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Development of a Biodegradable Composite Scaffold for Bone Tissue Engineering: Physicochemical, Topographical, Mechanical, Degradation, and Biological Properties

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Abstract The development of synthetic materials and their use in tissue engineering applications has attracted much attention in recent years as an option for trabecular bone grafting. Bioabsorbable polyesters of the poly(α-hydroxy acids) family, and specifically polylactic acid (PLA), are well known bioabsorbable materials and are currently used for numerous biomedical applications. The incorporation of an inorganic phase, such as a soluble calcium phosphate glass in the $P_2O_5 - CaO - Na_2O - TiO_2$ system, into the polymeric matrix enhances the mechanical integrity of the material. In fact, the flexural elastic modulus increases from 3.2 to 10 GPa with 50 wt/wt % of glass particles. It also improves the biological behavior and modifies the degradation pattern of the polymer. The presence of glass particles accelerates the material degradation and induces the formation of calcium phosphate precipitates in the surface of the composite. Therefore, the combination of a bioabsorbable polymer such as

PLA with a soluble calcium phosphate glass leads to a fully degradable composite material with a high bone regenerative potential. The success of a 3D scaffold depends on several parameters that go from the macro- to the nanoscale. The solvent and casting technique, together with particulate leaching, allows the elaboration of 95%-porosity scaffolds with a well interconnected macro- and microporosity. Factors such as surface chemistry, surface energy, and topography can highly affect the cell-material response. Indeed, the addition of glass particles in the PLA matrix modifies the material surface properties such as wettability AI (Area index or real-surface-area/nominal-area ratio) and roughness, improving the cell response and inducing morphological changes in the cytoskeleton of the osteoblasts. This study offers valuable insight into the parameters affecting cell-scaffold behavior, and discusses the special relevance that a comprehensive characterization and manufacturing control of the composite surface can have for monitoring the biological–synthetic interactions.

Keywords Bioabsorbable composite scaffold · Bone tissue engineering · Osteoblast cell culture · Protein adsorption · Wettability

Abbreviations

AI	Area index or real-surface-area/nominal area ratio
CaP	Calcium phosphate
E	Young's modulus
ECM	Extracellular matrix
FCS	Fetal calf serum
G5	$44{,}5P_2O_5 - 44{,}5CaO - 6Na_2O - 5TiO_2$ glass (molar composition)
HV	Vickers microhardness
ICP-MS	Inductively coupled plasma-mass spectroscopy
MTT	Tetrazolium-salt assay
Mw	Molecular weight
PLA	Polylactic acid
SBF	Simulated body fluid
S_a	Average 3D roughness
Sku	Kurtosis of the 3D surface texture
Ssk	Skewness of the 3D surface texture
T_g	Glass transition temperature
Wa	Work of adhesion

1 Introduction

Nowadays, autografts, allografts, and xenografts are used for the restoration of bone injuries. Although the use of these grafts has presented satisfactory results under certain conditions, there are some restrictions associated with donor site scarcity, rejection, diseases transfer, and elevated harvesting costs. Due to the numerous drawbacks these grafts present, research has focused on the development of alternative synthetic materials.

Bioabsorbable polymers such as aliphatic polyesters from the poly (α-hydroxy acids) family, especially polylactic acid (PLA), are well known bioabsorbable materials and are widely used for biomedical applications

such as sutures, pins, screws and drug delivery systems [1–4]. Given the biocompatibility and biodegradability features PLA presents, its use in tissue engineering applications has attracted much attention in recent years. Thereby, the development of PLA biodegradable porous scaffolds represents a promising alternative for trabecular bone grafting.

The incorporation of an inorganic phase into the polymeric matrix may enhance the mechanical integrity of the material, as well as its biological behavior, and can also modify the degradation mechanism of the polymer. Some calcium phosphate ceramics and biological glasses have been used with this aim [5–7]. Specifically, calcium phosphate (CaP) glasses are well suited for bone remodeling given that they possess a chemical composition close to that of the mineral phase of bone and that their solubility rate can be adjusted by controlling their chemical composition.

Therefore, the combination of a bioabsorbable polymer such as PLA with a soluble CaP glass leads to a fully degradable composite material with a high bone regenerative potential.

The success of a 3D scaffold depends on several parameters that range from the macro- to the nanoscale. Macro- and microporosity, as well as interconnectivity, are of great importance in promoting tissue ingrowth, vascularization, and the delivery of nutrients throughout the newly formed tissue. The attachment and adhesion of the cells on the material surface is also of paramount importance. These are protein-mediated processes, where factors such as surface chemistry, surface energy, and topography can affect the cell-material response [8]. Indeed, surface characteristics at all dimensional scales affect the adsorption of proteins. Differences in protein adsorption (type of adsorbed proteins, orientation and conformation, and the kinetics of adsorption) lead to variations in the number of cells and their force of adhesion to the substrate [9, 10]. This is a process mediated by the interactions between the adsorbed proteins and integrins, which are cell membrane proteins [11]. The cell adhesion process triggers different chemical and mechanical signals, thus influencing the regulation of cell survival, proliferation and differentiation, which in turn determine cell function within a defined tissue.

This review offers some insight into the parameters affecting the cell-scaffold behavior from the macro- to the microscale, from the bulk to the surface, and discusses the special relevance that a comprehensive characterization and manufacturing control of the composite surface might have in monitoring the biological–synthetic interactions.

2
Development of the Composite Material

The resorption rate of a biomaterial in vivo involves a very complex mechanism that depends on numerous variables and involves both the material

physicochemical features and biological events, including protein- and cell-mediated processes. Among the physicochemical properties, the solubility of the material plays an important role and significantly affects the biomaterial's stability in vivo. Thus, if the material's solubility rate is too high, it will be resorbed by passive dissolution due to the physiological fluids without stimulating tissue turnover, i.e., the resorption/regeneration process mediated by bone cells during bone remodeling. In contrast, if the solubility of the material is too low, it will remain in the body for a long period of time, and bone remodeling will not take place adequately. The use of materials with a moderate solubility rate induces an active resorption process, which is lead by cells and resembles the biological bone remodeling process. Hence, the control of degradation kinetics is a key point in the design of bioabsorbable materials for regenerative purposes.

2.1 Calcium Phosphate Soluble Glasses

Calcium phosphate glasses represent an interesting alternative, since the solubility of these glasses can be adjusted depending on their chemical composition. This fact presents an important advantage over crystalline calcium phosphates.

The structural unit of phosphate glasses is the PO_4 tetrahedron. The basic phosphate tetrahedra form long chains and rings that give rise to the 3D vitreous network [12]. These phosphate chains and rings may be interrupted by the incorporation of certain ions, generating nonbridging oxygens in the glass structure. The incorporation of other modifying ions can lead to the creation of ionic cross-links between nonbridging oxygens of two different chains, thus reinforcing the glass network. Therefore, depending on the modifiers present in the vitreous structure, long-term or short-term soluble phosphate glasses can be obtained [6, 13–15].

Previous studies show that the addition of CaO, Na_2O and TiO_2 into the phosphate network allows control of the solubility and mechanical properties of these glasses within certain ranges [15–17]. Both CaO and TiO_2 enhance glass stability, particularly TiO_2 given its small ionic radius and the large charge on the Ti^{4+} ion [18, 19]. The characteristics of the Ti^{4+} ion allow it to penetrate into the vitreous arrangement, inducing a higher degree of reticulation in the glass network.

The CaP glass of the $44{,}5P_2O_5 - 44{,}5CaO - 6Na_2O - 5TiO_2$ (molar composition) system, coded G5, is a good candidate since it presents a good chemical stability (see Fig. 1) as well as good mechanical properties.

In vitro degradation studies on the G5-glass were performed with SBF [20] (an acellular and aproteic fluid that has an ionic concentration similar to that of human blood plasma, see Table 1) at physiological temperature. ICP-MS analyses showed that G5-glass dissolution occurs uniformly, which means

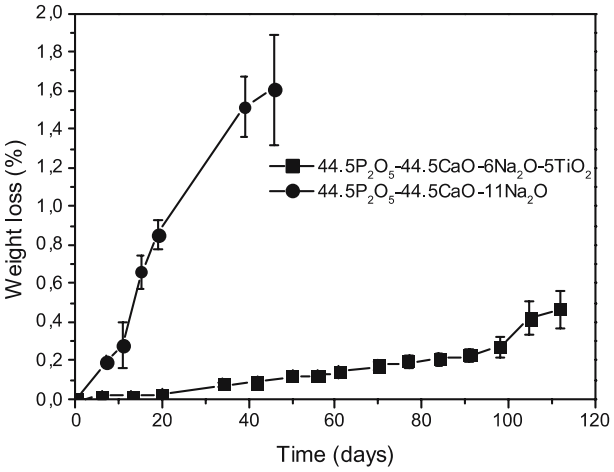

Fig. 1 Weight loss versus dissolution time for two different CaP glasses during degradation in SBF at 37 °C. Error bars not shown if smaller than symbols

Table 1 SBF and human blood plasma ionic composition and concentration (mM)

Ion	SBF	Human plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	147.8	103.0
HCO_3^-	4.2	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

that none of the ions conforming the glass network is released preferentially. In addition, in vitro analysis revealed that during dissolution water diffuses into the glass surface and surrounds the external PO_4 chains, creating a hydrated layer. When the phosphate polymeric chains have been completely surrounded by the aqueous medium, the hydrated chains separate from the bulk of the material and leach into the solution. Due to the homogeneous superficial dissolution process, the mechanical properties of the glass are maintained throughout the degradation period [21].

Biocompatibility studies of the G5-glass, performed with human skin fibroblasts and osteoblast-like human cells from a cell line coded MG63, have shown that this material as well as its degradation products are noncytotoxic [22].

Cell differentiation studies are used to follow the development of cell phenotype by analyzing the concentration of two proteins directly related to bone extracellular matrix mineralization: alkaline phosphatase and the osteocalcin. Cell differentiation studies performed on the G5 glass have shown that it induces an earlier differentiation of the osteoblastic cells than the polysterene plate controls (unpublished data) (see Fig. 2). Consequently, a faster bone formation could be obtained.

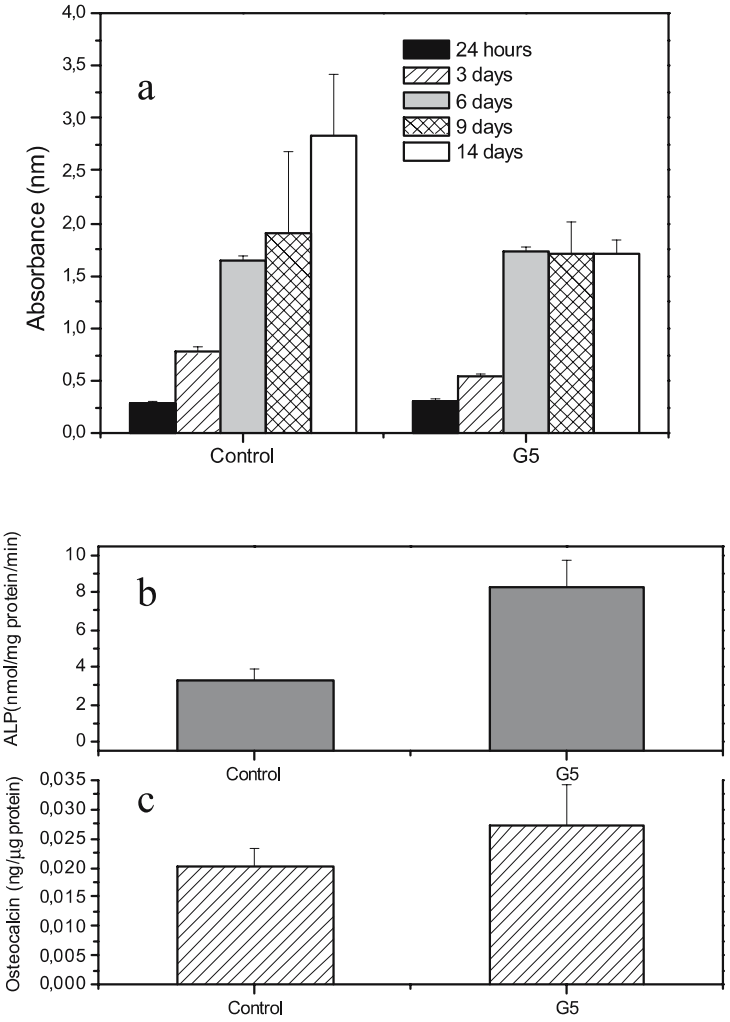

Fig. 2 **a** MTT results of the effect of the G5 glass on MG63 cells, showing cell proliferation. **b** Alkaline phoshatase activity (ALP) values of MG63 cells after 11 days of culture. **c** Osteocalcin concentration values of the MG63 cells after 11 days of culture

Table 2 Properties of the G5 glass (mean values ± standard deviation)

Properties	G5 glass
T_g [°C]	532.9
HV [HV0.2]	431.1 ± 7.8
E [GPa]	71.1 ± 1.7
Solubility rate in distilled water [g cm^{-2} h^{-1}]	$3.13.10^{-6} \pm 1.38.10^{-7}$
Solubility rate in SBF [g cm^{-2} h^{-1}]	$3.2.10^{-7} \pm 1.03.10^{-7}$

Recently, in vivo studies have also revealed a good biocompatibility and guidance of the newly formed tissue to the G5-glass surface, which confirms its osteoconductive potential. In an in vivo study using a rabbit model, the percentage of new bone formation with implanted glass particles was comparable to that obtained for the autologous bone (control), after 12 weeks of implantation [23]. The properties of the G5-glass are summarized in Table 2.

2.2 PLA/Calcium Phosphate Glass Composite Material

Given the advantages of incorporation of an inorganic phase into a polymeric matrix, the G5-glass has been combined with a 95L/5DL-PLA in order to develop a nonporous 2D fully resorbable composite material that could be used in different load-bearing bone-repairing situations.

In general, the incorporation of the G5-glass particles in the polymer improves the flexural mechanical properties of PLA, modifies its degradation behavior, and induces interesting changes in the material surface morphology.

PLA flexural mechanical properties are very low in comparison with cortical bone properties. Therefore, PLA properties are insufficient for high load-bearing applications. Addition of the inorganic phase into the PLA matrix leads to a rise in the mechanical properties of the material, to more nearly approach the mechanical properties of bone and, thus, allowing a better load-transfer to the newly formed tissue [24]. Former studies have shown that the mechanical properties of nonporous materials, especially the Young's modulus (E), undergo a significant increase (from 3.2 to 10 GPa) with the incorporation of 50% by weight of glass particles. However, the PLA matrix has a saturation limit for enveloping the particles, and the efficiency of the G5 particles seems to decrease as the percentage of particles exceeds this limit of approximately 60%.

The presence of glass particles modifies the in vitro degradation pattern of the polymer. In general, the degradation of PLA depends on several factors, which include its crystallinity, molecular weight, dimensions, composition,

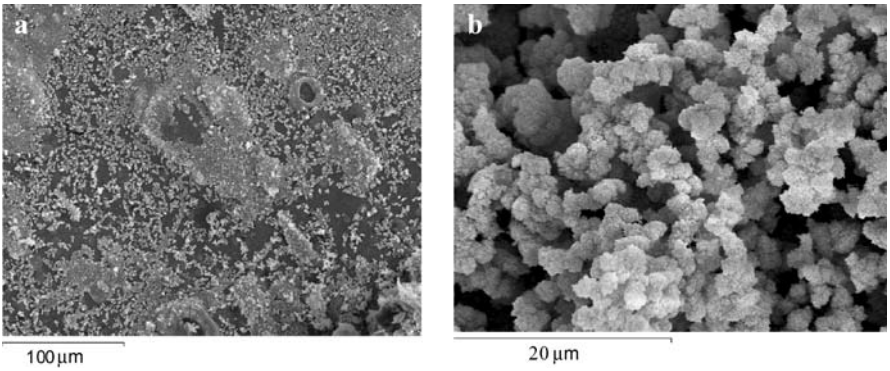

Fig. 3 Composite surface (**a**) and microstructure of the CaP precipitate (**b**) formed at the material surface after 6 weeks of immersion in SBF at 37 °C

and the pH of the surrounding medium. Nevertheless, in spite of the influence these factors may have on the degradation of PLA, it is well known that the degradation mechanism of this polymer is a bulk mechanism autocatalyzed by carboxyl end groups formed by chain cleavage [25, 26].

The addition of G5 particles into the polymer matrix implies the presence of PLA/G5 interfaces at the surface, which allows the penetration of the aqueous fluid into the interior of the composite. This fact, combined with the glass reactivity in aqueous media, induces the formation of surface microcracks. These facilitate both fluid penetration, which accelerates degradation of the polymer chains, and the release of the degradation by-products. At the same time, the degradation products of the glass act as buffering agents that interfere with the autocatalytic process. All these events lead to a higher mass loss and a higher crystallinity, and to a lower Mw loss of the PLA/G5 composite in comparison to the PLA polymer.

On the other hand, the G5 particles react with SBF, giving rise to a globular CaP amorphous structure (see Fig. 3) that emerges in the composite material (manuscript submitted), with a Ca/P ratio close to 1.5. This CaP precipitate could enhance the interaction between the bone cells and the material during bone regeneration since this amorphous CaP is a transient structure to hydroxyapatite, which is the mineral phase of bone with a higher Ca/P ratio.

3
Surface Characterization

The cell adhesion process is critical to most bone regeneration applications [27, 28]. In general, cell adhesion to synthetic substrates is a protein-mediated process. Thus, the amount, type, and activity of the adsorbed pro-

teins on the material surface are a key issue, though the individual role of each parameter is not clear. Numerous studies have shown that the characteristics of the adsorbed proteins and the cell behavior depend strongly on surface properties such as hydrophilicity, surface energy, and the topography of the substrate surface [8].

3.1 Roughness

Roughness and texture are two of the properties that most influence the biological behavior of synthetic materials. On one hand, it is well known that when the topographical features of the surface roughness follow a regular disposition (columns, grooves, etc.), cells are oriented by the pattern and have limited motility [29, 30]. This behavior, which is a consequence of the micro- and/or nanometer texture, is called cell guiding [31].

On the other hand, higher roughness in anisotropic-topographical surfaces is related to better attachment, adhesion, and differentiation of the osteoblast cells onto synthetic materials [8, 9]. This is because osteoblasts can extend from peak to peak and take optimal shapes for their "accommodation". These optimal shapes lead to changes in the cytoskeleton that also favor, via biochemical signals, osteoblast behavior. However, roughness must be of the order of cell dimensions for osteoblasts to "feel" the topographical features [32]. This means that roughness must be in the micrometer range with a maximum and minimum value for the height of and the distance between the peaks/valleys. Consequently, the calculation not only of amplitude roughness parameters but also of spatial and/or hybrid parameters is of paramount relevance.

The influence of roughness in the nanometer scale on cell behavior is controversial, and can be due to the changes that it induces in other physicochemical properties, such as wettability and Z-potential [33]. This will mainly influence the layer of proteins that are adsorbed on those surfaces. This

Table 3 Roughness parameters values (white light optical interferometry) of PLA and PLA/G5 before and after being polished (mean values ± standard deviation)

Material	S_a [μm]	Sku	Ssk	AI
PLA/G5				
Polished	0.238 ± 0.11	53.1 ± 27	− 5.25 ± 2.7	1.09 ± 0.02
Unpolished	0.411 ± 0.04	4.8 ± 2	− 0.40 ± 0.43	1.15 ± 0.10
PLA				
Polished	0.054 ± 0.01	11.2 ± 8	− 0.99 ± 0.7	1.01 ± 0.00
Unpolished	0.372 ± 0.12	8.3 ± 7	0.97 ± 1.1	1.19 ± 0.06

knowledge, and the comments of the previous paragraph, suggest the use of several roughness characterization techniques in order to cover all dimensional scales, from micro to nano.

Polished and unpolished PLA and PLA/G5 have nontextured surfaces with nanometer roughness (Table 3). Consequently, as explained above, different surfaces will influence their biological response by the changes that roughness provokes in properties such as wettability.

3.2 Wettability

According to some authors, contact angle and work of adhesion (Wa) are the best wettability properties to predict the material–cell interactions at the initial stages of contact [34, 35]. Therefore, contact angle measurements have been performed to evaluate the hydrophilicity of the composite material. The G5-glass possesses a hydrophilic surface, so the incorporation of glass particles in the PLA matrix reduces the hydrophobic behavior of the polymer (Table 4). Thus, depending on the quantity of glass incorporated into the PLA/G5 composite material, the biomaterial surface wettability can be adjusted to obtain different degrees of hydrophilicity.

There is some debate about the affinity of proteins to hydrophobic surfaces. Some authors sustain the hydrophobic affinity theory [36], while others prefer the hydrophilic affinity theory [37]. The results obtained from studies carried out with culture medium supplemented with 10% fetal calf serum (FCS) suggested that the complex mixture of proteins present in FCS presented a higher affinity for the hydrophilic surfaces. Furthermore, the Wa values suggested that the mixture of proteins adsorbed better on hydrophilic surfaces, though the type of protein and their adsorption speed onto the surfaces is still unknown. However, the use of dynamic contact angle techniques (as has been confirmed with other materials) could help identify the velocity of adsorption and the number of steps of adsorption, desorption, and/or adsorption/desorption that lead to the final interaction between the substrate and the proteins in the culture medium (Fig. 4). The contact angles obtained for the two different fluids are shown in Table 5.

Table 4 Effect of the weight percent of G5 glass on the polished composite wettability (mean values ± standard deviation)

Composition	Contact Angle with distilled water (°)
0 wt % G5 glass	73.56 ± 1.50
20 wt % G5 glass	72.86 ± 1.60
50 wt % G5 glass	67.56 ± 1.70

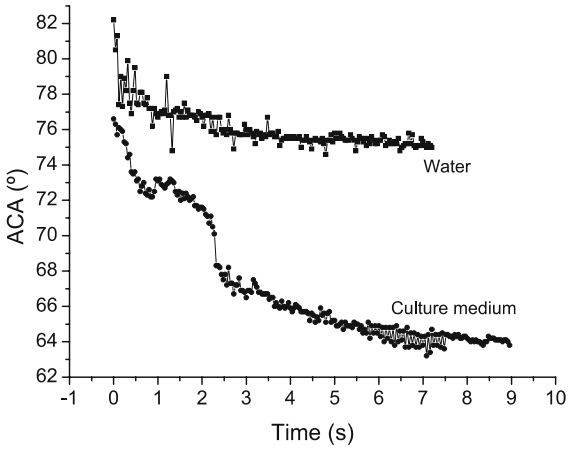

Fig. 4 Dynamic contact angles showing the different advancing contact angle (ACA) evolution during the time of interaction of the distilled water and the culture medium on titanium samples. The abrupt increase at $t \approx 1$ s and the abrupt decrease at $t \approx 2$ s of the ACA values indicate processes of adsorption, desorption or adsorption/desorption of proteins on the surface

Table 5 Contact angle values (°) at $t = 0$ s, with different fluids, on the surface of PLA, G5 glass, and the composite material (mean values ± standard deviation)

Material	Contact angle	
	Distilled water	Culture medium
PLA	73.59 ± 0.98	78.31 ± 0.84
PLA/G5	67.56 ± 1.71	68.82 ± 2.02
G5	29.80 ± 0.97	42.05 ± 1.76

The wettability of a surface is known to be affected by its topography, as discussed in the previous section. This statement has been corroborated in the case of the PLA/glass composite material (Table 6). Indeed, composite material specimens with a rough surface presented contact angle values significantly higher than the values reported for the polished materials. Thus, surface roughness leads to differences in the wettability of the surface. This change in the wettability behavior is dependant on the behavior of the ideally nonrough surface of the material studied [38]. For hydrophilic surfaces (contact angle < 90°), the higher the roughness, the lower the contact angle, which could be related to a more hydrophilic surface. For clearly hydrophobic surfaces (contact angle > 90°), the higher the roughness, the higher the contact angle, which could be related to a more hydrophobic surface. Never-

Table 6 Effect of roughness and sterilization on the composite wettability (mean values ± standard deviation) of PLA/G5

PLA/G5 material	Contact angle with distilled water [°]
Polished	67.56 ± 1.71
Rough	82.88 ± 4.03
Polished and sterilized	64.94 ± 1.79

theless, the limit value of 90° has been discussed and materials with contact angles close to 90° could not follow the general rule [39]. If the roughness is sufficiently high, the peaks of the roughness can retain fluid leading to metastable states of the drop that give a increasing value of contact angle. As a consequence, further studies must be made on the influence of roughness on wettability, which is a subject of special interest, as explained in previous sections.

On the other hand, the sterilization processes may somehow affect the surface structure of the material and, therefore, its wettability. In our case, ethylene oxide was chosen as the sterilization technique over autoclaving and gamma-irradiation, since this technique neither modifies the structure nor degrades the component materials of the PLA/G5 composite. The wettability results obtained for the sterilized materials showed a slight increment in the material hydrophilicity (Table 6). The mechanism by which the sterilization process modifies the material surface is still not clear. However, this may be due to a reaction between the sterilization agent and PLA, leading to a hydroxyl or similar group that would increase the hydrophilicity of the composite. For other materials studied in our laboratory, the changes in wetting behavior due to the sterilization treatment can be attributed to the changes that the small amount of contamination remaining on the biomaterial surface induces in the Lewis-basic component of the surface energy. Ethylene oxide sterilization changes the titanium surface from being an electron donor (nontreated) to bipolar (sterilized).

4 Protein Adsorption

Cell adhesion takes place in two different stages. The first stage consists of the adsorption of a layer of proteins that selectively adhere onto the biomaterial surface, and is completed in an interval from seconds up to a few minutes [40]. This is mainly mediated by the surface properties. The second stage involves cell adhesion onto the layer of proteins. This is a more complex process, mediated by extracellular matrix (ECM) proteins, cell mem-

brane proteins, and cytoskeletal proteins [41]. The cell membrane proteins, and in particular the integrins, interact with the layer of adsorbed proteins, the ECM proteins, and cytoskeletal proteins in order to promote the adhesion of cells to the materials. The interactions between ECM proteins and the integrin-receptor binding domains are of great importance since they have crucial effects on cell function. Indeed, the protein–integrin interactions can affect cell adhesion, motility, conformation, and differentiation. Thereby, the interactions between these proteins with the substrate and with the cells are of paramount importance [42].

Fibronectin, vitronectin, and type I collagen are some of the most representative ECM proteins involved in cell adhesion processes, therefore adsorption studies with these proteins and the PLA/G5 composite material have been performed. Preliminary studies have shown that all proteins adhere better to the G5 (the most hydrophilic material) than to the other materials. Vitronectin presented the best adhesion with PLA (the most hydrophobic material), and the PLA/glass composite presented an intermediate behavior. Further experiments are being conducted to evaluate the direct implication of the main proteins present in ECM to regulate cell proliferation and differentiation in the studied materials, and to obtain information on how the quality of the surface (physicochemical and topographical) influences the adsorbed protein layer.

5 Biological Behavior

In vitro models are the first approach used to understand the cell–substrate interaction and biocompatibility of the materials. Cell cultures are ideal systems for the analysis of a specific cell type under certain conditions because they avoid the complexity of the numerous variables involved in in vivo studies. It is not possible, however, to directly extrapolate in vitro results to in vivo results. Indeed, in a previous study performed with two CaP glass formulations with different solubilities, in vitro studies indicated that the differences in solubility affect cell cultures [23, 43]. However, in vivo, the differences in solubility were not evident and the two materials presented good biocompatibility.

Cell cultures performed with MG63 osteoblast-like human cells have indicated that the composite material is noncytotoxic and that the initial attachment of the cells to the PLA, G5, and PLA/G5 substrates, is better for the G5-glass (the most hydrophilic material) than for the other two materials, PLA (the most hydrophobic material) being the substrate with the lowest amount of attached cells. Besides, proliferation and differentiation assays have suggested that the most hydrophilic surface triggered the differentiation process earlier than the hydrophobic surfaces (Fig. 5). Furthermore, SEM

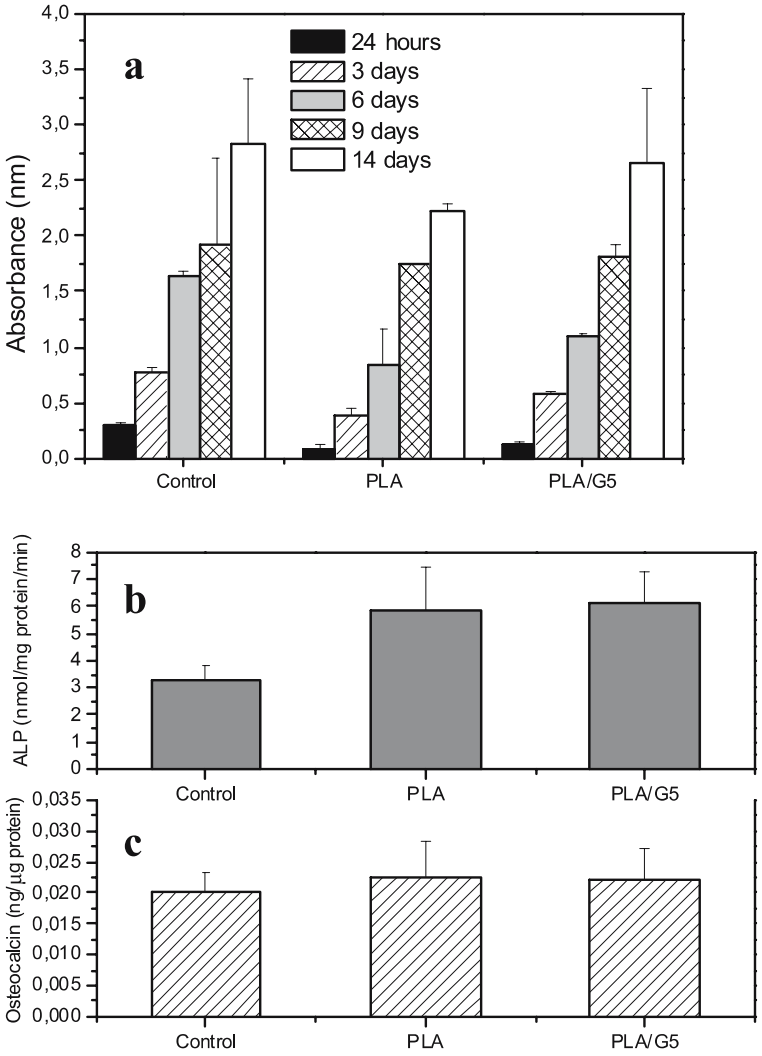

Fig. 5 **a** MTT results of the effect of the PLA and PLA/G5 composite material on MG63 cell proliferation. **b** Alkaline phoshatase activity (ALP) values of MG63 cells after 11 days of culture. **c** Osteocalcin values of the MG63 cells after 11 days of culture

images have shown significant differences in the morphology of the cells cultured on the substrates with flat or rough surfaces. PLA and G5 flat surfaces presented flat extended cells, while the composite rough material induced conformational changes in the cell cytoskeleton. These changes were mirrored in more rounded cells (Fig. 6).

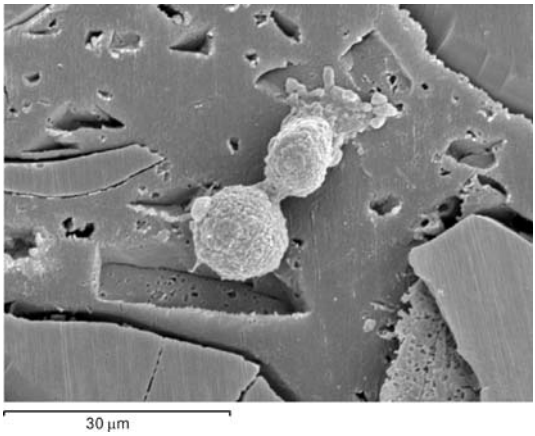

Fig. 6 MG63 osteoblast-like cells on a G5/PLA composite surface showing round shapes

6
Development of a Bioabsorbable Composite Scaffold

The composite material made of PLA and the G5-glass has been used to make scaffolds for tissue engineering. Tissue engineering can be briefly defined as the "… engineering of living tissues …" [44]. In other words, living cells are grown, either in vitro or in vivo, on degradable scaffolds. The scaffolds should offer:

1. A 3D highly porous interconnected network.
2. Adequate mechanical properties relative to the site of implantation and the cells' requirements.
3. Biocompatibility and bioresorbability.
4. A suitable surface quality – physical, chemical and topographical properties – for cell attachment [45]. Thus, the scaffolds should act as surrogate extracellular matrices until the cells create their own [46].

Various fabrication methods have been developed in order to attain the 3D scaffold characteristics. In the case of synthetic polymer or polymer-matrix composite scaffolds, the methods include [47]: solvent casting and particle leaching, phase separation, extrusion, gas foaming, and free form fabrication. Each method presents certain advantages with respect to others, ranging from ease of manufacture to control of the microstructure/nanostructure. Solvent casting and phase separation methods have been studied at our laboratory.

6.1
Solvent Casting

The solvent casting method was developed by Mikos et al. [48] amongst others for pure PLA, and several authors have used the method to manufac-

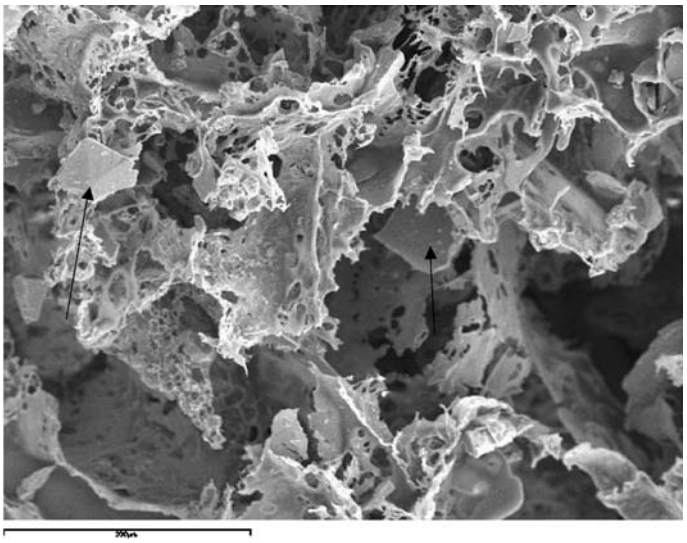

Fig. 7 SEM image of a composite scaffold produced by solvent casting and particle leaching. The black arrows indicate glass particles. The magnification bar corresponds to 200 μm

ture composite scaffolds [49–52]. The method consists of dissolving a polymer in a solvent, and adding particles of a leachable porogen: salt, glucose, etc. The mixture forms a thick paste, which is left to dry in air or under vacuum until the solvent has evaporated completely. The porogen is then dissolved in water by soaking the paste for several days, leaving behind a network of interconnected pores (Fig. 7). In the case of composites, the second phase (i.e., the glass particles) is added with the porogen and remains within the structure after the porogen is leached out. The advantage of the solvent casting method is that it is a simple and fairly reproducible method which does not require sophisticated apparatus. The disadvantages include thickness limitations intrinsic to the particle leaching process and limited mechanical properties. Further, some authors question the homogeneity and interconnection of the pores in the scaffolds, as well as the presence of residual porogen [53]. As with the solid composite material, the addition of glass particles is meant to increase bioactivity and reinforce mechanical properties.

6.1.1 Macroporosity

The morphology, magnitude, and interconnection of the scaffolds' porosity are critical factors in assessing their viability as tissue engineering devices. The structure of the scaffolds and their porosity should transmit the cues for

cell adhesion, proliferation, and differentiation, as well as allowing the delivery of nutrients and waste products. It is thus very important to quantify the porosity and to understand which factors play an important role in its tailoring.

A typical solvent cast scaffold is manufactured with approximately 90 wt % of porogen, which produces between 85 and 95% porosities. The influence of scaffold composition on its macroporosity was studied thoroughly at our laboratory using NaCl as a porogen (unpublished data). The magnitude of the porosity is mainly influenced by the wt % of NaCl particles, whereas the pore morphology is chiefly affected by the NaCl particle size (Fig. 8). Neither the wt % nor the size of the G5-glass particles affected the porosity of the composite scaffolds. The interconnection of the pores becomes obvious at high porosities, and is implicit to the particle leaching method if no NaCl remains. The solvent casting method produces a very homogeneous distribution of the glass particles, as can be seen in (Fig. 9).

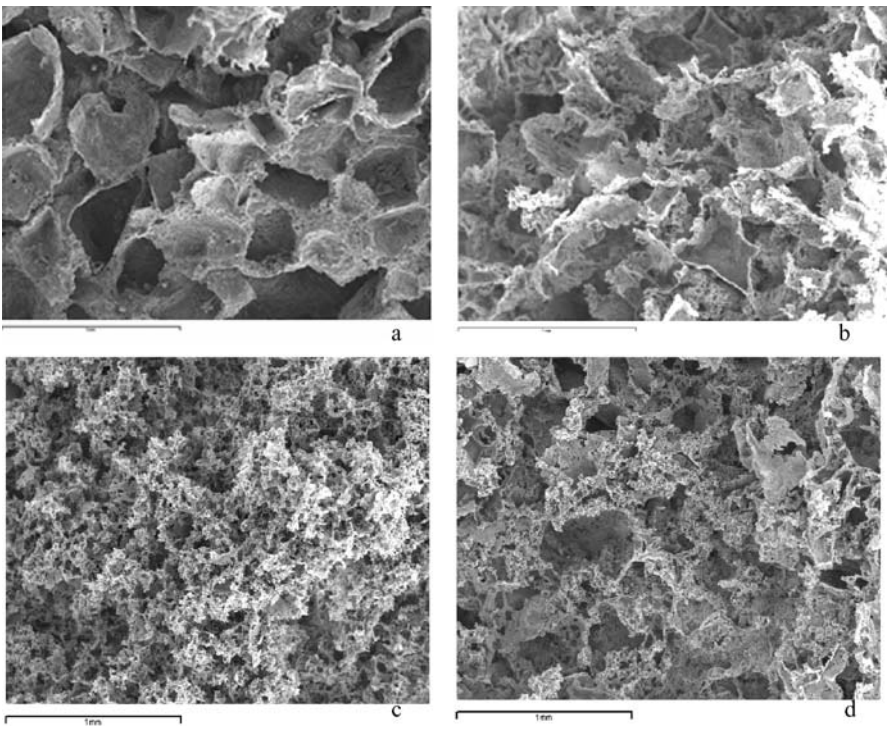

Fig. 8 SEM images of composite scaffolds made by solvent and casting illustrating the effects of changing porogen particle size and weight percent on the porosity. **a** 75 wt % and large particle size, **b** 94 wt % of porogen and large particle size, **c** 94 wt % and small particle size, and **d** 94 wt % of porogen and large particle size. All magnification bars correspond to 1 mm

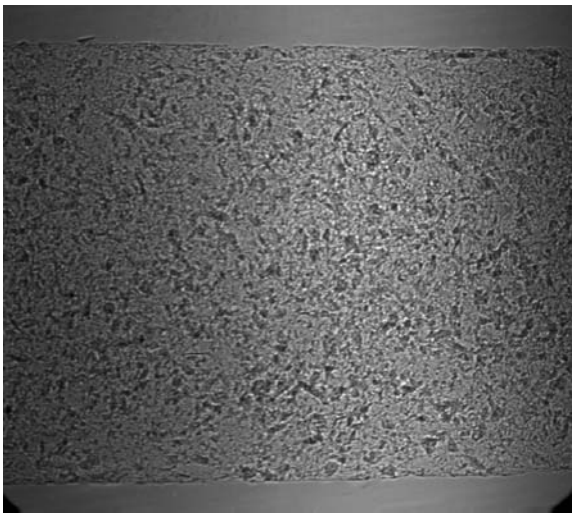

Fig. 9 Synchrotron radiation X-ray projection of a scaffold made by solvent casting. The image reveals the homogeneity of the glass particle distribution. The height of the sample seen in the image is approximately 1 mm and the length seen in the image is approximately 1.5 mm

6.1.2 Mechanical Properties

The mechanical properties of the scaffolds are usually measured by performing compression tests. For scaffolds with 85–95% porosities, stiffness ranged between 100 and 150kPa, yield stresses ranged between 25 and 35kPa, and yield strains ranged between 15 and 60%. Similar values for stiffness are reported in the literature for these porosity levels [54, 55]. Yield properties, however, are often not reported or are poorly defined, and are thus difficult to compare.

The stiffness of the scaffolds decreases as their porosity and wt % of the G5-glass phase increases. The negative effect of the porosity is logical since a higher porosity means less material is supporting the compressive force. The effect of the G5 particles on stiffness may seem surprising, though it is in accordance with composite material mechanics, in which an increase in Young's modulus is mainly attained by introducing a reinforcing phase in the form of fibers, not particles. This effect may be suppressed by improving the adhesion between the PLA matrix and the glass particles.

The glass phase does, however, reinforce the scaffolds' yield properties significantly (Fig. 10). The yield properties are perhaps the most critical mechanical properties because they guarantee the integrity of the macroporous network, which is vital for the cells.

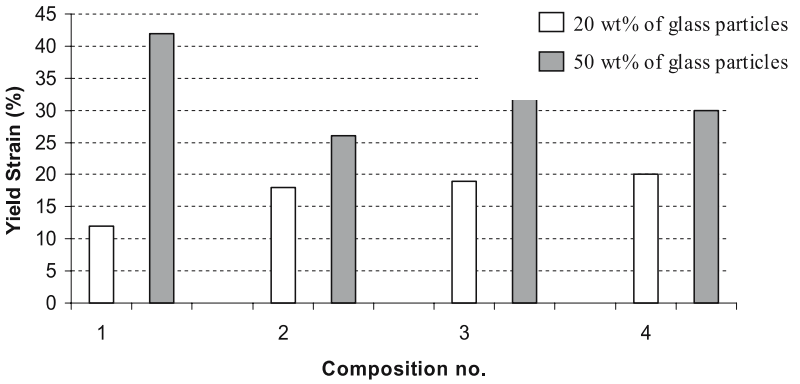

Fig. 10 Differences between yield strain for scaffolds with 20 and 50 wt % of glass particles. The size of the porogen and glass particles varies between compositions 1–4

As a consequence of these results and those of the previous section, the wt % of G5-glass particle can be increased to improve yield properties and potential bioactivity of the scaffolds, without affecting the scaffold's macroporosity.

6.2 Phase Separation

The phase separation technique may prove to be a useful alternative for manufacturing composite biodegradable scaffolds with specific properties. Phase separation of polylactide solutions was first developed by Schugens et al. [56, 57] to produce PLA scaffolds. Later, several authors applied this technique to composite scaffolds [58–61], and have even combined it with solvent casting [62]. The method consists of inducing a solid–liquid or liquid–liquid phase separation of a polylactide solution. The polymer is dissolved in a solvent, often dioxane, and quenched at a certain temperature ranging from 0 °C to – 196 °C. The solutions are finally freeze-dried for several days at around 10^{-2} Torr. This method creates a very distinct microstructure (Fig. 11), which can be controlled by varying certain processing parameters such as the quenching temperature, the freeze-drying temperature, and the polymer concentration.

Preliminary studies using phase separation for fabrication of G5/PLA scaffolds show promising results in certain critical aspects. The glass particle in Fig. 12 seems entrapped within the scaffold's microstructure and may contribute to its stiffness. The relative anisotropy of the microstructure may be exploited for specific applications such as nerve regeneration [56]. Furthermore, the phase separation method creates both macro- and microporosity, which would enable cell adhesion in the macropores, and allow the infiltra-

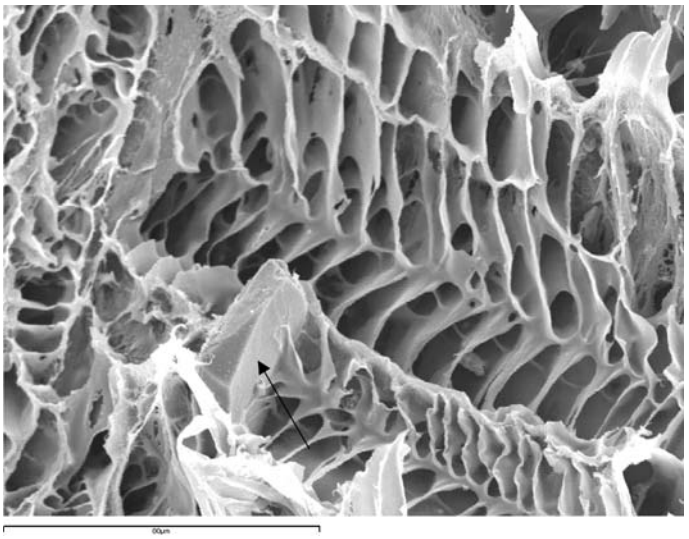

Fig. 11 SEM image of a composite scaffold produced by phase separation. The black arrow indicates a glass particle. The magnification bar corresponds to 80 μm

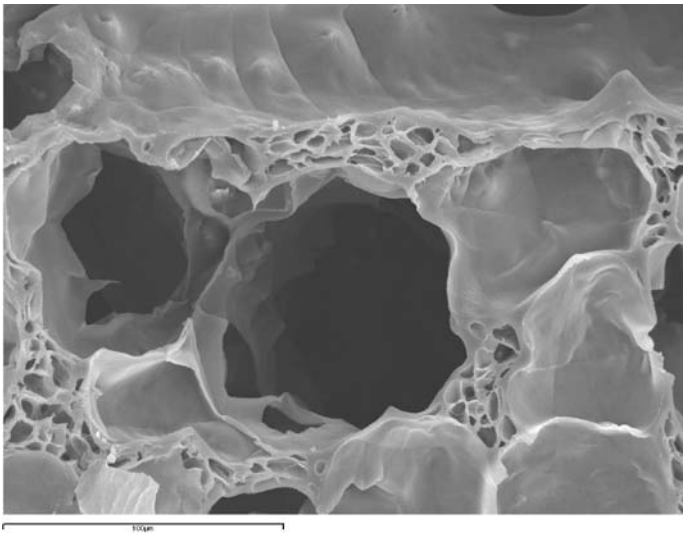

Fig. 12 SEM image of a phase separated scaffold. Two distinct pore sizes can be observed. The magnification bar corresponds to 100 μm

tion of vital blood vessels through the micropores (Fig. 12). From a topographical point of view, the phase separation technique also provides interesting results. Apart from controlling the macrostructure and porosity, the

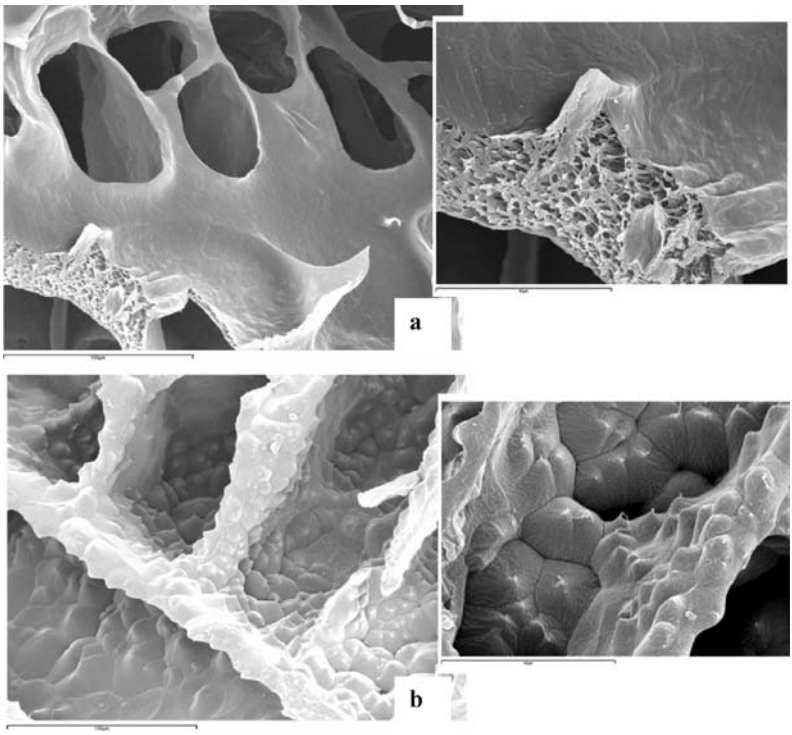

Fig. 13 SEM images of scaffolds prepared by phase separation with different topographical features. **a** Shows a scaffold with a relatively smooth surface even at high magnifications (image to the right). A microstructure within the pore walls is also visible. **b** Shows a scaffold with a distinct microporosity and nanosize wave-like features. The magnification bars of the images to the left correspond to 100 μm, those on the images to the right correspond to 40 μm

processing conditions produce a variety of micro- and nanotopographical features (Fig. 13). As explained before, taking the advantages related to both micro- and nanotopographical roughness, the cell behavior could be adequately affected and even effectively tailored.

7 Conclusion

The development of scaffolds made of a biodegradable composite (PLA/calcium phosphate glass) for bone tissue engineering applications is of major interest as an alternative to existing bone grafts, and is being pursued at our laboratory. The degradability, mechanical properties, and quality of the porosity of these scaffolds have been thoroughly characterized. Moreover,

surface properties such as topography, surface energy, and wettability in different dimensional scales were measured in order to correlate them to the biological response of the constructs. The interaction between the synthetic material and biological entities (proteins and cells) is the key issue in determining the success of the potential scaffold. Thus, control of the material's surface quality by means of the fabrication process is our main challenge in this exciting field of research.

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