REVIEW

Structure and functionalization of mesoporous bioceramics for bone tissue regeneration and local drug delivery

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This review article describes the importance of structure and functionalization in the performance of mesoporous silica bioceramics for bone tissue regeneration and local drug delivery purposes. Herein, we summarize the pivotal features of mesoporous bioactive glasses, also known as ‘templated glasses’ (TGs), which present chemical compositions similar to those of conventional bioactive sol–gel glasses and the added value of an ordered mesopore arrangement. An in-depth study concerning the possibility of tailoring the structural and textural characteristics of TGs at the nanometric scale and their influence on bioactive behaviour is discussed. The highly ordered mesoporous arrangement of cavities allows these materials to confine drugs to be subsequently released, acting as drug delivery devices. The functionalization of mesoporous silica walls has been revealed as the cornerstone in the performance of these materials as controlled release systems. The synergy between the improved bioactive behaviour and local sustained drug release capability of mesostructured materials makes them suitable to manufacture three-dimensional macroporous scaffolds for bone tissue engineering. Finally, this review tackles the possibility of covalently grafting different osteoinductive agents to the scaffold surface that act as attracting signals for bone cells to promote the bone regeneration process.

Keywords: mesoporous bioceramics; structure; functionalization; bone tissue regeneration; local drug delivery

1. Introduction

The progress regarding bioceramics for bone tissue repair and regeneration has experienced a great advance as a result of the scientific efforts aimed at improving the tissue–material response after implantation [1–6]. In the 1950s, the goal was to employ inert materials, whose main target was substitution with the

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lowest tissue response, maybe because the only expected tissue response was inflammation and material rejection. In this setting, the biology did not receive enough attention, and the only requirement was that the biomaterial did not react with the body.

However, the discovery of Bioglass by Hench in 1969 led to a shift in the perspectives regarding the reactivity at the tissue–implant interface which does not necessarily involve a risk to human health if the products of these reactions are beneficial [7]. In this sense, the scientific efforts made so far have resulted in a deep knowledge of biomaterials with the aim of controlling and improving their reactivity with the tissues and accelerating the healing process. Thus, the design, development and commercialization of bioactive and biodegradable materials began, and now they are commonly used in the repair of living tissues [7,8].

The evolution in the concept from substitution to repair has recently been replaced by the idea of regeneration [1,9–12]. This idea can be illustrated by focusing on third-generation bioceramics, where the aim is to provide appropriate scaffolding for cells, allowing them to perform their bone regeneration work. Bone tissue engineering is a powerful tool that is supported on three main ‘pillars’: cells, signals (biochemical factors) and scaffolds [13–15]. These three elements play key roles in the processes that promote bone formation, i.e. osteogenesis, osteoinduction and osteoconduction, whose concepts are displayed in figure 1. Nowadays, the challenge for the scientific community is the design of biocompatible and bioactive ceramics for the manufacture of osteoconductive three-dimensional macroporous scaffolds with appropriate macroporosity and mechanical properties that promote osteoinduction and osteogenesis of bone tissue.

The possibility of developing bioceramics with ordered mesoporous structures has represented a significant advance for the scientific biomaterials community [16]. In this sense, mesoporous bioactive glasses, also denoted as ‘templated glasses’ (TGs), which present similar compositions to conventional sol–gel glasses (SiO2–CaO–P2O5) and highly ordered mesoporous arrangements, exhibit accelerated bioactive responses [17–23]. The highly ordered mesoporous arrangement of cavities present in these nanostructured materials permits the confinement of different drug molecules to be subsequently released, acting as controlled delivery systems [24–34]. The chemistry of the silica surface is mainly governed by the presence of abundant silanol groups [25]. Thus, it is possible to functionalize the silanol-containing mesoporous walls with different organic groups [35,36] to achieve higher control over drug loading and release processes [26,27,33].

This double scope, bioactive response and controlled drug delivery capability, makes mesoporous materials good candidates to manufacture three-dimensional scaffolds for bone tissue engineering [11,33,37,38]. In addition, it is also feasible to attach osteoinductive agents, such as certain peptides, proteins and growth factors, to the external surface of scaffolds, which would act as signals to enhance or stimulate new bone formation [11,39].

This review shows in a relatively simple fashion that by tailoring the different parameters that govern the bioactive behaviour of mesoporous materials and their controlled drug delivery capability, it is possible to design three-dimensional macroporous scaffolds with superior healing and regeneration capability suitable for bone tissue engineering applications.
Figure 1. The three main pillars that support bone tissue engineering: cells, scaffolds and biochemical factors. (Online version in colour.)

2. Bioactive templated glasses: a new generation of nanostructured bioceramics

One of the current challenges for the biomaterials scientific community within the bioceramics field is the design of new synthetic approaches to combine the intrinsic properties of silica-based ordered mesoporous materials (SMMs), concerning their structural and textural features, with the bioactive response of conventional sol–gel glasses [17,19,40].

Initially, SMMs were designed for catalytic purposes owing to their exceptional structural and textural properties, offering a great number of advantages in host–guest systems [41–46]. It was in 2001 when SMMs were proposed for the first time as drug delivery systems [24]. Since then, SMMs have been widely reported as excellent matrices to load and locally release in a sustained fashion several kinds of drug molecules [25–28,30,32,33,47–49]. Furthermore, SMMs present in vitro bioactive behaviour [16,50], since they are able to develop an apatite-like layer onto their surfaces when soaked into a simulated body fluid (SBF) [51]. However, high surface areas and porosity alone are not sufficient to make these materials highly bioactive, showing apatite formation layer kinetics too slow compared with conventional sol–gel glass (60 days instead to 3 days for conventional glasses).

So, the real scientific advance has been the synthesis of the so-called TGs with similar composition to conventional sol–gel glasses and the structural and textural properties of the SMMs (figure 2). The synthesis of TGs is carried out through an
evaporation-induced self-assembly (EISA) method and using a non-ionic triblock copolymer (EO\textsubscript{20}PO\textsubscript{70}EO\textsubscript{20}) as structure-directing agent. These materials exhibit accelerated bioactive responses, as indicated by a much faster and more intense apatite-like phase formation upon reaction with SBF, compared with conventional sol–gel glasses with similar composition. The highly bioactive behaviour of TGs is due to their chemical composition, which is similar to the sol–gel bioactive glasses, together with their high surface areas and porosities as well as ordered pore structure [17,19,52].

(a) Tailoring the structural and textural properties of templated glasses

The possibility of obtaining different pore arrangements with different geometries and well-defined textural properties at the nanometric level in TGs offers a variety of reactive/bioactive responses depending on the required clinical needs. As in the case of SMMs, the formation of an ordered mesoporous arrangement is driven by different parameters such as surfactant nature, surfactant concentration, solvent, use of additives, pH, temperature, etc. [53]. In the particular case of TGs, the presence of CaO and P\textsubscript{2}O\textsubscript{5} in the silica network as well as solvent evaporation temperature also play important roles in the textural and structural properties of the final matrix.
Figure 3. Influence of (a) CaO and (b) P$_2$O$_5$ contents on the structural features of TGs. TEM images and their corresponding three-dimensional reconstruction of TG structures. $^{31}$P solid-state NMR spectrum corresponding to TG–SiO$_2$–CaO–P$_2$O$_5$ showing the formation of ACP clusters. Schematic depiction as an inset of ACP distribution into the pore wall of the corresponding TG.

(i) Influence of CaO and P$_2$O$_5$ content

The CaO content in TGs is a very important factor, since it not only acts as a modifier of the silica network [54,55] but also influences the mesopore structure formed after the surfactant removal [19]. Therefore, in previous work, it was demonstrated that decreasing the CaO amount in TG–SiO$_2$–CaO–P$_2$O$_5$, the porous structure led to an evolution from three-dimensional cubic structures to two-dimensional hexagonal structures [19,52] (figure 3a). These structural modifications can be explained by the influence of Ca$^{2+}$ ions on the silica condensation, since Ca$^{2+}$ ions act as network modifiers decreasing the silica network connectivity. As a result, the inorganic/organic volume ratio of the micelle is increased, thus decreasing the curvature radius of the surfactant micelles, which favours the formation of hexagonal phases rather than cubic ones.

In addition, it has been experimentally demonstrated that the P$_2$O$_5$ content also exerts an important influence on the mesoporous structure. To determine such influence, two TGs with compositions 85SiO$_2$–10CaO–5P$_2$O$_5$ and 90SiO$_2$–10CaO (mol%), i.e. in presence and absence of phosphorus, respectively, were prepared under the same experimental conditions. Moreover, the molar ratio between the network formers (SiO$_2$ + P$_2$O$_5$) and surfactant was always kept constant [56]. Transmission electron microscopy (TEM) studies evidenced that in the presence of phosphorus, the mesoporous arrangement consisted of a three-dimensional bicontinuous cubic structure while in the absence of phosphorus,
the structure evolved to two-dimensional hexagonal (figure 3b). To explain this structural change, an in-depth study by solid-state nuclear magnetic resonance (NMR) was performed. $^1\text{H} \rightarrow ^{31}\text{P}$ CP/MAS and SP/MAS solid-state NMR measurements evidenced that the presence of CaO and P$_2$O$_5$ in TG–85SiO$_2$–10CaO–5P$_2$O$_5$ leads to the formation of amorphous calcium phosphate (ACP) clusters that are accumulated onto the material surface [57]. Therefore, calcium cations do not contribute to decrease the silica connectivity, and consequently, the inorganic/organic volume ratio of the micelle is decreased forming cubic structures. On the contrary, in the absence of phosphorus, such as in TG–90SiO$_2$–10CaO, the total calcium content provoked a disruption in the silica network, as confirmed by $^1\text{H} \rightarrow ^{29}\text{Si}$ CP/MAS NMR spectra. This can be ascribed to the higher inorganic/organic volume ratio of the micelle, which would increase the curvature radius of the surfactant micelles and contribute to the formation of hexagonal phases rather than cubic ones.

Regarding the textural properties, TGs possess higher surface areas and pore volumes than conventional sol–gel glasses owing to the presence of the surfactant during the synthesis of the former, which drive the final porosity characteristics. Although CaO and P$_2$O$_5$ contents do not directly control the textural properties of TGs, they are key parameters that modulate the structure of TGs, which is in turn associated with determined textural features.

(ii) Influence of solvent evaporation temperature

The evaporation temperature used during the EISA process also affects the final mesostructure of TGs [56]. It has been observed that the structure of a TG evolves from three-dimensional bicontinuous cubic structure to two-dimensional hexagonal structure when decreasing the solvent evaporation temperature. The dependence of a mesoporous structure on the evaporation temperature can be explained in terms of a reduction of hydrogen interactions. In the case of non-ionic triblock copolymers such as Pluronic P123, the micelle size is strongly dependent on the hydrogen-bond interactions with the solvent, which becomes greater when hydrogen interactions are reduced. Consequently, the hydrophilic/hydrophobic ratio is reduced, favouring hydrophobic mesostructures such as cubic $I_a$-3d, as previously indicated by Zhao and co-workers [58] for pure silica mesoporous materials obtained via the hydrothermal method.

(b) In vitro bioactivity of templated glasses

The textural and structural characteristics of TGs provide them with accelerated in vitro behaviour compared with conventional sol–gel glasses with similar compositions. In fact, TGs exhibit the fastest bioactive response ever reported for bioactive materials [6]. Despite it being well established that both textural and chemical composition exert influence over bioactive processes of TGs, investigations regarding the different parameters that govern such processes are still ongoing.

Contrary to what occurs in the conventional sol–gel glasses, where a higher amount of CaO leads to lower network connectivity and improves the glass reactivity and bioactivity, TGs apparently do not exhibit the same trend. In TGs, an increase in the CaO content provokes a decrease in the apatite crystallization...
rate from newly formed ACP layer, which is evidenced by scanning electron microscopy and Fourier transform infrared spectroscopy techniques [19]. Figure 4 shows the bioactive study by TEM in two different settings; one represented by a TG with high calcium content and two-dimensional hexagonal structure, TG–58SiO₂–37CaO–5P₂O₅, and another represented by a TG with lower calcium content exhibiting three-dimensional bicontinuous cubic structure, TG–85SiO₂–10CaO–5P₂O₅. In the case of TG–58SiO₂–37CaO–5P₂O₅, TEM images reveal that after 1h soaked in SBF, such surface generates a large amount of newly formed ACP, which is transformed after 4h into nanocrystalline oval nuclei of octacalcium phosphate (OCP). Finally, the transformation from these oval OCP nuclei to needle-shaped apatite nanocrystals takes place after 8h in SBF. These results evidence that this composition of TG exhibits a sequential transition from ACP to OCP and to calcium-deficient carbonate hydroxyapatite (CHDA) similar to the natural bone biomineralization process. Usually, all bioactive materials obtained to date form a CHDA phase through the direct crystallization of previously precipitated ACP without previous formation of metastable OCP phase. These results could be explained as owing to the synergy of both high calcium content (58 mol% of CaO) and high surface area (195 m² g⁻¹) which provokes a larger ion exchange between Ca²⁺ and H₃O⁺. This would lead to an instantaneous drop in the local pH value to 6.5, which would permit the formation of metastable OCP phase [52].

On the contrary, in the case of TG–85SiO₂–10CaO–5P₂O₅, with lower CaO content and three-dimensional bicontinuous cubic structure, TEM images evidence that only after 1h in SBF, a nanocrystalline apatite layer is formed onto the material surface. This has been the fastest apatite kinetic formation observed...
so far. The three-dimensional pore system provides not only high surface area and pore volume, but also allows easier ionic exchange with the surrounding medium by increasing the mass transport and diffusion processes. Thus, cubic TG–85SiO$_2$–10CaO–5P$_2$O$_5$ shows the fastest crystalline apatite formation rate because of its excellent intrinsic textural and structural features.

Finally, the role of P$_2$O$_5$ in the bioactive behaviour of TGs has been also studied by comparison with a P$_2$O$_5$-free composition. A preliminary bioactive study corresponding to TG–90SiO$_2$–10CaO and TG–85SiO$_2$–10CaO–5P$_2$O$_5$ showed that the P$_2$O$_5$-free composition exhibited slower \textit{in vitro} bioactive response than P$_2$O$_5$-containing TG, although the network former/modifier molar ratio and the textural properties are identical. The difference could be reflected in the local structure in both materials. TG–90SiO$_2$–10CaO contains Ca$^{2+}$ cations distributed within the silica network (see §2a(i)), whereas TG–85SiO$_2$–10CaO–5P$_2$O$_5$ contains Ca$^{2+}$ as ACP clusters at the wall surface [56,57] that could act as nucleation sites for the newly formed apatite.

3. Mesoporous materials for local drug delivery

As above mentioned, SMMs have been widely proposed as local delivery systems of pharmaceutical molecules owing to their improved drug adsorption features and predictable release kinetics [25–28,30,32,33]. It has been demonstrated that both small drugs, such as alendronate (250Da), and large molecules, such as peptides and proteins (ca 70kDa), can be loaded within the mesopores by adsorption processes, using an impregnation method, to be subsequently released via diffusion-controlled mechanisms. Recently, the direct detection of drug molecules in the inner part of mesopore channels has been reported for the first time by using spherical aberration correctors incorporated in a scanning transmission electron microscope [59].

SMMs are excellent matrices to host various guest molecules owing to their structural (ordered pore structure), textural (narrow pore diameter distributions and high surface areas and pore volumes) and chemical (presence of abundant silanol groups that permit covalently anchoring organic functions) properties, which play key roles in the loading and release of molecules (figure 5) [27]. The pore diameter acts as size selective modulator for the confinement of guest molecules within the mesoporous channels. Moreover, this parameter is also a limiting factor for the diffusion of the molecules to the surrounding medium, thus regulating the release rate. The adsorption of molecules into mesoporous matrices depends on the adsorptive properties of the silica surface and consequently the number of molecules loaded will depend on the surface area of the matrix, i.e. the higher the surface area, the higher the contact surface and consequently the higher the amount of molecule loaded. An increase in the filling of the mesopores can be ascribed to an increase in the drug–drug intermolecular interactions within the pore voids. In this case, the pore volume may increase the drug loading.

Regarding the chemical properties, as previously commented, the chemistry of the silica surface is mainly governed by the presence of abundant silanol groups [25]. The interaction of these carriers with the guest molecules would be via weak interactions such as van der Waals forces or hydrogen bonds. However, it is possible to functionalize the silanol-containing mesoporous walls.
with different organic groups via sol–gel chemistry, giving rise to a full family of organic–inorganic mesoporous materials [35]. There are two main routes used to functionalize mesoporous materials, post-synthesis (silylation or grafting) and one-pot synthesis (co-condensation). In the former, the functionalization process is performed by grafting the organic functions, under anhydrous conditions, to the previously formed pure inorganic mesoporous silica matrix. The grafting process is usually performed by reaction of organosilanes of the type $(R'O)_3SiR$, or less frequently chlorosilanes $ClSiR_3$ or silazanes $HN(SiR_3)_3$, with the free silanol groups of the pore surfaces. The co-condensation method involves the simultaneous condensation of the corresponding silica and organosilica precursors, $(R'O)_3SiR$, in the presence of structure-directing agents during the mesoporous synthesis, and all the functionalization process is carried out in one step.

The main difference between both functionalization methods concerns the maximum achievable degree of organic modification. When using the co-condensation method, the organic groups are grafted to the outer and the inner part of the silica walls. Consequently, the degree of mesoscopic order of the products decreases with increasing the concentration of the organosilica precursor in the reaction mixture, which ultimately leads to totally disordered products. Consequently, the content of organic functionalities in the modified silica phases does not normally exceed 40 mol% to avoid disordering of the mesoporous matrix.

**Figure 5.** Parameters that govern the loading and release rate of drug molecules in silica-based ordered mesoporous materials. Functionalization of mesoporous materials with functional alkoxysilanes ($R$ is the organic group). (Online version in colour.)
On the other hand, by using the post-synthesis method, the organic functions are located in the outer surface of the already formed mesopore silica walls, enabling a higher functionalization degree. However, post-synthesis method is accompanied by a reduction in the textural properties (pore diameter, surface area and pore volume) of the hybrid material, albeit depending on the size of the organic residue and the degree of occupation.

Organic modification of the silica walls leads to functionalized mesoporous silica materials containing functional groups able to undergo attracting interactions with the guest molecules, allowing a fine tuning of the drug loading and release kinetics \[27\]. In the resulting organic–inorganic hybrid mesoporous matrices, these interactions are usually established through electrostatic attractive interactions, hydrophilic–hydrophobic forces or electronic interactions \[25,60,61\].

Functionalization has been revealed as the cornerstone in the development of SMMs as controlled delivery systems with improved loading and release features \[27\]. The organic modification of the mesoporous silica surface will depend on the chemical nature of the targeted drug. In this review, we will tackle two of the main distinctive groups, functionalization with hydrophobic groups and functionalization with amine moieties.

(a) Functionalization of mesoporous materials with hydrophobic groups

The functionalization of the mesoporous silica surface with hydrophobic groups is the best choice when the aim is to confine hydrophobic drugs into the mesopore channels. For instance, SBA-15 materials functionalized using hydrophobic groups have been proposed as controlled delivery systems of L-tryptophan (L-Trp), a hydrophobic model amino acid. L-Trp exhibits a hydrophobic aromatic indole ring that makes it necessary to increase the hydrophobicity of SBA-15, which contains high density of silanol groups. Loading assays were performed at pH 10, where L-Trp exhibits an overall negative charge owing to the deprotonation of the carboxylic group present in the amino acid \[62\]. Actually, unmodified SBA-15 loaded less than 5 mg g\(^{-1}\) of L-Trp, probably owing to the extremely different chemical nature of the hydrophobic amino acid and hydrophilic SBA-15. The post-synthesis functionalization of SBA-15 using quaternary amines with different alkyl lengths, methyl and octadecyl, led to L-Trp loads of 43 and 82 mg g\(^{-1}\), respectively. The highest amino acid load obtained for SBA-15 functionalized with the longest hydrocarbon chains can be explained by the interactions taking place between the hydrophobic chains of the host and the indole group of the L-Trp guest. Using long hydrocarbon chains resulted in about two-thirds of the silica surface being functionalized, which increased the surface hydrophobicity, allowing an increased L-Trp loading. On the other hand, using short alkyl chains, such as methyl groups, allows coulombic control of the confinement owing to the interaction between the negatively charged L-Trp with the more accessible positively charged quaternary amines. The L-Trp release patterns from both functionalized matrices displayed an initial burst effect, and the rest of the amino acid was released in a sustained manner, following first-order and zero-order or linear kinetics from SBA-15 modified with quaternary amines with methyl or octadecyl alkyl length, respectively.

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Table 1. Amount of ipriflavone (IPF) loaded in each sample and percentage of IPF released after 10 days of in vitro delivery assay.

<table>
<thead>
<tr>
<th>material</th>
<th>IPF loaded (mg g⁻¹)</th>
<th>IPF released (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>12.9</td>
<td>n.a.</td>
</tr>
<tr>
<td>TG−Pr−SH</td>
<td>40.5</td>
<td>13</td>
</tr>
<tr>
<td>TG−Pr−OH</td>
<td>60.8</td>
<td>6.0</td>
</tr>
<tr>
<td>TG−Pr−NH₂</td>
<td>61.4</td>
<td>7.3</td>
</tr>
<tr>
<td>TG−Ph</td>
<td>117.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Recently, López-Noriega et al. [63] have reported the organic functionalization of TGs with hydrophobic groups to incorporate ipriflavone (IPF), a highly hydrophobic drug with oestrogenic properties. This research work shows that by tailoring the hydrophobicity of the surface, it is possible to fix a drug to the surface of a TG to achieve long-term drug delivery. The different functionalizations used in this work were mercaptopropyl (−Pr−SH), hydroxypropyl (−Pr−OH), aminopropyl (−Pr−NH₂) and phenyl (−Ph). Significant differences among functionalized materials were observed in the amount of drug adsorbed, which are summarized in table 1. TG−Ph sample loads ca 120 mg g⁻¹ drug, TG−Pr−OH and TG−Pr−NH₂ load ca 60 mg g⁻¹ and TG−Pr−SH loads ca 40 mg g⁻¹. In all cases, the amount of drug loaded into non-functionalized TG (ca 13 mg g⁻¹) was insignificant compared with the functionalized samples. Two main factors determine the degree of drug loading: the hydrophobicity of the surface and the presence of a chemical group able to form a stable interaction with the drug molecule. However, hydrophobicity is not sufficient to achieve an appropriate bond to IPF, since samples functionalized with butyl and chloropropyl groups did not exhibit significant drug loads, being about the same order as non-functionalized TG (ca 11 mg g⁻¹). Samples functionalized with −Pr−OH, −Pr−NH₂ and −Pr−SH bond to IPF by means of hydrogen bonding interactions, while Ph-functionalized samples bond to IPF through π–π stacking interactions. The drug release profiles are in agreement with IPF loading capability (table 1), evidencing the high chemical affinity of the drug for the new functionalized surface. The TG−Ph sample releases less amount of drug than samples exhibiting hydrogen bonds with the drug (table 1) since, as also observed from drug loading, π–π stacking is more efficient at linking this molecule to the material surface. This work clearly evidences that the appropriate chemical functionalization of the surface is essential when using TGs as hydrophobic drug carriers. It is also demonstrated that by choosing a specific functionalization agent, it is also possible to modulate the amount of IPF incorporated into the material.

The functionalization of the mesoporous silica surface with hydrophobic species is an interesting strategy used not only to increase the host–guest matrix–drug interactions, but also to decrease the wettability of surfaces. Thus, the drug transport out of the matrix is impeded as the aqueous medium does not easily penetrate inside the mesopores. This approach has been applied by several authors. For instance, the controlled release of the macrolide antibiotic erythromycin from SBA-15 functionalized with hydrocarbon chains...
has been reported [64]. The organic modification was performed with C8 and C18 hydrocarbon chains by post-synthesis functionalization of SBA-15 with octyltrimethoxysilane and octadecyltrimethoxysilane, respectively. It is worth mentioning that functionalization resulted in a decrease of the textural properties of SBA-15, such as the surface area, which led to a decrease in the amount of drug loaded compared with pure silica SBA-15. Functionalization also resulted in a decrease of the effective pore diameter and a decrease of the wettability of the SBA-15 surface by aqueous solution. This resulted in a clear decrease in the drug release kinetics from functionalized samples compared with pure silica SBA-15. For instance, erythromycin showed release rates from samples functionalized with C18 that were one order of magnitude lower than that from pure silica SBA-15.

(b) Functionalization of mesoporous materials with amine moieties

Functionalization of SMMs with amino groups (−NH2) is another approach used to promote the host–guest interactions with drugs containing functional groups such as phosphonate or carboxylate, which can be electrostatically attracted to ammonium groups (−NH3+). Amino groups can be protonated during the load process by reacting with the acid group of the drug in a non-polar aprotic solvent, or by loading the drug at pH below the pKa value of primary amines (9–11). Subsequently, the release process takes place at physiological pH of 7.4, at which the amino groups remain protonated.

This strategy usually leads to higher amounts of drug loaded and to a decrease in the initial amount of drug released, i.e. the burst effect is minimized. Moreover, this approach also permits one to achieve higher control over drug release kinetics. The improvements are mainly owing to the strong attractive ionic interaction taking place between phosphonate or carboxylate groups of the drug and protonated amino groups of the functionalized materials. Such attracting interactions are stronger than the weak hydrogen-bonding interaction between the silanol groups covering the pure silica surface and the functional groups present in the drug.

The success of this approach was evidenced when MCM-41 and SBA-15 were functionalized with aminopropyl groups by using the post-synthesis method and these matrices were tested as alendronate local delivery systems [26]. Alendronate is a potent bisphosphonate that inhibits bone resorption by osteoclasts, and which is widely employed in osteoporosis treatments by oral administration of the drug. Both mesoporous matrices have two-dimensional hexagonal structures but differ in the pore diameter (3.8 nm for MCM-41 and 9 nm for SBA-15) and in the surface area (1157 m2 g−1 for MCM-41 and 719 m2 g−1 for SBA-15). The amount of alendronate loaded in MCM-41 was higher than in SBA-15 owing to the higher surface area of the former. During the drug release assays, it was observed that in both cases, there was an initial burst effect, followed by first-order kinetics for MCM-41 and zero-order kinetics for SBA-15. The differences in release behaviour can be explained by the smaller surface areas and larger pore diameters of the latter.

Aminopropyl functionalized materials exhibited a general trend, but the stronger attractive interaction resulted in improved load and release behaviours. Thus, the amount of drug loaded on amino-modified matrices was almost
threefold greater than that on unmodified matrices. For instance, the amount of alendronate loaded was 23 mg g\(^{-1}\) for SBA-15 and 83 mg g\(^{-1}\) for amino-functionalized SBA-15.

There was also a noticeable drop in the initial burst effect. Thus, for instance, \(ca\) 55 per cent of the alendronate loaded into the unmodified SBA-15 was released to the medium during the first 24 h of assay, falling to \(ca\) 11 per cent in the amino-modified SBA-15. Moreover, the release rates of functionalized materials were smaller than those of non-modified matrices.

Once demonstrated that amine functionalization modulates adsorption and release behaviours of certain drugs from SMMs, the next step would be to achieve different drug dosages attending to a concrete clinical need. With this goal in mind, a first approach could be using different functionalization degrees of the silica mesoporous matrix. The post-synthesis functionalization method allows achieving a gradual coverage of the surface by adjusting parameters such as the amount of modifying agent used. The first research work tackling this matter consisted of gradually increasing the nominal degree of surface functionalization from 25 up to 100 per cent, taking into account the total amount of silanol groups present in SBA-15 [65]. The obtained experimental range of amine functionalization degree permitted a gradual loading of alendronate, i.e. the higher the content of grafted amino groups, the greater the amount of alendronate loaded. The drug delivery rate was independent of the functionalization degree, although the maximum delivered amount of drug could be correlated with the number of aminopropyl groups grafted onto the SMM surface. Nonetheless, following this strategy, the experimental functionalization degree reached a maximum value of \(ca\) 50 per cent, involving a limited number of grafted aminopropyl groups.

A ground-breaking strategy to achieve a higher quantity of functional groups on SBA-15 matrices without the restrictions of one functional group per functionalization agent molecule consists of covalently anchoring dendrimers, i.e. highly branched monodispersed macromolecules that possess a large number of functionalities. Dendrimers have many biomedical applications, for instance, as contrast agents in magnetic resonance imaging, in neutron capture and gene transfection therapies and in drug delivery [66–69].

The use of surface-tethered dendrimers to tailor the density of functional groups of mesoporous silica has been demonstrated as a good strategy to adjust the drug dosage [70]. Thus, the first (G1), second (G2) and third (G3) generations of poly(propyleneimine) dendrimers, which, respectively, possess 4, 8 and 16 primary amine functional groups on their periphery, were attached to the surface of the channels of SBA-15 through covalent linkages. To attain this goal, dendrimers were provided in a first step of synthesis with a reactive trialkoxysilane group, \(\text{−Si(OR)}_3\), that allows the amine-functionalized dendrimers to covalently bond to the silica surface of SBA-15 via post-synthesis grafting. Reactive triethoxysilane moieties were introduced into the dendrimer structure through the condensation reaction of primary amines with isocyanates by using the bifunctional molecule 3-isocyanatopropyltriethoxysilane. The reaction conditions were selected to obtain one linker on average per dendrimer.

This novel two-step synthetic path for the preparation of dendrimer-functionalized SBA-15 materials was optimized, leading to a homogeneous surface coverage on the inner surface of the mesoporous channels (figure 6).
successive dendrimer generations gave rise to gradual changes in all the properties of the final functionalized matrices. For instance, there was a gradual decrease of the textural properties of SBA-15 (surface area, pore volume and pore diameter), with a dramatic decrease in the surface area of SBA-15 from 806 to 16 m² g⁻¹ after organic modification with the third-generation dendrimer being particularly worth mentioning. Moreover, there was a gradual increase of the organic matter content, although in all cases, the experimental organic contents were smaller than the theoretical ones, and this effect was more noticeable with the increase of the dendrimer generation. As expected, the steric hindrance was more pronounced for higher generations, and led to a limiting coverage value for each generation.

To evaluate the application of these novel materials as controlled delivery devices, adsorption and in vitro release assays were carried out using the anti-inflammatory ibuprofen, which contains a carboxylic acid within its structure, as model drug. The amount of ibuprofen loaded into these materials gradually increased with the dendrimer generation used to functionalize the silica pore walls (figure 6). The drug amount loaded ranged from 21.5 per cent in pure SBA-15 to 28.8, 40.8 and 48.0 per cent for SBA-15 functionalized with the first, second and third dendrimer generations, respectively. Ibuprofen release mechanism from different mesoporous matrices was purely Fickian diffusion controlled and could
be fitted to the Higuchi model [71]: \[ [\text{IBU}] = k \cdot t^{1/2} \]
where \([\text{IBU}]\) is the amount of ibuprofen released at time \(t\) and \(k\) is the kinetic constant. The fit of experimental data to this model exhibited a linear relationship versus the square root of time, as displayed in figure 6. This linear range, associated with ideal diffusion drug release conditions, gradually increased when increasing the dendrimer generation. Moreover, the release rate constant values exhibited the same trend, i.e. the higher the dendrimer generation the greater the kinetic constant. All these findings show that the effective control over drug dosage can be modulated by selecting the appropriate dendrimer generation to functionalize the SBA-15.

4. Macroporous scaffolds for bone tissue engineering

In the previous sections, we have shown that mesoporous materials can be designed to possess a dual function: \textit{in vitro} bioactive behaviour and controlled delivery capability. However, when aiming at bone tissue regeneration applications, it must be highlighted that the main role will be played by cells. In this sense, one can easily realize that the mesoporous cavities of mesoporous materials (2–50 nm) are too small to host bone cells, which need dimensions of the order of micrometres. Furthermore, one should carefully consider the application of these materials. Bone pores, whose size ranges between 1 and 3500 μm, are necessary for several physiological functions carried out by bone [72]. Consequently, three-dimensional scaffolds suitable for bone tissue engineering demand porosity similar to that of natural bone (figure 7). Such macroporosity is essential to allow bone cell penetration, adherence, growth and proliferation that lead to bone in-growth and thereafter vascularization on implantation. Hence, it is necessary to apply conformation methods that preserve the mesoporosity of mesoporous materials while providing interconnected porosity [15,73].

The capability of mesostructured bioceramics to act as drug delivery systems as well as their bioactive behaviour have been demonstrated, as above described, and therefore they seem good candidates to be used as starting materials for the manufacture of three-dimensional scaffolds for bone tissue engineering. So the current challenge is to design and prepare scaffolds that combine hierarchical macroporosity to allow bone oxygenation, nutrient supply and vascularization, and mesoporosity to permit the load and release of drugs to treat bone pathologies.

Several research works have been reported concerning the preparation of three-dimensional scaffolds based in mesostructured bioceramics. For instance, Yun et al. [74,75] reported the fabrication of three-dimensional TG-based scaffolds exhibiting hierarchical giant- (300–1000 μm), macro- (10–30 μm) and meso- (ca 5 nm) porosity by using a combination of sol–gel, double polymer templating and rapid prototyping methods. The \textit{in vitro} tests in SBF revealed that the bioactive behaviour of TGs after conformation as three-dimensional scaffolds was preserved, showing the presence of an apatite layer on their surface after 24 h in SBF. More recently, our research group has reported the setting up of the conditions for the manufacture process of hierarchical mesoporous–macroporous scaffolds when using the above-mentioned synthetic approach [76].

Li et al. [77] prepared hierarchical TG-based scaffolds using P123 surfactant as mesostructure template and polyurethane foam (PUF) as template for the...
macroporous structures. The resulting materials exhibited hierarchical porosity with interconnected macropores of 200–400 μm or 500–700 μm and uniform mesopores of 3.7 nm. These TG-based scaffolds were bioactive after 4 h of soaking in SBF.

Zhu et al. [78] have reported the use of P123 and PUF to synthesize hierarchically structured three-dimensional TG-based scaffolds with four different chemical compositions and their in vitro bioactivity and cell adherence evaluation. Such scaffolds showed similar mesostructural and textural features, exhibiting interconnected macroporous networks with pore diameters in the 200–400 μm range and mesopores of 4.9 nm in size. Cell cultures demonstrated that primary human bone-derived cells were able to attach and spread to different degrees on the different scaffolds. These authors also indicated that the differences in supporting cell growth and differentiation observed for different scaffolds could be related to the apatite formation on the surface of scaffolds, which has been reported to affect cell activity [79,80]. Additionally, the release of calcium and silicon ions may be involved in the modulation of cellular attachment [81].

The manufacture of three-dimensional scaffolds able to drive cell in-growth is an important challenge in bone tissue engineering. The aim is to design and fabricate pieces that support and structure the newly formed tissue and such pieces must be made starting from the most suitable material. One of the most employed strategies in bone tissue engineering involves the initialization of the

Figure 7. Orders of magnitude for different elements involved in bone tissue engineering. The sizes of osteoinductive agents and biological nanoapatites are in the nanometre scale. Cells and bone porosity fall in the micrometre scale. Bone scaffolds must be designed and fabricated with pore sizes similar to those existing in natural bone. (Online version in colour.)

Phil. Trans. R. Soc. A (2012)
regeneration process in vitro by soaking the scaffold in appropriate cell culture and in the presence of osteogenic agents. Then, the scaffold is implanted in the patient [13,82]. However, the current challenge in bone tissue engineering is to chemically graft appropriate osteogenic agents, such as certain peptides, proteins and growth factors, into the three-dimensional scaffold to be directly implanted in the patient. Such osteogenic agents would act as signals to induce cells to regenerate new bone.

When mesostructured bioceramics are used as starting materials for the fabrication of scaffolds, such osteoinductive agents should be covalently grafted to the external surface of silica. This approach would allow one to ‘decorate’ the scaffold with potent osteoinductive signals able to promote the appropriate bone cellular functions where needed (figure 8) [1,11,39,83]. Hence, the first step would consist of optimizing the functionalization method to specifically modify the external surface of mesoporous silica with appropriate organic groups and afterwards grafting the osteoinductive agents. It is known that during the post-synthesis functionalization of mesoporous silica using appropriate silylation agents, the external surface is more accessible to organic modification than the internal surface of the channels. In this sense, the reaction of the calcined material with a highly reactive functionalization agent would result in the preferential functionalization of the external mesoporous surface, which undoubtedly would favour the subsequent grafting of the osteoinductive signals. Another alternative to achieve selective functionalization consists of starting from the material still containing the surfactant in the mesoporous cavities, which would allow
functionalizing the external mesoporous silica surface. The last step would consist of surfactant removal by using solvent extractions to avoid destroying the incorporated organic functions [84,85].

In addition, it should be mentioned that it is also feasible that such peptides, proteins or growth factors can be incorporated inside the mesoporous channels. This possibility would involve the non-covalent interaction between the mesoporous inner matrix surface and the osteoinductive agent. Thus, if the osteoactive factors are released in the implant surrounding when needed they could also help the bone regeneration process [86]. However, in this case, the leaching of the osteoinductive factor would be indiscriminate, which may involve the use of relatively high amounts of expensive osteoactive agents. On the contrary, the covalent grafting of such osteogenic signals would allow appropriately designing three-dimensional scaffolds attending to concrete clinical needs.

5. Concluding remarks

Mesostructured materials have emerged as a full family of bioceramics able to fulfil the requirements for bone tissue regeneration purposes with local controlled drug delivery capability. Among them, TGs constitute a new class of nanostructured materials with accelerated bioactive behaviour compared with conventional bioactive glasses. This improved bioactive behaviour has been attributed to the highly ordered arrangement of uniform-sized mesopores. Moreover, the possibility of tailoring the structural and textural characteristics of TGs 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, cancer, etc. The proper functionalization of the inner surface of mesopore channels allows for modulating drug loading and plays a key role in sustained drug release.

This double scope of mesostructured bioceramics, improved bioactive behaviour 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 (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.

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