate an apatite-like phase on their surface when immersed in SBF for 12–24 h. As a result, the mechanical properties of the immersed material were increased by PDMS incorporation while the calcium served to promote HCA formation by both providing physical sites for HCA nucleation and through contribution of the crystal growth thermodynamically; the net result being an increase in in vitro bioactivity. In instances where hydrophobicity is an issue, other less hydrophobic organic components may also be added [471].

The hybrids of sol–gel BGs with natural polymers including collagen, chitosan and gelatin, have also been researched targeting bone matrix and even for eluting drugs. Several characteristics have been suggested for the tissue-engineered constructs, including porosity, suitable pore size and shape, fiber alignment and orientation, fiber density, internal and external architecture, hydrophilicity, and hydrophobicity, including water uptake, binding, and delivery [472,473]. Regardless of the composition of the scaffolds, the abovementioned characteristics are crucial for their osteoconductive properties [474]. Since the materials should be eventually substituted by new bone, another important criterion is their resorbability. Degradation of the polymers is based on enzymatic or hydrolytic pathways [474,475]. Polymeric scaffolds have unique properties, such as biodegradation. Natural polymers are considered as the first biodegradable biomaterials, while synthetic biodegradable polymers can be produced under controlled conditions [474,476]. Bioactive ceramics, such as HA, TCP (TCP is more quickly biodegraded than HAP), and BGs, react with physiological fluids [476]. However, their biodegradability is often insufficient, limiting their potential clinical use. This issue can be overcome by blending synthetic and natural polymers or using composite materials that improve the scaffold properties such as biodegradability. These products are often named ‘hybrid’ materials [477–479]. Recently, a novel nanocomposite hydrogel made of collagen and mesoporous BG nanoparticles with surface amination has been developed [480]. The addition of BG nanoparticles into the collagen hydrogel significantly increases the bioactivity of the scaffold and improves its mechanical properties; this novel strategy would therefore be suitable for bone tissue engineering applications [480]. Moreover, the BG foam produced by sol–gel method is an osteoinductive material with a network of interconnected macropores necessary for cell colonization. It has been shown that BG can differentiate human adipose-derived stem cells into osteoblasts, in vitro [481]. When BGs were implanted, they bind to collagen, growth factors, and fibrin to form a porous matrix to allow infiltration of osteogenic cells [482].

BGs and their hybrid matrices exhibit unique properties for hard tissue regeneration due to their osteoconductive and osteoinductive behaviors. In addition to this, they also show an ability to bond to soft tissue [483,484]. The rapid formation of a thick HCA layer is responsible for the strong bonding between BGs and bone [483,484]. For soft tissue applications, the bioactive layer prevents the formation of fibrous encapsulation, thus enabling better integration between an implanted material and the tissue itself. More recently, it was observed that ionic dissolution products from 45S5 Bioglass® and other silicate-based glasses stimulate expression of several genes in osteoblastic cells [370]. Furthermore, some BGs were shown to stimulate angiogenesis both in vitro and in vivo [485,486], whilst possible antibacterial [487,488] and anti-inflammatory [489] effects of BGs have also been investigated. Increasing evidence in the literature indicates that ionic dissolution products from inorganic materials are key to understanding the behavior of these materials within the context of tissue engineering.

One of the more interesting aspects of materials produced by the sol–gel method is the relatively high surface area that results from condensation of the gel network. As noted above, sintering is often applied in order to produce a more condensed material. However, this high surface area may also present significant benefits concerning the release of desired ionic species. With adequate control over the dissolution rate as a whole, the efficiency of the material as a vehicle for the delivery of active agents would be far greater in materials produced via sol–gel methodologies. Some of the recent studies have shown the potential of the sol–gel BGs and BG-based hybrids with organic materials in eluting drug molecules that have therapeutic actions [476,477–482]. Since studies have only just begun to emerge, more exciting areas in tissue repair and regeneration are warranted for further investigation and should be explored in the near future.
5.1.2. Matrices with other sol–gel compositions

The development of bone implant devices has resulted in an increase in our understanding of the microenvironment where the replacement has occurred. This evolution can be measured by defining three different generations [105]. First generation bone graft substitutes required the biomaterial to match the physical properties of the tissue to be replaced while maintaining a degree of inertness with the tissue microenvironment. These include metals such as stainless steel, titanium, and alloys; ceramics such as alumina and zirconia; and polymers such as silicone rubber, polypropylene, and poly(methyl methacrylate). A common occurrence for all these biomaterials when implanted, is the formation of fibrous tissue at the biomaterial–tissue interface. This eventually encapsulates the entire implant and can subsequently lead to aseptic loosening. This occurs as a non-specific immune response to a material that cannot be phagocytosed and an inflammatory response persists until the foreign body becomes encapsulated by fibrotic connective tissue, shielding it from the immune system and isolating it from the surrounding tissues [490]. To avoid this non-specific immune response, second-generation bone graft substitutes were developed with bioactive interfaces to elicit a specific biological response (i.e. osteoconduction) and to avoid the formation of the fibrous layer and improve osseointegration. The overall strategy has been to modify first generation biomaterials with coatings that are bioactive or biodegradable. This bioactivity is provided by a surface chemistry that allows either mineralization through heterogeneous nucleation and crystallization of HA or covering of the biomaterial surface with bioactive ceramics such as HA, β-TCP, or bioactive glass. Another type of second-generation bone graft substitutes has been designed as biodegradable with the aim that the rate of degradation matches the healing rate of the injured bone tissue. These materials are based on the use of synthetic or natural polymers that can provide a controlled chemical breakdown under physiological conditions into inert products that can be resorbed by the body. Third generation bone graft substitutes aim to get closer to the autograft standard by using biomaterials capable of inducing specific cellular responses at the molecular level by integrating the bioactivity and biodegradability of second generation devices. This type of bone graft is based on the concept of bone tissue engineering which focuses on creating devices that enhance bone repair and regeneration by incorporating bone progenitor cells and growth factors to stimulate cells into a scaffold made of various natural or synthetic biomaterials or combination thereof, and with sufficient vascularisation to allow access to nutrients to support this process [491]. This method of bone regeneration is osteoconductive in which MSCs are recruited and stimulated to differentiate into pre-osteoblast and depends on the microenvironment.

From a material science perspective, scaffolds are an important area in tissue engineering and can be divided into two main categories including biological (natural or organic) and synthetic (artificial) materials [490–493]. However, for the regeneration of load-bearing bones, the use of biodegradable polymer scaffolds is challenging because of their low mechanical strength and lack of biological activity. Attempts have however been made to reinforce the biodegradable polymers with a biocompatible inorganic phase such as HA [494–497]. Although brittle, scaffolds fabricated from inorganic materials such as calcium phosphate-based bioceramics and bioactive glass can provide higher mechanical strength than polymeric scaffolds. Biodegradable metals are currently under investigation [498], but their corrosion behavior in vivo remains a key concern.

To produce a scaffold, important information can be gained by deconstructing biologic tissues to their basic components and then reconstructing the critical component according to the tissue engineering goals [499,500]. Such constructs are specific for one or two materials (e.g., collagen + hydroxyapatite, collagen + chitosan) and therefore have low antigenicity compared with cadaveric grafts [499,501]. Human primary osteoblasts have been seeded onto innovative collagen–gelatin–genipin–HAp scaffolds. In vitro attachment, proliferation, and colonization of human primary osteoblasts on collagen–genipin–HAp scaffolds with different percentages of HA (10%, 20%, and 30%) all increased over time in culture, but comparing different percentages of HA, they seemed to increase with decreasing HA content [502]. A tricomponent osteogenic composite scaffold made of collagen, HA, and poly(ε-lactide-co-ε-caprolactone) has been recently developed, and this composite scaffold was combined with human osteoblast-like cells. The composite was highly porous and enabled osteoblast-like cell adhesion and growth [503]. Jung et al. [504] elucidated the role of collagen membranes when used in conjunction with bovine hydroxyapatite particles incorporated with collagen matrices for lateral
onlay grafts in dogs. This strategy leads to superior new bone formation and bone quality compared to bone graft alone.

CaP ceramics, specifically \(\beta\)-tricalcium phosphate (\(\beta\)-TCP) and synthetic HA, have recently been used in composites and in fibrous composites formed using the electrospinning technique for bone tissue engineering applications. CaP ceramics are often sought because they can be bone bioactive, so that apatite forms on their surface, facilitates bonding to bone tissue, and is osteoconductive [505]. In a recent study, the bioactivity of electrospun composites containing CaPs and their corresponding osteogenic activity was investigated. Electrospun composites consisting of (20/80) HA/TCP nanoceramics and poly(\(\varepsilon\)-caprolactone) were fabricated, and the results demonstrated that after seeding the hybrid scaffold with human MSCs, the cells not only showed greater osteogenic differentiation but also proliferated and produced more bony matrix \textit{in vitro} [505].

5.2. Nanocarriers to deliver biomolecules

The goal of an ideal drug-delivery system is to deliver the therapeutic amount of a drug to the proper site in the body to achieve promptly, and to maintain, the desired drug concentration [75]. Drug-delivery carriers have attracted interest during the past decades, since they can deliver low-molecular-weight drugs, as well as large biomacromolecules such as proteins and genes, either in a localized or in a targeted manner. Many newly designed drugs are also based on biomolecules such as peptides, proteins, and DNA. These molecules are by and large chemically unstable, thus encapsulating and protecting them can be useful in order to deliver drug molecules to the target tissues. Various drug delivery carriers such as micelles, dendrimers, liposomes, emulsions, and porous materials have been showing great promises [506–508]. In particular, porous materials that can be easily processed by the sol–gel approach have attracted greater attention because of their high surface area, stability, uniform porous structure, and tunable pore size [509,510]. In this part, we discuss how sol–gel nanocarriers are evolving as DDSs targeting the repair and regeneration of tissues and the treatment of diseases like cancer.

The essential function of DDS is the ability to increase drug solubility, securing the biological activity from harsh environmental factors like acidic and enzymatic degradation; as well carriers can change drug metabolism and pharmacokinetics to increasing drug accumulation in the targeted location and decrease the off-target distribution and unwanted toxicities. Incorporation of drugs into mesoporous silica materials was introduced as early as 1983 and developed by Kortesuo et al. [284] who reported a sustained release of toremifene citrate from implanted silica sol–gel glass discs with no tissue irritation at the site of the implantation over 42 days. In a logical development from this, mono-dispersed mesoporous silica microspheres were produced by Grun et al. [101]. Following this, increasingly narrow size distributions were developed by fine-tuning the reaction conditions and using various surfactants [511,512]. The uniformity of these materials was identified at an early stage of development in order to ensure a controlled rate or dose of drug release, however, the exact nature of the material must also be controlled. As noted above, particle size can influence both drug release kinetics and the entire loading capacity of MNPs and sol–gel formulated mesoporous nanocarriers have shown excellent capacity to deliver genetic molecules intracellularly, including small interfering RNA (siRNA), micro-RNA (miRNA), messenger RNA (mRNA), and plasmid DNA (pDNA), etc. Compared with viral carriers, non-viral gene delivery systems are much safer and exhibit low-immunogenicity, but the main roadblock of current non-viral carriers is their low gene transfection efficiency. MSNs are considered to be promising candidates for high efficiency gene delivery. The mesoporous structure and tailorable pore size provide space to accommodate gene molecules. Sterically protected in the mesopores, these payloads can escape from nuclease degradation during delivery [513].

To increase the loading capacity of electronegative nucleic acids, well-established surface chemistry allows easy surface modification of MSNs with polycationic groups. Polycation polymers including PAMAM [514], PEI [515], and mannosylated PEI [516] have been functionalized onto MSNs for gene delivery. The positive surface not only increases electrostatic interactions between negatively charged genes, but also facilitates escape from intracellular endosomes through the “proton sponge effect”. Xia et al. [517] found that non-covalent attachment of PEI to the surface of MSNs not only generated a cationic surface for DNA/siRNA constructs attachment, but also increased MSN cellular
uptake. Recently, direct packaging of siRNA or DNA within the mesoporous structure of MSNs has also been achieved without the need for surface modification [100,237]. In this case, the main driving forces for siRNA and DNA adsorption into mesopores were intermolecular hydrogen bonds instead of the electrostatic interactions that associated with surface functionalisation. Similar to the delivery of small pharmaceutical drugs, the loading of genes in MSNs can also be controlled by pore size [205,209]. For example, MSNs with large pores (≥23 nm) and functionalised with amino groups have been synthesized for the delivery of pDNA to human cells [209]. Large pores can protect the plasmid against degradation by nucleases in an intact supercoiled form, whereas plasmid vectors loaded in MSNs with 2 nm pores were completely released or degraded because the majority of the vector was loaded on the outer surface of the MSN particles [205].

Nel et al. [517,518] utilized PEI to improve the gene transfection efficiency of MSN because PEI is known to intracellularise effectively. PEI was first incorporated into MSNs, and siRNA and DNA subsequently adsorbed. Particle to nucleic acid ratios of 10:100 were needed for complete adsorption. As expected, the high positive charge density of the PEI layer was beneficial for adsorption and showed high transfection efficiency. With non-porous silica NPs, the surface modification showed also some effects on the DNA adsorption and the gene transfection, in which case however the DNA may not be fully protected against degradation by nucleases in the plasma [519,520]. Two main approaches can thus be proposed to enhance the adsorption of genes onto the mesopores of silica NPs: optimization of the loading conditions and increasing mesopore dimensions. In a recent report, Li et al. [209] demonstrated the exclusive adsorption of short salmon derived DNA (20–250 base-pairs with an average chain length of 50 nm) onto the mesopores of small-pore MSNs (pore diameter 2.7 nm) under chaotropic salt conditions where the diameter of the particles was about 70 nm and where the core was composed of a 10 nm super-paramagnetic iron oxide crystallite. Using longer calf thymus DNA (20,000 base-pairs) the maximum loading capacity was reduced to about 22 mg DNA/g MSN, ascribed to the stiffness of DNA under the conditions used. The same group also studied the packaging of siRNA into the mesopores of MSNs under the same conditions as those used for the adsorption of salmon DNA but virtually no adsorption of siRNA was observed [205]. However, performing the siRNA adsorption under more dehydrating conditions (66.7% ethanol) led to siRNA loadings of 13.5 mg siRNA/g MSN at an equilibrium siRNA concentration of 80 µg/ml regardless as to whether a negative control siRNA or expressed green fluorescent protein (eGFP) siRNA was used thus highlighting the importance of optimizing the adsorption conditions to the hydrophilicity of the cargo. The benefit of using MSNs with larger pores was also demonstrated by Solberg and Landry [521]. Using amino-functionalised MSNs containing much larger cage-like pores, with an inner diameter of 20 nm and a particle size of 70–300 nm, Gao et al. [100] were able to adsorb up to about 150 µg/g MSN of firefly luciferase plasmid DNA (5256 base-pairs) from PBS buffer. The surface amino-groups allowed for an attractive interaction with the negatively charged DNA. The adsorbed plasmid was protected against enzymatic degradation, although the exact location of the plasmid, inside the mesopores and on the outer particle surface, remained unclear.

However, many cancer drugs have poor solubility, instability and poor cellular uptake that hamper their therapeutic effects. Due to frequent occurrence of side effects, the therapeutic window of many drugs is narrow. Therefore, the development of delivery systems that can carry a high payload of drug, protect the drug from degradation, facilitate cellular uptake and target specific cell populations is necessary for the clinical applicability of many drugs. MSN-based carriers have thus been highlighted to be especially suited for delivery of small molecules with the advantage of being able to carry a high load of poorly soluble drugs [522]. As more than 40% of substances discovered through combinatorial screening programs have poor solubility in water [523] it comes as no surprise that MSNs based materials have attracted a high interest as drug carriers. The majority of studies to date have focused on the delivery of anticancer drugs such as DOX. Lee et al. [421] delivered DOX to tumor sites and observed DOX-originating fluorescence as well as apoptosis ex vivo in the harvested tumor cells 48 h post-injection. However, no free drug was used as control in this study, and hence, the therapeutic benefit of particle-mediated delivery remained unclear. Hillegass et al. [524] applied similar sol–gel based silica microparticles as carriers for DOX to treat malignant mesothelioma. DOX-loaded and empty microparticles were injected both subcutaneously and directly into the tumor or intraperitoneally in an in vivo mouse model. Particle-mediated delivery showed enhanced intracellular uptake of
DOX, improved therapeutic efficacy. Enhanced efficacy by incorporation of DOX in MSNs has also been demonstrated in Hep-A-22 liver cancer [525]. For example, Meng et al. [526] demonstrated enhanced particle-mediated DOX efficacy by PEG–PEI coated 50 nm MSNs after intravenous administration. Tumor regression was significantly higher as compared with free drug. Importantly, the particle-encapsulated DOX demonstrated reduced systemic, hepatic and renal toxicity. The particles accumulated at tumor sites due to the enhanced permeability and retention effect. Active targeting achieved by specific tumor targeting ligands to the particles can further enhance therapeutic efficacy.

Along with genetic molecules, small drugs have also been delivered substantially through sol–gel NP systems. In particular, anticancer drugs have been the most potentially studied in this regime. Combination therapies with two or more therapeutic agents with complementary or synergistic effects have been used in a variety of diseases especially in cancer therapy. It has been one of the most promising areas in nanomedicine to develop efficient DDSs that could simultaneously deliver two or more kinds of therapeutic molecules in a coordinated manner. Because small-molecule drugs and macromolecular drugs differ greatly, conventional DDSs have difficulty in co-delivery. With tunable pore size, MSNs are excellent candidates for drug co-delivery systems [375,527,528]. They offer both interior pore and exterior particle surface for loading different guest molecules, which is particularly useful for controlling the release sequence of different cargos. Lin et al. [529] designed glucose-responsive double delivery system for both modified insulin and cAMP. These two kinds of molecules had a precise releasing sequence by immobilising gluconic acid-modified insulin (G-Ins) proteins on the exterior surface of MSNs as caps to encapsulate cAMP molecules inside the mesopores. The release of both G-Ins and cAMP from MSNs can be triggered by the introduction of saccharides, such as glucose. Chen et al. utilized MSNs to co-deliver doxorubicin and Bcl-2 siRNA into multidrug-resistant cancer cells to reverse drug resistance [375]. Zhu et al. designed enzyme-triggered drug and gene co-delivery systems by combining hollow mesoporous silica with enzyme degradable PLL polymer [528,530].

More recently, Ashley et al. [531] fused supported lipid bilayers onto MSNs to construct a “proto-cell” (Fig. 5.2A). The organic–inorganic hybrids were able to synergistically combine the advantage of MSNs with extraordinarily high drug loading capacity with liposomes for enhanced lateral bilayer fluidity. They enabled targeted delivery and controlled release of high concentrations of multicomponent cargos within the cytosol of cancer cells. The hybrids were used to deliver drugs and drug cocktails, siRNAs, and protein toxins (Fig. 5.2B). When delivering a cocktail of DOX, 5-fluorouracil and Cisplatin, the protocells had a $10^6$ times higher killing effect on multidrug resistant cells over comparable liposomes (Fig. 5.2C and D). In one instance, by co-delivering Bcl-2 siRNA and DOX, the anticancer efficiency was increased 132 times for drug resistant human ovarian cancer cells compared with free DOX [375]. Lindén and co-workers have delivered γ-secretase inhibitors (GSIs) encapsulated into folate bioconjugated PEI coated MSNs to inhibit Notch signaling, which plays a significant role in tumor angiogenesis, maintenance and progression, and is a specific cancer stem cell signaling for maintaining stem cell characteristics [532]. They showed effective Notch inhibition and significantly enhanced breast tumor therapy efficacy [533].

One important concept in MSN-based DDS is ‘gatekeeping’, which hybridises another type of molecules or NPs (much smaller than MSNs) on the surface of MSNs. The gatekeeping material is primarily sensitive to internal or external stimuli to enable stimulus-responsive opening of mesopore gates to release drug molecules on demand. Acid-decomposable inorganic materials are examples of gatekeepers that are able to control drug release offering opportunities to design promising carriers for specific therapeutic agents (Fig. 5.3A). One of the more elegant examples of this was demonstrated by Rim et al. [534] who introduced inorganic CaP as a pore blocker through enzyme-mediated mineralization on MSN surfaces. This block could then be dissolved in intracellular endosomes as nontoxic ions to initiate drug release. The construction of the nanoparticle involves urease functionalisation of MSN surfaces and subsequent enzyme-mediated surface calcium phosphate mineralization in the presence of urea under mild conditions within a short time. For pH-controlled DOX release from mineralized MSNs, pH variation between physiological pH and low pH is employed. The results show that a large amount of DOX was released after 24 h under low pH conditions (Fig. 5.3B). Furthermore, the pH-dependent dissolution kinetics of HA-like coating support the DOX release profiles from CaP-capped MSNs (Fig. 5.3C), which confirms that the dissolution of pore blocks results in the opening
of the pore and then triggers DOX release. In breast cancer MCF-7 cells, DOX-loaded mineralized MSNs carry DOX in nanopores effectively before endocytosis. DOX release can also be facilitated in lysosomes by the dissolution of mineral coatings (Fig. 5.3D).

An alternative strategy was also employed by Muhammad et al. [535]. These authors made use of acid-decomposable ZnO QDs to seal the nanopores of MSNs and inhibit premature drug (DOX) release. After internalization into HeLa cells, the ZnO QD lids are dissolved rapidly in the acidic intracellular compartments, therefore enabling intracellular drug release. In this pH-responsive DDS, ZnO QDs behave as a dual-purpose entity that not only serves as a lid but also imposes a synergistic anti-tumor effect on cancer cells. Another modification of pH-responsive MSNs, able to overcome drug resistance, was published by He et al. [536]. Their composite NPs consisted of MSN carriers still containing the porogen, CTAB, and DOX, where the surfactant served as a chemosensitizer, thus enhancing drug efficacies whilst also acting secondary offensive against multi-drug resistant cancers. It should also be noted that such pH-sensitive DDSs are not only of use in cancer research, but may also have applications in other circumstances where the diseased state can be delineated by a change in pH.
Diagnosis of diseases at an early stage is a key factor in medicine and improves the chance for a successful treatment. Successful diagnosis is in turn dependent on the clinician ability to determine the exact nature of the diseased state. It therefore stands to reason that tools that are able to improve the clinician ability to make such deductions would aide treatment in their own right. Imaging modalities in the clinic generally include optical imaging, MRI, CT, ultrasound and PET or single photon emission computed tomography (SPECT). Fig. 5.4 shows the characteristics of these imaging modalities currently used in the clinical field [18]. Each imaging modality has its own unique advantages along with intrinsic limitations (such as insufficient sensitivity or spatial resolution) which make it difficult to obtain accurate and reliable information at the disease site [537]. To compensate for this problem, the combination of imaging modalities, such as PET and CT or PET and MRI, have gained attention to improve currently used imaging instruments for diagnosis. Multimodal imaging is a powerful method that can provide more reliable and accurate detection of disease sites. Some inorganic NPs also exhibit...
intrinsic imaging abilities, such as MNPs for MRI, gold NPs for CT, and QDs for optical imaging. These can be combined with different imaging agents by co-encapsulation or conjugation to develop multimodal imaging platforms.

Commonly used imaging modalities have varying capabilities based on individual mechanisms of tissue contrast or function, specific sensitivity, spatial and temporal resolution in relationship to diseases, as well as biological processes. Multimodality imaging with two or more imaging modalities allows integration of the strengths of individual modalities while overcoming their limitations. Anatomical imaging technologies such as CT and MRI provide unparalleled structural detail whereas functional modalities such as PET and SPECT provide insight into morphological and functional behaviors. By incorporating anatomical and functional imaging in a common hybrid imaging platform, a synergism in the imaging capabilities can be achieved, thus making it possible to visualize and delineate precise structural and functional information. Multimodality imaging thereby ensures better elucidation of physiological mechanisms at molecular and cellular levels. Therefore, multimodality imaging has an immensely beneficial role for improved diagnosis and therapeutic planning of a disease. The field of multimodality imaging has seen rapid progress in the last decade, with its value having been demonstrated in numerous studies. Multifunctional NPs can thus enable multimodal imaging with the combination of two or more imaging modalities for simultaneous imaging and therapy. These techniques are expected to overcome the hurdles of traditional diagnosis and therapy through

Fig. 5.4. Characteristics of imaging modalities currently used for biomedical applications [18].
optimized therapy called “theranostics”. The sol–gel silica, with its abundant surface silanol groups, a mesoporous structure, and the facile sol–gel synthetic method, provides a matrix for integrating other NPs or chemicals to form nanohybrids.

Generally, silica NPs themselves do not have characteristics for imaging. Therefore, encapsulation with silica matrix is one of the most widely used methods for surface modification of inorganic NPs [538], because the unique properties of the NPs can be preserved by silica shells and various high-quality NPs synthesized in organic media can be readily transferred to aqueous media. Controlled sol–gel reactions generate amorphous silica shell around the NPs as described in Fig. 5.5 for the carrier system.

The formation of mesoporous silica instead of a dense silica shell can impart additional drug delivery functionality onto silica-based nanocomposite NPs. Instead, they afford an excellent platform that allows facile loading of a broad range of imaging and therapeutic functions thus making them a good candidate for theranostic purposes. Aside from small molecules, NPs can also be easily incorporated into silica matrices and reports on using such technology to encapsulate inorganic materials (such as MNPs, gold NPs, and QDs) have been well documented [113,540–542]. Moreover, several functionalities can be encapsulated into a single silica particle simultaneously [113,541,542]. A theranostic NP can be dissected into at least three basic components, i.e. biomedical payload, carrier, and surface modifier, depending on their roles and physical locations (Fig. 5.5) [539]. Nanocarriers should provide sufficient physical protection for the biological payloads under physiological conditions during delivery to the desired target site. Various sol–gel based inorganic carriers have been developed. The carrier might be designed to become cancer-specifically disintegrated for better imaging or therapeutic efficacies. Finally, modifiers are attached to the surface of the carrier which are expected to provide theranostic nanomaterials with additional properties, for example, long circulation time, barrier-penetrating ability, and target-specific binding ability. The components for generating a theranostic nanoparticle along with material classifications and functions are listed in Table 5.1. Sathe et al. [543] utilized silica to encapsulate both MNPs and QDs, creating hybrids that retained both magnetic and optical properties. Koole et al. [544] reported a dual-function core–shell–shell nanoparticle, where silica NPs were loaded with both QDs and gadolinium complexes. Both of which served to illustrate this point.

One of the most widely studied theranostic hybrid NPs is magnetic-based because the MRI applications facilitate detection of even the earliest stages of disease, monitoring of tumor response to drug therapies, and tracking cell migration [21]. Surface modifications may be easily introduced through conjugation with targeting moieties, fluorescence dyes, genes, or drugs to provide multimodal functionalities [21,545]. For soft tissue imaging, researchers commonly employ gadolinium ions as chemical contrast agents. In addition to gadolinium, MNPs of elements such as iron, nickle, cobalt, manganese, chromium, as well as their chemical compounds are also used in MRI.

![Fig. 5.5. Schematic illustration of a multifunctional nanocomposite [539].](image)
Magnetic nanocrystals coated by hydrophobic surfactants cannot be used directly for biomedical applications, because intravenously injected nanocrystals tend to agglomerate thereby blocking blood vessels or resulting in nonspecific accumulation in nontarget organs. Many efforts have been made to avoid aggregation of NPs by increasing colloidal stability. Encapsulation by silica is one such example. In 2006, the Hyeon group reported a simple and general method for encapsulation of magnetic oxide NPs in mesoporous silica shell [546]. In this method, pre-synthesized uniform magnetic oxide NPs stabilised with oleic acid were transferred to aqueous media by capping with CTAB. Subsequent silica sol–gel reaction followed by the removal of surfactants resulted in the production of mesoporous silica spheres embedded with iron oxide NPs. This simple and highly reproducible synthetic process can serve as a standard protocol for the fabrication of uniform-nanoparticle possessing mesoporous-silica core–shell nanostructures for multifunctional theranostic applications.

Using this synthetic method, uniform iron-oxide–nanoparticle with mesoporous-silica core–shell nanocomposite NPs were synthesized for imaging and therapy [547]. Particle size strongly affects the efficiency of in vivo delivery and cellular uptake [548,549]. For effective systemic delivery, it is desirable to keep the size of therapeutic nanocomposite particles smaller than 100 nm because of their high colloidal stability in a physiological environment and long blood circulation time. The overall particle size could be controlled from 45 to 105 nm by varying the concentration of iron oxide NPs (Fig. 5.6A). The resulting core–shell NPs were monodisperse and maintained discrete form without aggregation, which is highly desirable for in vivo applications. Because these nanocomposite NPs are composed of superparamagnetic iron oxide nanoparticle core and mesoporous silica shell, they can be used not only as an MRI contrast agent but also as a drug delivery vehicle. Furthermore, organic fluorescence dyes such as fluorescein isothiocyanate and rhodamine B isothiocyanate can be readily immobilised in mesopores rendering them useful as fluorescent imaging probes. Surface modification with PEG imparted biocompatibility and colloidal stability under physiological conditions. Even after PEG-coating, the hydrodynamic diameter of nanocomposites was kept below 100 nm and they were easily internalised into cells by endocytosis. DOX loaded nanocomposite NPs were internalised by cancer cells and induced cell death (Fig. 5.6B). The applicability of nanocomposite for in vivo cancer imaging was also demonstrated. Fig. 5.6C shows in vivo MR and optical images of subcutaneously injected cancer cells labeled with magnetic iron oxide nanocomposites. The labeled cells appeared as dark contrast in the MRI scan due to enhanced T2 relaxation, and intense red emission, due to rhodamine B isothiocyanate, was also observed in the fluorescence image. Such multimodal imaging capability is very useful for both noninvasive diagnosis and guidance to surgical treatment [550]. The size and shape of the nanocomposite NPs also enable systemic delivery to the tumors. When these NPs were administered to the tumor-bearing mice via intravenous injection into the tail vein, they were accumulated at the tumor sites and detected via MRI 2 h after the injection (Fig. 5.6D). The multifunctional capability of the nanocomposite NPs as MRI and fluorescence imaging probes, along with their potential as drug delivery vehicles, makes them novel candidates for simultaneous cancer diagnosis and therapy.

### Table 5.1
Materials used in multifunctional NPs for biological imaging, DDSs, and tissue engineering.

<table>
<thead>
<tr>
<th>Component</th>
<th>Material</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomedical payload</td>
<td>Imaging agents for optical, CT, MRI, PET, ultrasonic imaging (organic dye, QDs, UCNPs, magnetic materials, metal NPs with SPR, CNT)</td>
<td>Imaging enhancement</td>
</tr>
<tr>
<td></td>
<td>Therapeutic agents (anticancer drugs, DNA, siRNA, hyperthermal/photodynamic materials)</td>
<td>Cancer cell death induction, gene up/downregulation</td>
</tr>
<tr>
<td>Carrier</td>
<td>Inorganic (hollow metal NPs, hollow metal oxide NPs, carbon nanostructures, porous Si or SiO2 NPs)</td>
<td>Multifunctional (imaging ability added to above functions)</td>
</tr>
<tr>
<td>Surface modifier</td>
<td>Antibody, Aptamer, Peptide/protein, Small molecules, Charge-balancing molecules</td>
<td>Molecular imaging, Target specific delivery, Uptake enhancement, Penetration of barrier, Signaling transduction, Stimuli responsiveness</td>
</tr>
</tbody>
</table>

Magnetic nanocrystals coated by hydrophobic surfactants cannot be used directly for biomedical applications, because intravenously injected nanocrystals tend to agglomerate thereby blocking blood vessels or resulting in nonspecific accumulation in nontarget organs. Many efforts have been made to avoid aggregation of NPs by increasing colloidal stability. Encapsulation by silica is one such example. In 2006, the Hyeon group reported a simple and general method for encapsulation of magnetic oxide NPs in mesoporous silica shell [546]. In this method, pre-synthesized uniform magnetic oxide NPs stabilised with oleic acid were transferred to aqueous media by capping with CTAB. Subsequent silica sol–gel reaction followed by the removal of surfactants resulted in the production of mesoporous silica spheres embedded with iron oxide NPs. This simple and highly reproducible synthetic process can serve as a standard protocol for the fabrication of uniform-nanoparticle possessing mesoporous-silica core–shell nanostructures for multifunctional theranostic applications.
Along with magnetic-NPs, gold NPs have also been popularly researched in the hybrid materials with MSNs to provide multifunctionality for theranostics, because of their intrinsic optical properties to convert near infrared (NIR) light into heat and CT image [551, 552]. Current clinical therapy investigations have shown that when combined with chemo-thermotherapy, additional enhancements result in anticancer efficacy, because hyperthermia can promote drug delivery into tumors, which increase drug toxicity [553, 19]. However, gold NPS with nonporous structures exhibit low loading capacities and limited elasticity, restricting their application in effective drug delivery. Therefore, development of multifunctional NIR absorbing nanomaterials that can deliver both drugs and heat, are highly desired. Simple sol–gel methods have been reported for the synthesis of mesoporous silica-encapsulated gold nanorods [554]. The obtained materials have high biocompatibility and stability in biological environments. Most importantly, multifunctional hybrids have specific advantages for combinations of chemo-therapy and photothermal-therapy. Up to now, the use of both therapies applied to cancer cells has been rarely reported while their biomedical applications mainly remains in fluorescence imaging [555], drug release [3] or in vitro cancer research [556] rather than dual-model therapy (chemo-photothermal therapy in vivo and CT image) [555]. Thus, these in vivo studies allow research to elucidate the validity of drug-loaded gold nanorods encapsulated within silica, where safety is extremely significant.

Zhang et al. [556] reported that the development of mesoporous silica-coated gold nanorods as a novel cancer theranostic platform. The large specific surface area of mesoporous silica guarantees a high drug payload. To demonstrate the multiple functionality of new platforms, a well-known

![Fig. 5.6.](image-url)
chemotherapy drug, DOX, was employed as a model drug. The theranostic principle based on the unique properties of Au@SiO₂ is illustrated in Fig. 5.7 (a–e) [556]. One imaging modality of the silica-coated gold nanorods, two-photon imaging, was employed to visualize the intracellular colocalisation of material with DOX and some cellular compartments. NIR laser irradiation at a low intensity as used to enhance drug release from DOX-loaded silica-coated gold nanorods for chemotherapy, while at higher irradiation intensity it also gave rise to hyperthermia, which can kill the surrounding cells. Silica-coated gold nanorods can thus provide two therapeutic modes for cancer treatments.

As one of the most widely used diagnostic tools in hospitals today, X-ray CT is an imaging technique based on X-ray absorption difference (absorption contrast) of the body compositions. Due to their high atomic weight and X-ray absorption coefficient, small iodinated molecules are generally used as contrast agents to improve the visibility of internal body structures in X-ray CT procedures. However, these contrast agents only allow very short time imaging due to rapid renal excretion and may also cause renal toxicity. Recently, silica-coated gold nanorods have attracted much attention...
for the development of new CT imaging agents, because gold has a higher atomic weight and X-ray absorption coefficient than iodine. Luo et al. [555] reported indocyanine green-loaded silica-coated gold nanorods for the dual capability of X-ray CT and fluorescence imaging. It was demonstrated that nanorod system could enhance CT contrast significantly and multiplexed images can be easily obtained by using this dual mode imaging agent. In another paper, the same group also showed that X-ray imaging could be employed to monitor the tumor targeting ability of folic acid-conjugated silica-coated gold nanorods during the whole blood circulation (Fig. 5.7F) [430]. The sol–gel chemistry offers a simple method to coat and build in chemical functionality to NP’s.

5.4. Sol–gel based sensing devices

Biosensors rely on the interfacing of a biorecognition element to a physical transducer that converts the biological reaction into a measurable signal. Key attributes that can be optimized include response time, initial and long-term biological activity, analytical range, sensitivity, selectivity, detection limit, and reproducibility. To a certain extent, many of these parameters are determined by the biorecognition element on the basis of its binding constant to the analyte of interest but also by the specific detection method employed. Substantial changes in analytical performance can therefore be derived from changes in these parameters [557–559]. Typically, reduced accessibility or activity leads to poorer sensitivity and may alter the analytical range. Furthermore, analyte–matrix interactions can lead to an expanded analytical range, but lower sensitivity, although this can be controlled to some extent by the addition of charged polymers [560]. Other approaches include the ability to make the matrices either electrochemically active or optically transparent, the ability to achieve high protein loading, to tune the polarity and porosity of the matrix to selectively modulate enzyme activity, the potential to modulate the material composition to maximize the biocompatibility of the matrix, and the reduced leaching of biomolecules [561,562]. Some of the more recent articles that have aimed to review the development of these devise have focused on the application of hybrid sol–gel films and monoliths for optical and electrochemical sensing of inorganic species [563], MSNs for biosensing [299,427], zeolites and mesoporous silicates for electrochemical detection [564], and sol–gels and templated mesoporous materials for fluorescence-based sensing [565,566]. The versatility of mesoporous silicates has resulted in their application to sensors for a wide range of analytes in liquid and vapor phase environments. However concerns that often need to be addressed in the utility of these materials are ever present and include reversibility and reproducibility, selectivity, response and recovery time, and ease of application. These factors are especially important when considering the use of sol–gel based sensing devices for medical diagnostics or managing treatment.

Several properties of sol–gel-derived materials, most notably, those that are silica-based, render them particularly compatible with biosensor development. Firstly, they are transparent in the UV and visible spectral range, making them amenable to common detection techniques such as absorption, reflection, fluorescence, chemiluminescence, and bioluminescence [564,567–569]. Secondly, as a solid-phase support, the materials are mechanically robust, relatively chemically inert, resistant to thermal degradation, photochemical degradation, and biodegradation [564,569–571]. Thirdly, they can be doped with a wide variety of sensing elements because the silica framework grows around the guest biomolecules, stretching the upper limits on dopant size [569,572]. The interpenetrating networks of silica effectively serve to “cage” biomolecules, preventing them from leaching. While global biomolecule dynamics are restricted, the cage is still sufficiently loose to allow for local rotational and translational motions, including those required for substrate binding [196,573,574]. At the same time, this tight fitting precludes macromolecular exchange while allowing for unrestricted transport of small molecules including buffer ions, substrates, and products of reactions in and out of the porous structure as may be required in sensing applications [196,574].

Recent progress in the development of “protein friendly” sol–gel processing methods has allowed these materials to be utilized as components of numerous biosensors, using delicate biomolecules such as luciferase and kinases, or even membrane-bound receptors as biorecognition elements. Among the different nanoparticle biosensors, enzyme immobilised NPs have been reported in numerous publications [575]. While enzyme-based sensors show great specificity and low concentration detection limits, they suffer from denaturation and inactivation, plus they lack two important
characteristics held by mesoporous silicas: (I) high porosity and (II) optical transparency. The former property allows for the location of sensing molecules not only on the external surface of the material but also inside of the pores, which enables the loading of large amounts of sensing molecules giving fast responses and low detection limits. Silicate glass obtained by the sol–gel process has been used for biosensing purposes [576–578]. Microporous silica NPs have long been used for the creation of biosensors utilizing drugs, enzymes, antibodies [579–581] and DNA [582] as recognition elements. However, pores in sol–gel derived silica lack a high degree of order, which results in nonlinear paths, and consequently slow diffusion of the analytes to the sensing molecules. Some fraction of the sensing molecules might even be unreachable leading to less than optimal response. In contrast to microporous silica, mesoporous silica has much more available surface area due to its larger and more ordered pores. This allows for larger concentrations of accessible receptor molecules, the possibility to detect larger molecules, and a faster diffusion to the sites where the receptors are located, which altogether results in even faster and better response of the sensors.

Proteins have been used largely as active elements for the selectivity of mesoporous silica supported biosensors. For example, mesoporous silica was loaded with glucose oxidase and horseradish peroxidase and used as a sensor for glucose [583,584]. In another study myoglobin and hemoglobin were immobilised in mesoporous silica modified electrodes to be used as a sensor for H₂O₂ and NO₃⁻/CO₃⁻ [585,586]. The main reason for using enzymes and proteins as the recognition unit is their high specificity for substrates. However, these biomolecules are relatively expensive and lack long-term chemical stability. To avoid these disadvantages, while maintaining high analyte selectivity, research as a whole has pursued a new approach. Unlike the molecular imprinting approach, selectivity is attained not by synthesizing size or shape selective recognition receptors, but by controlling the diffusional penetration of analytes into the surface-functionalised mesopores. As described below, new mesoporous silica-based selective sensory systems have been reported by using this strategy.

To develop a biosensor suitable to distinguish between several structurally similar neurotransmitters, such as dopamine, tyrosine and glutamic acid, selective functionalisation of the exterior and interior surfaces can be exploited (Fig. 5.8) [514]. PLLA coated MCM-41-type mesoporous silica nanosphere (PLA-MSN) material can serve as a fluorescence sensor system for detection of amino-containing neurotransmitters in neutral aqueous buffer. Utilizing the PLLA layer as a gatekeeper, several structurally simple neurotransmitters can react with primary amine groups and form the corresponding fluorescent isoindole product. The observed large difference in the rates of diffusion is most likely due to the different electrostatic, hydrogen bonding, and dipolar interactions between these neurotransmitters and the PLLA layer. The isoelectric points of dopamine, tyrosine, and glutamic acid are 9.7, 5.7, and 3.2, respectively, whereas the isoelectric points of PLLA is typically below 2.0 which means the dopamine will be positively charged and the others will be negatively charged under physiological conditions. The PLLA layer of the PLA-MSN sensor shows a unique “sieving” effect that regulates the rates of diffusion of the amino acid-based neurotransmitters into the sensor mesopores of the material.

Another biosensor that was synthesized and studied by Descalzo et al. [587,588] involved aminomethylanthracene groups grafted onto MSNs and used for the recognition and detection of anions. The bulk of the grafted group combined with the steric restrictions provided by the pore size of the material led to the ability of the material to respond in different degrees to adenosine tri-, di-, and monophosphate. Adenosine triphosphate was able to quench the fluorescence of the material to a larger degree than the other two species, and small anions such as chloride, bromide and phosphate did not produce any response of the sensor. These authors compared the sensitivity toward Adenosine triphosphate of the aminomethylanthracene MSN with aminomethylanthracene grafted on fumed silica and observed a 100-fold improved sensitivity when the matrix was mesoporous [587,588].

A growing number of reports have demonstrated the versatility of mesoporous silicates as active components in pH sensing. These applications typically involve immobilising an indicating dye on a mesoporous surface or encapsulating it in silicate walls. In living organisms, intracellular pH plays key roles in enzyme, cell, and tissue activities, and microenvironments in endosomes (pH 5.5–6) and lysosomes (pH 4.5–5) are mildly acidic. Besides, some tumor cells possess lower extracellular pH (pH 6.4–6.9) compared to normal tissues (pH 7.2–7.4). Thus, monitoring pH changes and gradients are quite important to determine the efficiency of pharmaceutical therapies, diagnose certain cancer
diseases, and investigate cellular internalisation pathways. If MSN-based drug nanocarriers are endowed with pH-sensing ability, they might be useful in practical applications for simultaneous chemotherapy and the monitoring of therapeutic efficiency. Moreover, this novel type of multifunctional MSN-based nanocarriers can be quite advantageous for investigating drug nanocarriers–cell interactions and understanding the underlying mechanism. The fabrication of fluorescent pH-sensing organic–inorganic hybrid MSN capable of tunable redox-responsive release of embedded guest molecules [589]. The reversible addition–fragmentation chain transfer copolymerization of N-(acryloxy)succinimide, oligo(ethylene glycol) monomethyl ether methacrylate, and 1,8-naphthalimide-based pH-sensing monomer at the surface of MSN led to fluorescent organic–inorganic hybrid MSN. The obtained hybrid MSN exhibits excellent water dispersibility and acts as sensitive fluorescent pH probes in the range pH 4–8 due to the presence of 1,8-naphthalimide moieties. After loading with rhodamine B as a model drug molecule, copolymer brushes at the surface of hybrid MSN were cross-linked with cystamine to block nanopore entrances for the effective retention of guest molecules. Taking advantage of disulfide-containing cross-linkers, the release rate of rhodamine B can be easily adjusted by adding varying concentrations of dithiothreitol, which can cleave the disulfide linkage to open blocked nanopores. The increase of dithiothreitol concentration from 0 to 20 mM led to 20–30 times enhancement of rhodamine B release.

FITC has also been modified with an amino-bearing siloxane to provide a precursor for direct mesoporous material synthesis [590]. The result is a material with fluorescein isothiocyanate on the pore walls, which is responsive to pH changes in the range from 3.1 to 11.2 that can be interrogated through laser excited photoluminescence. Grafting of silane-modified 5-methoxy-2-(pyridyl) thiazole onto SBA-15 has been applied to sensing pH and cupric ion detection [21]. The material exhibited dual fluorescence emission bands. The first at 420 nm was quenched while the second at 448 nm increased in intensity as pH decreased in the range from 5.7 to 1. Addition of cupric ions at pH 6.0 quenched and blue-shifted fluorescence. An impressive detection limit of $3.2 \times 10^{-6}$ M was obtained. This response was found to be somewhat selective for copper with smaller responses to ferric iron and mercuric mercury. Encapsulation of bromothymol blue provided a material that yielded visual color changes from orange–yellow to royal blue across pH values ranging from 2 to 12.

Fig. 5.8. Schematic representation of the synthesis of PLA-coated MSN-based fluorescence sensor system for detection of amine-containing neurotransmitters, i.e., dopamine, glutamic acid, and tyrosine (R–NH$_2$) (5,6-epoxyhexyltriethoxysilane = EHTES, cetyltrimethylammonium bromide (CTAB) surfactant = ~ ~ ~ ~ ~ ~). Kinetic measurements of the fluorescence detection of dopamine (●), tyrosine (----- - - - - - - -), and glutamic acid (-----) with OPTA-SS (a) and PLA-MSN (b). Fluorescence increase of OPTA-SS (c) and PLA-MSN (d) as a function of dopamine (●), tyrosine (○), and glutamic acid (▲) concentrations. The fluorescence intensities were measured 5 min after the introduction of every concentration of each neurotransmitter. Reprinted with permission from Ref. [514].
Evanescent wave absorbance spectroscopy (600 nm) was also used for interrogation [591]. Various sulfonephthalein indicators have also been encapsulated in hybrid xerogel films synthesized from mixtures of tetraethoxysilane and vinyltriethoxysilane for applications in pH sensing [592]. From a biomedical perspective, effective means of determining local changes in pH can be extremely useful. For example, microbial infections are often accompanied by a reduction in pH due to anaerobic respiration during sepsis. The ease with which trace element or drug concentrations within biological samples can be determined would also stand to provide great benefits to medical diagnostics and, ultimately, the care received by the patient.

6. Conclusions and future perspectives

In conclusion, the sol–gel synthesis method offers a molecular-level mixing and is capable of improving the chemical homogeneity of the resulting composite. This opens new doors not only to previously unattainable material compositions but also unique structures. Sol–gel derived bioceramics have a great potential for application as a coating on metallic substrates to provide a high degree of biocompatibility and promote a rapid healing response with minimal adverse biological events. Without a doubt, one of the main benefits of applying sol–gel approaches to the production of bioactive coatings is the relatively low temperatures under which synthesis can be achieved. However, compatibility is but one facet of sol–gel derived biomedical applications. Low temperature synthesis also allows for the inclusion of materials that would otherwise be destroyed by excessively high temperatures. Since sol–gel processes are carried out at such low temperatures, they can also allow the inclusion of biomolecules, therapeutic agents including drugs, growth factors, and proteins. These can be loaded during the sol–gel preparation process and released in a controlled manner. Further to this, the relatively low synthesis temperatures provide further benefits in that complications that can arise with the application of bioactive coatings can be avoided, such as mismatched thermal expansion coefficients found in conventional coatings, which can lead to the formation of cracks and poor interfacial bonding as a result of shear stresses induced upon cooling. The sol–gel method should be simple, stable, cost-effective, and scalable for facilitating future industrial production and clinical translocation.

As well as tunable chemistry, further advantages of the sol–gel synthesis process are the tunable structures that allow control over the morphology, porosity, size, and the ability to modify the surface for biomedical applications. The surface area and pore diameter are critical factors for drug adsorption and release in DDSs. MSNs have been the subject of significant research in drug-delivery applications with the possibility of embedding various protein molecules into these materials for biosensing purposes. The surface of MSNs can also be modified to promote their ability to deliver drugs or biomolecules to desired sites in a sustained manner that makes them interesting candidates for cancer therapy. Functionalisation can also be exploited in the development of sensing devices to allow not only therapy, but simultaneous diagnosis or so called theranostic purposes. Aside from direct loading of MSNs with biosensing molecules or methods of increasing the capacity thereof, the same steric properties responsible for the localization of elements of the sensing system can also be used to be filter substrates and thus add further specificity. It is the sol–gel process that underpins these new and advanced bioactives.

Concerns associated with non-viral gene delivery systems are also much lower than the viral vector counterpart. Whilst the relative merits of either approach are yet to be evaluated in full, non-viral DDSs are likely to experience less resistance in their journey from initial conception to mechanistic studies, clinical trials, and ultimate acceptance by the general public. This is not to say that alternative means should not be sought rather that non-biological delivery poses less of a potential hazard and thus the development of sol–gel based materials as DDSs may be a more attractive option.

The effect of shape is an area ripe for further research and from this, in vitro experiments will provide the basis for in vivo responses to be better predicted. However, it was also shown that many different structural features of MSNs could lead to different biological activities for these materials. Thus, probing and summarizing the overall biological responses induced by these materials, which possess a range of structural features and compositions, could be challenging. Nevertheless, in light of their
great potential applications in biomedical areas, many experts in the field seem to agree that the biological activities of these materials need to be fully assessed and evaluated in order to determine the proper physical or chemical traits that could make MSNs highly biocompatible.

A broad evaluation at the interface between the material’s properties and its biological surroundings is crucial to fully determine whether a given material is biocompatible. This task, however, is very complicated and challenging in the case of engineered nanomaterials such as MSNs because they possess a variety of physical properties and chemical compositions, which could lead to a diverse range of biological responses and trigger a cascade of different biological events. While a large number of studies are being conducted by various researchers worldwide to fully understand these structure-related biological effects of MSNs, including their possible negative impacts on human health, some of the studies also revealed contradictory results about the biocompatibility (or cytotoxicity) of various MSNs, as we discussed in this review.

The chemical modification, along with the original synthetic condition, can tailor the particle size, shape, or surface properties of the MSNs. This, in turn, could influence the biological properties and activities of the nanomaterials. This aspect of functionalized MSNs was also considered in this review. In particular, examples illustrated that the favorable size, shape, or surface property of organic-functionalized and nonfunctionalized MSNs alike that leads to insignificant interference in a given biological system can generally be attributed to those that cost the least in terms of interfacial energy at the nano-bio boundary. Specifically, in contact with cell or intracellular organelles, MSNs of a particular size and shape with larger surface area can be thermodynamically favored in their cellular interactions, leading to more intracellular accumulation but reduced cell injury. Thus, proper surface modification of MSNs (e.g., making MSNs with positively charged surfaces) can enhance their endocytosis as well as exocytosis, while also mollifying their intracellular trafficking. However, size may play a more significant role compared to shape in causing toxicity in vivo, if any, as the body generally recognizes a certain dimension of foreign particles, allowing or disallowing their further penetration into different organs or sites in the body to enable it to take part in some biological processes. At the same time, different surface properties regulate particle circulation in the bloodstream, whereas different affinities to certain biological receptors due to functionalization can dictate the final fate or biocompatibility of the particles.

A new class of nanostructured materials, which has accelerated or improved bioactive behavior compared with conventional bioactive glasses are being developed. This improved bioactive behavior has been attributed to the highly ordered arrangement of uniform-sized mesopores. Moreover, the possibility of tailoring the structural and textural characteristics of bioactive glasses offers numerous advantages to modulate their bioactive responses. The structural and textural properties of mesoporous materials also permit the development of bioceramics with controlled delivery capability able to load and locally release drugs to treat different bone pathologies such as infection, osteoporosis and cancer. The appropriate functionalization of the inner surface of the mesopore channels allows for modulating drug loading and plays a key role in offering sustained drug release.

Improved bioactive behavior and controlled drug delivery capability, makes them excellent candidates to be used as starting materials for the manufacture of three-dimensional macroporous scaffolds for bone tissue engineering. Within this application, special attention should be given to the possibility of covalently grafting osteoinductive agents, such as peptides, proteins and growth factors) to the surface of the three-dimensional scaffolds, which would act as attractive signals for bone cells and promote the bone regeneration process.

For cancer therapy, MSNs show obvious advantages over other nanoparticulate DDSs because of their extraordinarily high drug loading, potential for controlled drug release behavior, and ability to delivery more than one component. However, great challenges still exist. To overcome the in vivo physiological barriers and achieve efficient delivery of nanoparticles to cancer cells, MSNs as nanocarriers should integrate the requirements of long circulation time, low reticuloendothelial system sequestration, ability to extravasate into tumor tissue, and cancer cell specific internalization. The properties of nanoparticulate DDSs and the physiological microenvironment in bodies are co-determinants for the final therapeutic benefits. Even if with efficient delivery to tumor sites, the physiological nature of cancer brings tremendous difficulties in efficient therapy. With the diversity and multifunctionality of MSN-based nanocomposites, it may be a promising way to develop
cooperative therapies such as utility of the synergistic effect of photothermal/magnetohyperthermal therapy and chemotheraphy, or simultaneous utility of several nano-based formulations targeting different populations of cancer cells with different phenotypes. Certainly, a greater understanding about cancer biology and physiology is essential for more specific targeting options.

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References


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porous thin films by polyethylene glycol templating and


Morales Bokhimi A, Novaro O, Lopez T, Sanchez E, Gomez R. Effect of hydrolysis catalyst on the Ti deficiency and


Singh RK, Kim TH, Patel KD, Knowles JC, Kim HW. Biocompatible magnetite nanoparticles with varying silica-coating


Matijević E, Budnik M, Meites L. Preparation and mechanism of formation of titanium dioxide hydrogels of narrow size


Eiden-Assmann S, Widonijak J, Maret G. Synthesis and characterization of porous and nonporous monodisperse colloidal


Morales Bokhimi A, Novaro O, Lopez T, Sanchez E, Gomez R. Effect of hydrolysis catalyst on the Ti deficiency and


Scolan E, Sanchez C. Synthesis and characterization of surface-protected nanocrystalline titania particles. Chem Mater


Khanna PK, Singh N, Charan S. Synthesis of nano-particles of anatase-TiO2 and preparation of its optically transparent


Sugimoto T, Sakata K. Preparation of monodisperse pseudocubic α-Fe2O3 particles from condensed ferric hydroxide gel. J

Colloid Interface Sci 1992;152:587–90.


Lin Y, Wu GS, Yuan XY, Xie T, Zhang LD. Fabrication and optical properties of TiO2 nanowire arrays made by sol–gel


Bu SJ, Jin ZG, Liu XX, Yang LR, Cheng ZJ. Synthesis of TiO2 porous thin films by polyethylene glycol templating and


into the 2000's 1999. p. 73

Yan X, Yan ZS, Shi YF, Ran GX. Synthesis of zinc oxide nanotubes within ultrathin anodic aluminum oxide membrane


Fu YS, Du XW, Kulinich SA, Qiu JS, Qian WJ, Li R, et al. Stable aqueous dispersion of ZnO quantum dots with strong blue


Xiong HM, Wang ZD, Liu DP, Chen JS, Wang YG, Xia YY. Bonding polyether onto ZnO nanoparticles: an effective method


Process 1995;10:205–16


G.J. Owens et al. / Progress in Materials Science 77 (2016) 1–79
Liang X, Yue X, Dai Z, Kikuchi J. Photoresponsive liposomal nanohybrid cerasomes. Chem Commun (Camb) 2011;47:

Yamamoto S, Miyamoto T, Kokubo T, Nakamura T. Preparation of polymer-silicate hybrid materials bearing silanol groups

Kamitakahara M, Kawashita M, Miyata N, Kokubo T, Nakamura T. Bioactivity and mechanical properties of

Pereira APV, Vasconcelos WL, Orefice RL. Novel multicomponent silicate-poly(vinyl alcohol) hybrids with controlled


hybrid materials synthesized by sol–gel route: in vitro


Song JH, Yoon BH, Kim HE, Kim HW. Bioactive and degradable hybridized nanofibers of gelatin–siloxane for bone

Catauro M, Bollino F, Papale F, Gallicchio M, Pacifico S. Influence of the polymer amount on bioactivity and


Li X, Xie QR, Zhang J, Xia W, Gu H. The packaging of siRNA within the mesoporous structure of silica nanoparticles.


Li X, Xie QR, Zhang J, Xia W, Gu H. The packaging of siRNA within the mesoporous structure of silica nanoparticles.

Catauro M, Bollino F, Papale F, Gallicchio M, Pacifico S. Influence of the polymer amount on bioactivity and


Catauro M, Bollino F, Marciano S, Pacifico S. TiO2/PEG hybrid materials synthesized via sol–gel technique for


Li X, Zhang J, Gu H. Adsorption and desorption behaviors of DNA with magnetic mesoporous silica nanoparticles.


Woignier T, Duffours L, Colombel P, Durin C. Aerogels materials as space debris collectors. Adv Mater Sci Eng 2013;2013:


Klein LC. Sol–gel optics: processing and applications. US: Springer; 1994


Pelleg J. Mechanical properties of ceramics. Springer; 2014.


Pelleg J. Mechanical properties of ceramics. Springer; 2014.


Gorustovich AA, Roether JA, Boccaccini AR. Effect of bioactive glasses on angiogenesis: a review of in vitro and in vivo
Hulbert SJ, Young FA, Mathews RS, Klawitter JJ, Talbert CD, Stelling FH. Potential of ceramic materials as permanently
Rivera-Chacon DM, Alvarado-Velez M, Acevedo-Morantes CY, Singh SP, Gultepe E, Nagesha D, et al. Fibronectin and
Akkouch A, Zhang Z, Rouabba M. Engineering bone tissue using human dental pulp stem cells and an osteogenic
Patiola A, Arinezh TL. Evaluating apatite formation and osteogenic activity of electrospin composites for bone tissue
Torrey F, Trewyn BG, Lin VSY, Wang K. Mesoporous silica nanoparticles deliver DNA and chemicals into plants. Nat
Radu DR, Lai CY, Jeftinija K, Rowe EW, Jeftinija S, Lin VS. A polyamidoamine dendrimer-capped mesoporous silica
Horn C, Lu J, Liang M, Luo H, Li Z, Zink JI, et al. Mesoporous silica nanoparticles facilitate delivery of siRNA to shutdown
Park HY, Kim Y, Yoo MK, Choi YJ, Cho MH, Cho CS. Mannosylated polyethyleneimine coupled mesoporous silica


Moon JH, McDaniel W, Hancock LF. Facile fabrication of poly(p-phenylene ethynylene)/colloidal silica composite for...

Zong S, Cao Y, Zhou Y, Ju H. Zirconia nanoparticles enhanced grafted collagen tri-helix scaffold for unmediated biosensing...


Corrie S, Lawrie G, Battersby B, Trau M. Organosilica particles for DNA screening applications 2006 [Ieee].


