Control of the pore architecture in three-dimensional hydroxyapatite-reinforced hydrogel scaffolds

Jesús Román¹, María Victoria Cabañas¹, Juan Peña¹ and María Vallet-Regí¹,²

¹Departamento de Química Inorgánica y Bioinorgánica, Facultad de Farmacia, Universidad Complutense, 28040-Madrid, Spain
²Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Madrid, Spain
E-mail: vallet@farm.ucm.es

Received 11 February 2011
Accepted for publication 7 June 2011
Published 27 July 2011
Online at stacks.iop.org/STAM/12/045003

Abstract
Hydrogels (gellan or agarose) reinforced with nanocrystalline carbonated hydroxyapatite (nCHA) were prepared by the GELPOR3D technique. This simple method is characterized by compositional flexibility; it does not require expensive equipment, thermal treatment, or aggressive or toxic solvents, and yields a three-dimensional (3D) network of interconnected pores 300–900 µm in size. In addition, an interconnected porosity is generated, yielding a hierarchical porous architecture from the macro to the molecular scale. This porosity depends on both the drying/preservation technology (freeze drying or oven drying at 37 °C) and on the content and microstructure of the reinforcing ceramic. For freeze-dried samples, the porosities were approximately 30, 66 and below 3% for pore sizes of 600–900 µm, 100–200 µm and 50–100 nm, respectively. The pore structure depends much on the ceramic content, so that higher contents lead to the disappearance of the characteristic honeycomb structure observed in low-ceramic scaffolds and to a lower fraction of the 100–200-µm-sized pores. The nature of the hydrogel did not affect the pore size distribution but was crucial for the behavior of the scaffolds in a hydrated medium: gellan-containing scaffolds showed a higher swelling degree owing to the presence of more hydrophilic groups.

Keywords: biomaterials, tissue engineering, pore hierarchical architecture

1. Introduction
Tissue engineering has become an interdisciplinary field in which the principles of engineering and the life sciences are applied toward the generation of biological substitutes aimed at the creation, preservation or restoration of lost organ functions [1]. More recently, Williams has defined tissue engineering as ‘the creation (or formation) of new tissue for the therapeutic reconstruction of the human body, by the deliberate and controlled stimulation of selected target cells through a systematic combination of molecular and mechanical signals’ [2]. Although no biomaterial is mentioned in these definitions, tissue engineering does need a biomaterial as a framework for single cells to build a vital and well-functioning tissue [3]. These scaffolds act as an extracellular matrix whose three-dimensional architecture organizes the cells, directing the growth and formation of a desired tissue [4], and facilitates the delivery of molecular and mechanical signals [5].

This 3D architecture should include a hierarchical porosity ranging from the nanometer to the millimeter scale [6–8]. These different pore size domains affect such processes as vascularization, which ensures the transport of oxygen, nutrients and waste, and migration of different cell populations, which is necessary for cell survival and an adequate implant-tissue attachment [3, 9, 10].
The current trend in tissue engineering revolves around the implantation of 3D macroporous scaffolds, which function as templates for cellular activities to provide initial attachment and subsequent regeneration of damaged or lost tissues [11]. Nature shows several examples of lightweight, high-performance structural materials with outstanding strength and toughness [12]. In this sense, biology has long developed the ability to combine brittle minerals and organic molecules into hybrid composites with exceptional fracture resistance and structural capability [13–17].

Unfortunately, these complex structures are extremely difficult to replicate in a controlled manner. In the case of hard tissue substitution, a reasonable strategy is to design ceramic-polymer materials that combine the strength of single components and minimize undesirable drawbacks [18–26]. Bioceramics, such as hydroxyapatite, bioactive glass and tricalcium phosphate, impart high biocompatibility and the ability to induce bone formation while mechanically reinforcing the scaffolds [27–30]. In contrast, natural and synthetic hydrogels mimic the extracellular matrix owing to their high water content and chemical similarity. In addition to their use as scaffolds or bioreactors, reinforced hydrogels that can endure significant mechanical loads are expanding their use, Sigma-Aldrich, Steinheim, Germany) and gellan (Gelrite Riedel-de-Häen) at 37 °C [61]. The pH of the solution was maintained at 9.2 by adding an NH₄OH solution. The resulting suspension was vacuum-filtered, and the product was freeze-dried. The obtained nCHA powder had a CO₂ content of 8.0 ± 0.3% (determined by CHN Elemental Microanalysis) and a high specific surface area of 120 m² g⁻¹; it was composed of nanocrystals (15–20) × (3–5) nm in size. We used the following polyomers: agarose (Agarose for routine use, Sigma-Aldrich, Steinheim, Germany) and gellan (Gelrite Gellan Gum powder, Sigma-Aldrich, Steinheim, Germany).

2. Experimental details

2.1. Materials

Nanocrystalline carbonated hydroxyapatite (nCHA) was prepared by precipitation from an aqueous solution of Ca(NO₃)₂·4H₂O, (NH₄)₂HPO₄ and (NH₄)₂CO₃ (Puriss, Riedel-de-Häen) at 37 °C [61]. The pH of the solution was maintained at 9.2 by adding an NH₄OH solution. The resulting suspension was vacuum-filtered, and the product was freeze-dried. The obtained nCHA powder had a CO₂ content of 8.0 ± 0.3% (determined by CHN Elemental Microanalysis) and a high specific surface area of 120 m² g⁻¹; it was composed of nanocrystals (15–20) × (3–5) nm in size. We used the following polyomers: agarose (Agarose for routine use, Sigma-Aldrich, Steinheim, Germany) and gellan (Gelrite Gellan Gum powder, Sigma-Aldrich, Steinheim, Germany).

2.2. Scaffold preparation

Three-dimensional interconnected porous polymer/nCHA scaffolds were fabricated using the shaping process GELPOR3D patented by the authors [60, 62]. The fabrication procedure consists of the following steps: the gelling powder is suspended in deionized water and heated to 85 °C with continuous stirring; once a translucent sol is formed, the temperature is gradually decreased to 40–45 °C and then a ceramic powder (nCHA) is added under continuous stirring. The obtained slurry is poured into a designed mold containing a network of rigid filaments (1 mm in diameter) that can be removed after 1–2 min. After a few minutes the complete
consolidation of the bodies allows the unmolding of the material, which, freshly prepared, can be easily handled and shaped in the desired form. The 3D perforated scaffolds were preserved by different drying procedures: in an oven at 37 °C and by freeze drying.

Two natural polysaccharides, agarose and gellan, were used as gelling agents for the scaffold fabrication. Perforated scaffolds with different polymer and nCHA contents were prepared (table 1).

2.3. Characterization

The scaffolds were analyzed by x-ray diffraction (XRD) with a Philips X-Pert MPD diffractometer and by scanning electron microscopy (SEM, JEOL 6400). Fourier transform infrared (FTIR) spectra were recorded with a Nicolet Nexus spectrometer equipped with a Smart Golden Gate infrared (FTIR) spectrometer. Thermogravimetry analysis (TGA) was performed with a Perkin Elmer Pyris 1 spectrometer equipped with a Smart Golden Gate. Electron microscopy (SEM, JEOL 6400) was used to examine the possible interactions between the ceramic component and the gelling agents. Figure 1 and 2 show, respectively, the XRD patterns and FTIR spectra of the GL80-F scaffold prepared by the GELPOR3D method. The XRD patterns are very similar for different types of hydrogel or ceramic presences. However, even in these cases, the ceramic presence does not affect the gelation process, which is carried out within a few minutes.

Table 1 lists the compositions of the slurries used to prepare the scaffolds, demonstrating the versatility of the GELPOR3D technique. Note that low agarose contents may result in an insufficient interaction between the ceramic particles and polysaccharide binder that may cause particle migration after implantation. On the other hand, slurries with high ceramic loads may be too viscous for pouring into the mould and fill the interstices between the rigid filaments. Scaffolds with a non designed porosity have been prepared at higher ceramic loads [64]. However, in these cases, the ceramic presence does not affect the gelation process, which is carried out within a few minutes.

Table 1 lists the compositions of the slurries used to prepare the scaffolds, demonstrating the versatility of the GELPOR3D technique. Note that low agarose contents may result in an insufficient interaction between the ceramic particles and polysaccharide binder that may cause particle migration after implantation. On the other hand, slurries with high ceramic loads may be too viscous for pouring into the mould and fill the interstices between the rigid filaments. Scaffolds with a non designed porosity have been prepared at higher ceramic loads [64]. However, even in these cases, the ceramic presence does not affect the gelation process, which is carried out within a few minutes.

Figures 1 and 2 show, respectively, the XRD patterns and FTIR spectra of the GL80-F scaffold prepared by the GELPOR3D method. The XRD patterns are very similar for the scaffold and the nCHA powder, with all the diffraction maxima attributed to a poorly crystallized apatite phase (JCPDS card 24–0033), whereas the amorphous background due to gellan is hardly visible. No significant differences were observed in the XRD patterns of the samples prepared using different types of hydrogel or ceramic/polymer ratios.

Although the shaped ceramic maintains its nanoscale crystallinity and texture, as demonstrated below, it does not remain inert and interacts with other components in the scaffold. FTIR spectroscopy has been employed to examine the possible interactions between the ceramic component and the gelling agents. Figure 2 compares the spectra of the GL80-F scaffold, pure gellan and nCHA, revealing a weak interaction between the carboxylate and hydroxyl groups of the gellan with the nCHA. In particular, the broad peak at 3317 cm\(^{-1}\) in the gellan, related to the hydroxyl groups of the glucopyranose ring with various degrees of

### Table 1. Nominal compositions of the scaffolds obtained with agarose.

| Scaffold | Polymer solution conc. (%) | Polymer : nCHA ratio | Composition of hydrated scaffolds (%)<sup>a</sup> |
|----------|---------------------------|----------------------|--------------------------------------------------|
|          | (w/v %)                   |                      | Water  | Ceramic | Agarose |
| A<sup>b</sup> L80-(O/F)<sup>c</sup> | 2.5                       | 20 : 80              | 88.10  | 9.30    | 2.6     |
| AM50-(O/F) | 3.5                       | 50 : 50              | 93.10  | 3.45    | 3.45    |
| AH20-(O/F) | 5.0                       | 80 : 20              | 93.30  | 1.30    | 5.40    |

<sup>a</sup>Composition determined by thermogravimetry; theoretical values are indicated in brackets.<br>
<sup>b</sup>A or G stands for the type of gelling agent used: agarose or gellan, respectively.<br>
<sup>c</sup>-O or -F represents the drying procedure: in an oven or freeze drying, respectively.

3. Results and discussion

One of the main advantages of our preparation method is the possibility of shaping any kind of ceramic without modifying its original texture and microstructure, because the process is carried out at room temperature and in the absence of aggressive or toxic solvents. Other shaping methods of the nanocrystalline carbonated hydroxyapatite may reduce its high surface area (120 m\(^2\) g\(^{-1}\)), increase the crystal size and dissolve it. This would decrease its reactivity and thus osteogenic capacity, thereby affecting the bioactivity of this apatite when implanted in living organisms [63].
hydrogen bonding [65], is shifted to 3351 cm$^{-1}$ in the GL80-F scaffold. The bands at 1605 and 1412 cm$^{-1}$ in the gellan, due to asymmetric and symmetric stretching vibrations of the carboxylate group, appear at 1621 and 1421 cm$^{-1}$, respectively, in the scaffold; the latter peak interferes with the contribution of the CO$_3$$^-$ groups of the ceramic, which has signals at 1474, 1419 and 873 cm$^{-1}$ [61]. The shift of the hydroxyl band was also observed in agarose-containing scaffolds. A similar interaction between chitosan and a nanohydroxyapatite has been described, where the polymeric component not only served as a matrix but also provided an anchoring site for the ceramic particles [28].

Figure 3 shows the 3D porous scaffolds GH20, GM50 and GL80, obtained from slurries of three different compositions, in a hydrated state and dried by two different methods. The freshly prepared samples (top row) show a rubber-like texture and can be easily cut with a knife and shaped by hand. The preservation of these materials was carried out by freeze drying or in an oven at 37 °C. The freeze drying procedure results in the complete removal of water and a weight loss of 90–95%, as determined by thermogravimetry, but only in a 5–10% volume reduction. Drying in an oven causes a similar weight loss but with a much larger volume change (50–60%), while maintaining the body shape as shown at the bottom row of figure 3. Scaffolds with higher ceramic content shrink less regardless of the drying procedure, whereas the shrinking is independent of the gelling agent.

These clear differences between freeze-dried and oven-dried scaffolds markedly affect the microstructure and texture of the scaffolds. We used SEM to measure the diameter of the bigger pores—those that are generated when the rigid filaments of the mold are removed, yielding a 3D interconnected porosity, and those that are visible to the naked eye (figure 3). Such pores have been termed as ‘giant’ by several authors [66] to stress that they are large enough to allow the vascularization of the scaffold.
Figure 5. SEM images of the AH20-F, AM50-F and AL80-F scaffolds.

Figure 6. SEM images of the GH20-F scaffold at different magnifications.

The final size of these giant pores depends on the drying method, and is 600–800 µm for oven drying and 700–900 µm for freeze drying. The spread for a given drying method is related to the ceramic content—the largest contraction is observed in scaffolds with the lowest nCHA contents, which have giant pores with a diameter between 600 and 700 µm depending on the drying method. Figure 4 shows one of these giant pores in an AH20-F scaffold together with another, network like microstructure. This network is characteristic of freeze-dried materials and has been described by several authors as a honeycomb or sponge-like structure. This sponginess can be explained considering the elimination of water from the freshly prepared scaffold. During the freezing process, ice crystals nucleate from the solution and grow along the lines of thermal gradients; the subsequent lyophilization generates a porous material [67]. This type of structure, with 100–200-µm-sized pores, is clearly observed in freeze-dried scaffolds with low ceramic contents (figure 5). This characteristic honeycomb-like structure progressively transforms with increasing ceramic content, yielding a dense, pore-free surface (figure 5).

SEM observations (figure 6) clarified the physical interaction between the gelling agent and the nCHA particles. In the characteristic honeycomb structure of the polymeric network, a homogeneous distribution of the ceramic within the organic lamella that constitutes the channels (figures 6(a) and (b)) can be observed. A magnification of one of these walls shows how the particles are embedded in the polymeric matrix (figures 6(c) and (d)). Higher nCHA contents cause such a thickening of the channels walls, which may result in their partial or total occlusion.

The intimate ceramic–polymer interaction can modify the nCHA surface, thereby affecting its dissolution and the ability to generate new bones. However, N₂ adsorption porosimetry reveals a progressive decrease in the original surface area of the powdered nanohydroxyapatite from ∼120 to 58 m² g⁻¹.
in AL80-F, 28 m² g⁻¹ in AM50-F and 12 m² g⁻¹ in AH20-F, which correlates with the decreasing ceramic content in these scaffolds. In addition, the monomodal pore size distribution peaking around 50–100 nm in the ceramic is maintained in all these scaffolds.

The oven-dried scaffolds show a completely different morphology consisting of a wrinkled collapsed structure (figure 7). In this case, the water elimination causes a ceramic-dependent volume reduction and does not lead to the formation of the characteristic honeycomb structure of freeze-dried samples.

Hg intrusion porosimetry and He picnometry have been employed to complement the porosity characterization of the different pore size domains already described. Figure 8(a), which represents the quantity of Hg adsorbed on each material, allows the quantification of the differences, in terms of porosity, between the scaffolds. Increasing the ceramic contents decreases the adsorption capacity of the scaffolds (figure 8(a)). Note that the spongy structure of the freeze-dried scaffolds strongly depends on the ceramic content. As shown in figure 8(b), the GH20-F sample shows the highest Hg adsorption in pores of ~200 µm size; these pores seem to shrink to ~100 µm at higher ceramic contents, as can be observed for sample GM50-F, and disappear for sample GL80-F.

Analysis of the mercury intrusion at higher pressures confirms the presence of a third type of pore with diameters of 50–100 nm (figure 8(b), inset) detected by N₂ adsorption porosimetry. These pores can be clearly observed in samples GM50-F and GH20-F owing to their lower ceramic content and strong low-pressure signals (figure 8(a)). This observation can be explained as follows: the low ceramic/polymer ratio implies a thorough polymer coating of each ceramic particle, which destroys pores present at the interparticle areas in the original material.

Figure 8(a) contains the Hg adsorption curve of a GM50-O sample to illustrate the difference between the oven-dried and lyophilized scaffolds, namely, the absence of 100–200-µm-sized pores in the GM50-O scaffold, which are otherwise generated following the water extraction during the freeze-drying procedure. To understand this difference, as well as the effect of the composition on the microstructure of the obtained scaffolds, their densities were measured by three methods. The bulk (also known as envelope) density indicates the volume resulting from close-fitting imaginary envelopes completely surrounding each scaffold (ASTM D3766 standard). This volume was determined by two methods: manual measurement with a micrometer, by Hg porosimetry and He-picnometry, with the standard deviations of 0.02, 0.02 and 0.01, respectively. The porosities \( P_{SKE} \) and \( P_{SOL} \) were calculated using equation (1) (SD = 0.1) with \( d_b \) placed in the numerator and \( d_{SKE} \) or \( d_{SOL} \) in the denominator, respectively.

The densities \( d_b \), \( d_p \) and \( d_{SOL} \) were measured with a micrometer, by Hg porosimetry and He-piconometry, with the standard deviations of 0.02, 0.02 and 0.01, respectively. The porosities \( P_{SKE} \) and \( P_{SOL} \) were calculated using equation (1) (SD = 0.1) with \( d_b \) placed in the numerator and \( d_{SKE} \) or \( d_{SOL} \) in the denominator, respectively.

Table 2. Bulk, skeletal and solid densities and porosities of lyophilized and oven-dried agarose scaffolds.

|        | Bulk | Skeletal | Solid | \( P_{SKE} \) | \( P_{SOL} \) |
|--------|------|----------|-------|---------------|---------------|
| AL80-F | 0.14 | 0.18     | 2.32  | 3.20          | 92.2          |
| AM50-F | 0.08 | 0.09     | 1.45  | 2.70          | 93.8          |
| AH20-F | 0.07 | 0.07     | 1.26  | 2.04          | 94.4          |
| AL80-O | 0.67 | 0.61     | 1.81  | 3.10          | 66.3          |
| AM50-O | 0.82 | 0.73     | 1.72  | 2.57          | 57.5          |
| AH20-O | 1.19 | 0.92     | –     | 1.77          | 48.0          |

Figure 8. (a) Hg cumulative intrusion and (b) pore size distribution of the GH20-F, GM50-F, GM50-O and GL80-F scaffolds and nCHA.
Figure 9. Porosities of the different pore size domains in freeze-dried scaffolds.

Figure 10. Water uptake of some 3D scaffolds after 24 h averaged over four measurements.

densities of agarose scaffolds. No significant difference is observed between the bulk densities measured manually or by Hg intrusion porosimetry, in agreement with a previous study [68]. All the densities of freeze-dried samples increase with the ceramic content. The considerable volume reduction resulted in higher bulk densities in oven-dried than in freeze-dried scaffolds, and these densities decrease with the ceramic content. The higher values of $d_{\text{SKE}}$ and $d_{\text{SOL}}$ than the bulk densities ($d_5$ or $d_p$) can be explained by the different measured volumes. We also note the difficulties in the Hg intrusion porosimetry analysis of samples with the lowest ceramic content, as their collapsed structure hindered the penetration of mercury inside the dried scaffolds.

Two types of porosity values, $P_{\text{SKE}}$ and $P_{\text{SOL}}$, were calculated with equation (1) [69]; the bulk densities $d_5$ were placed in the numerator ($d_5$) and the skeletal ($d_{\text{SKE}}$) or solid ($d_{\text{SOL}}$) densities in the denominator ($d_5$).

$$P = 100 \times (1 - d_5/d_5).$$

Similar porosities of over 90% were calculated for all lyophilized scaffolds; these high values can be explained considering that the water content of the freshly prepared scaffolds varied between 88.1 and 93.30% (table 1). For samples M50-F and H20-F, regardless of the gelling agent, the porosity values relate to the amount of water eliminated during preservation. However, the sample with the highest ceramic content shows a considerable difference between the calculated porosities (92–94%) and the water percentage determined by thermogravimetry (88%). This additional porosity can be justified considering the pores left among the ceramic particles, and these pores have been observed by N$_2$ adsorption and Hg intrusion porosimetry. The latter technique allowed us the estimation of this interparticle porosity as 30–40% of the total porosity.

The calculated porosities were lower for oven-dried than for lyophilized scaffolds in all the cases, probably because the water elimination led to a considerable shrinkage, which increased with the polymer content, and did not create the 100–200 $\mu$m channels.

To summarize and clarify the pore characterization results, the estimated percentages of different pore size domains for lyophilized scaffolds are presented in figure 9. The ‘giant’ pore content (∼30%) relates to the amount of filaments employed to prepare the scaffolds. As previously established [60], the GELPOR3D method yields porosities of 10–40%, depending on the size of the filaments and their arrangement. The remaining porosity (70%) corresponds to the pores within the solid skeleton of the scaffold, and most of it can be related to water elimination. In the case of lyophilized scaffolds, the porosity values are equal to or greater than the water content, determined by thermogravimetry. In scaffolds with the highest ceramic content the porosity can be related to the pores generated between the ceramic particles or within them; this has been estimated by Hg intrusion porosimetry. The presence of this type of porosity cannot be ruled out in other samples.

A close examination of the pore size distributions obtained by Hg intrusion porosimetry reveals a weak maximum in the corresponding region, but its quantification yielded impractical values below 1%. As for oven-dried samples, their porosities were difficult to estimate because of the considerable volume shrinkage and unreliable Hg-intrusion porosimetry results.

The behavior of these reinforced hydrogels in hydrated media is of critical importance for all potential applications. The swelling behavior is intimately related to the fluid intake and, consequently, with the material porosity. Therefore, the different factors that affect porosity, such as the ceramic content and the preservation procedure, should affect the hydration rate and the final swelling degree. In addition, the nature of the polymer is responsible for the hydrogel behavior and determines the transition from the glassy structure in the dehydrated state to the formation of elastic gels. This behavior is attributed to the polymer component because, owing to the hydrophilic nature of its chains, the network can absorb water within its structure, swell without destruction, and maintain its overall structure [70].

Figure 10 depicts the final swelling degree of the 3D scaffolds in terms of water uptake, which was calculated from...
the gravimetry data as

\[ \text{% water uptake} = 100 \left( \frac{W_f - W_i}{W_i} \right). \]  

(2)

Here, \( W_i \) corresponds to the weight of the desiccated samples and \( W_f \) to the weight after swelling.

The capability of these scaffolds to adsorb fluids several times their own weight can be explained considering these materials as ceramic-reinforced hydrogels. Their swelling degree decreases with the nCHA content. A similar trend in water absorption has been reported for nCHA/chitosan [28] and β-TCP/agarose composites [64], indicating that the addition of ceramic decreases the water absorption. Not only the quantity but also the nature of the polymer affects the swelling degree; the water uptake is higher for scaffolds prepared with gellan than with agarose because of the more hydrophilic character of gellan. Finally, the scaffolds dried in an oven shrink more and absorb less water than freeze-dried scaffolds. This fact can be related with the higher porosity of lyophilized systems, and it has been observed in other scaffolds [42].

While swelling, the scaffolds increase their volume, by 20–40% for lyophilized samples containing agarose, 25–50% for samples containing gellan and more than 100% for oven-dried scaffolds. This capability to quickly rehydrate in the presence of any aqueous solution such as plasma or blood should facilitate the surgical applications of the scaffolds; it should also increase the biomineralization in the cell culture medium [71]. Finally, the fixation of the scaffold within the defect to be repaired is improved by swelling, which also ensures a constant pressure and an intimate contact stimulating bone regeneration.

4. Conclusions

Preservation techniques (freeze or oven drying) and the ceramic/polymer ratio have a critical effect on the pore architecture of the nCHA-gellan and nCHA-agarose scaffolds obtained by the GELPOR3D method. In this work, the interconnected porosity was designed to have 900 \( \mu \text{m} \) ‘giant’ pores, which yield a 30% porosity or void space. The porosity within the ceramic–polymer material that constitutes the scaffold has been characterized and quantified. Lyophilized scaffolds have two pore-size domains. One is parallel channels with diameters between 100 and 200 \( \mu \text{m} \) depending on the ceramic content, which can be attributed to the extraction of ice crystals during the freeze drying procedure. The other pore size distribution, centered between 50 and 100 nm, depends on the ceramic microstructure. Despite their small total volume, these pores may be important for the migration of fluids from or to the cells or for the controlled release of bioactive molecules. Oven-dried scaffolds are characterized by two types of pore: ceramic-dependent nanosized pores and a tunable network of interconnected giant pores. The hydration behavior of the preserved scaffolds depends on both the pore hierarchy and nature of the hydrogel, i.e. the more hydrophilic structure of gellan than of agarose results in a higher swelling degree in terms of weight and volume.

Acknowledgments

This work was supported by CICYT, Spain (Project MAT2008-00736) and by the Comunidad de Madrid, Spain (S2009/MAT-1472). The XRD and SEM measurements were performed at C.A.I. Difracción de Rayos X and Microscopía Electrónica (UCM), respectively.

References

[1] Langer R and Vacanti J P 1993 Science 260 920
[2] Williams D F 2009 Biomaterials 30 5897
[3] Eisenbarth E 2007 Adv. Eng. Mater. 9 1051
[4] Yang S F, Leong K F, Du Z H and Chua C K 2001 Tissue Eng. 7 679
[5] Willie B M, Petersen A, Schmidt-Bleen K, Cipitria A, Mehta M, Strube P, Lienau J, Wildemann B, Fratzl P and Duda G 2010 Soft Matter 6 4976
[6] Vallet-Regí M 2008 Chem. Eng. J. 137 1
[7] Hollister S J 2005 Nat. Mater. 4 518
[8] Vallet-Regí M 2010 J. Int. Med. 267 22
[9] Guobao W and Ma P X 2008 Adv. Funct. Mater. 18 3568
[10] Yuan H P, Kurashina K, de Bruijn J D, Li Y B, de Groot K and Zhang X D 1999 Biomaterials 20 1799
[11] Hollister S J 2009 Adv. Mater. 21 3330
[12] Hukins D W L, Leaby J C and Mathias K J 1999 J. Mater. Chem. 9 629
[13] Launey M E, Munch E, Alsem D H, Barth H B, Saiz E, Tomsis A P and Ritchie R O 2009 Acta Mater. 57 2799
[14] Munch E, Launey M E, Alsem D H, Saiz E, Tomsis A P and Ritchie R O 2008 Science 322 1516
[15] Huebsch N and Mooney D J 2009 Nature 462 426
[16] Vallet-Regí M, Colilla M and González-Valdés B 2011 Chem. Soc. Rev. 40 596
[17] Sundar S, Kundu J and Kundu S C 2010 Sci. Technol. Adv. Mater. 11 014104
[18] Vallet-Regí M 2006 Dalton Trans. 2511
[19] Ramakrishna S, Mayer J, Wintermantel E and Leong K W 2001 Compos. Sci. Technol. 61 1189
[20] Barrere F, Mahmoud T A, de Groot K and van Blitterswijk C A 2008 Mater. Sci. Eng. R.—Rep. 59 38
[21] Hing K A 2004 Philos. Trans. R. Soc. Lond. A 362 2821
[22] Vallet-Regí M 2010 C. R. Chim. 13 174
[23] Sánchez-Salcedo S, Nieto A and Vallet-Regí M 2008 Chem. Eng. J. 137 62
[24] Juhasz J A, Best S M and Bonfield W 2010 Sci. Technol. Adv. Mater. 11 014103
[25] Furuchi K, Oaki Y, Ichimiyi H, Komotori J and Imai H 2006 Sci. Technol. Adv. Mater. 7 219
[26] Cabañas M V, Veja J, Román J and Vallet-Regí M 2006 J. Biomed. Mater. Res. Part A 78A 508
[27] Vallet-Regí M and González-Calbet J M 2004 Prog. Solid State Chem. 32 1
[28] Thein-Han W W and Misra R D K 2009 Acta Biomater. 5 1182
[29] Sopyan I, Mel M, Ramesh S and Khalid K A 2007 Sci. Technol. Adv. Mater. 8 116
[30] Zhang L L, Hanagata N, Maeda M, Minowa T, Ikom T, Fan H and Zhang X D 2009 Sci. Technol. Adv. Mater. 10 025003
[31] Lee K Y and Mooney D J 2001 Chem. Rev. 101 1869
[32] Peppas N A, Hilt J Z, Khademhosseini A and Langer R 2006 Adv. Mater. 18 1345
[33] Schmidt J J, Rowley J and Kong H J 2008 J. Biomed. Mater. Res. A 87 1113
[34] Van Tomme S R, Storm G and Hennink W E 2008 Int. J. Pharm. 355 1
[35] Klouda L and Mikos A G 2008 Eur. J. Pharm. Biopharm. 68 34
[36] Slaughter B V, Khurshid S S, Fisher O Z, Khademhosseini A and Peppas N A 2009 Adv. Mater. 21 3307
[37] Calvert P 2009 Adv. Mater. 21 743
[38] Rutolf M P 2009 Nat. Mater. 8 451
[39] Annabi N, Nichol J W, Zhong X, Ji C D, Koshy S, Khademhosseini A and Dehghani F 2010 Tissue Eng. B 16 371
[40] Rinaudo M 2008 Polym. Int. 57 397
[41] Tabata M, Shimoda T, Sugihara K, Ogomi D, Serizawa T and Akashi M 2003 J. Biomed. Mater. Res. B 67 680
[42] Cabañas M V, Peña J, Román J and Vallet-Regí M 2009 Eur. J. Pharm. Sci. 37 249
[43] Fialho A M, Moreira L M, Granja A T, Popescu A O, Hoffmann K and Sa-Correia I 2008 Appl. Microbiol. Biotechnol. 79 889
[44] Gong Y H, Wang C M, Lai R C, Su K, Zhang F and Wang D A 2009 J. Mater. Chem. 19 1968
[45] Oliveira J T, Martins L, Picciocchi R, Malafaya I B, Sousa R A, Neves N M, Mano J F and Reis R L 2010 J. Biomed. Mater. Res. A 93 852
[46] Smith A M, Shelton R M, Perrie Y and Harris J J 2007 J. Biomat. Appl. 22 241
[47] Ho M-H, Kuo P-Y, Hsieh H-J, Hsien T-Y, Hou L-T, Lai J-Y and Wang D-M 2004 Biomaterials 25 129
[48] Zmora S, Glicklis R and Cohen S 2002 Biomaterials 23 4087
[49] Landi E, Valentini F and Tampieri A 2008 Acta Biomater. 4 1620
[50] Pancrazio J J, Wang F and Kelley C A 2007 Biosens. Bioelectron. 22 2803
[51] Deville S, Saiz E, Nalla R K and Tomsia A P 2006 Science 311 515
[52] Gutierrez M C, Ferrer M L, del and Monte F 2008 Chem. Mater. 20 634
[53] Lozinsky V I, Galiev I Y, Plieva F M, Savina I N, Jungvid H and Mattiasson B 2003 Trends Biotechnol. 21 445
[54] Plieva F M, Galiev I Y, Noppe W and Mattiasson B 2008 Trends Microbiol. 16 543
[55] Lozinsky V I, Plieva F M, Galiev I Y and Mattiasson B 2001 Bioseparation 10 163
[56] Wegst U G K, Schecter M, Donius A E and Hunger P M 2010 Philos. Trans. R. Soc. A 368 2099
[57] Mallick K K 2009 J. Am. Ceram. Soc. 92 S85
[58] Qian L, Ahmed A, Foster A, Rannard S P, Cooper A I and Zhang H F 2009 J. Mater. Chem. 19 5212
[59] Buckley C T and O’Kelly K U 2010 J. Biomed. Mater. Res. B 93 459
[60] Peña J, Román J, Cabañas M V and Vallet-Regí M 2010 Acta Biomater. 6 1288
[61] Padilla S, Izquierdo-Barba I and Vallet-Regí M 2008 Chem. Mater. 20 5942
[62] Vallet-Regí M, Peña López J, Román Zaragoza J and Cabañas M V 2010 Método para la preparación a baja temperatura de piezas de biocerámicas con porosidad tridimensional diseñada e interconectada. Spain. ES2333851
[63] Alcaide M, Serrano M-C, Román J, Cabañas M-V, Peña J, Sánchez-Zapardiel E, Vallet-Regí M and Portolés M-T 2010 J. Biomed. Mater. Res. A 95 793
[64] Román J, Cabañas M V, Peña J, Doadrio J C and Vallet-Regí M 2008 J. Biomed. Mater. Res. A 84 99
[65] Agnihotri S A, Jawalkar S S and Aminabhavi T M 2006 Eur. J. Pharm. Biopharm. 63 249
[66] Yun H S, Kim S E, Hyun Y T, Heo S J and Shin J W 2007 Chem. Mater. 19 6363
[67] Francis Suh J K and Matthew H W T 2000 Biomaterials 21 2589
[68] Xu H H K, Quinn J B, Takagi S, Chow L C and Eichmiller F C 2001 J. Biomed. Mater. Res. 57 457
[69] Karageorgiou V and Kaplan D 2005 Biomaterials 26 5474
[70] Morris V J, Brownsey G J, Chilvers G R, Harris J E, Gunning A P, Ridout M J and Stevens B J H 1990 Carbohydr. Polym. 13 165
[71] Madhumathi K, Kumar P T S, Kavya K C, Furuike T, Tamura H, Naik S V and Jayakumar R 2009 Int. J. Biol. Macromol. 45 289