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Acrylic scaffolds with interconnected spherical pores and controlled hydrophilicity for tissue engineering

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Polymer scaffolds are obtained in which the geometric characteristics (pore size, connectivity, porosity) and the physico-chemical properties of the resulting material can be controlled in an independent way. The interconnected porous structure was obtained using a template of sintered PMMA microspheres of controlled size. Copolymerization of hydrophobic ethyl acrylate and hydrophilic hydroxyethyl methacrylate comonomers took place in the free space of the template, different comonomer ratio gave rise to different hydrophilicity degrees of the material keeping the same pore architecture. The morphology of the resulting scaffolds was investigated by scanning electron microscopy (SEM), the porosity of the material calculated, and the mechanical properties compared with those of the bulk (non porous) material of the same composition.

1. Introduction

Tissue engineering is a field in rapid expansion [1]. Scaffolds are used as cellular culture supports; for example, chondrocyte cells are seeded and cultured *in vitro* and later implanted in the damaged area [2–6]. In this technique, the scaffold must perform a number of critical functions for cellular growth to take place. First of all, it should provide the cells with a suitable surface for attachment [7, 8]. The hydrophilic/hydrophobic balance in the material influences the adherence of cells to the scaffold walls, which is the first step in the cellular growth process. When cells are cultured *in vitro*, an adequate chemical environment must be provided as to keep the differentiated cell function, and this depends also on the surface properties of the scaffold. Surface free energy, electric charge and morphology may all affect cell attachment and its behaviour either indirectly, e.g., by controlling the adsorption of proteins present in the cultured medium, or directly, e.g., by guiding cell spreading with adequate surface topography [9–13]. In this sense it is found that cell adsorption is relatively large on hydrophobic surfaces and small on more hydrophilic ones [9, 13]; however, cell culture has also been performed on hydrophilic materials [14]. Improved cytocompatibility in terms of cell adhesion is described when surface hydrophobicity of the material increases [15], or even when the hydrophilic material is mechanically reinforced with an inorganic phase [16].

This work is focused on non-biodegradable scaffolds. Although many times tissue engineering is associated with the idea of *in vivo* tissue regeneration, where new tissue is growing and, at the same time, the scaffold is degrading there are applications where non-biodegradability is required, and they are not scarce. One of the possible application fields of these permanent polymer sponges is the anchoring ring of a cornea prosthesis. The cells of the host stroma are expected to grow into the pore structure of the scaffold and fix the synthetic lens avoiding its extrusion [17, 18]. Nerve regeneration is a promising field where permanent scaffolds are used *in vivo* [19, 20]; however the structures developed in this work are focused on *in vitro* cultures. Here also non - degradability is no inconvenience, since the scaffold must provide a permanent environment for the adequate phenotype development, and later the cultured cells are recollected and transplanted.

On another hand, the three-dimensional (3D) scaffold construct must have an interconnected porous structure in order to allow the cell development through all the network, maintain their differentiated function, as well as to allow the entry and exit of nutrients and metabolic waste removal [2, 21, 22]. The scaffold 3D structure can be obtained by different methods known to generate a porous structure in a polymer matrix: using gases [23], fiber templates [24], employing water soluble particles such as NaCl as porogen [25],

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solvent casting [26], polymerization in the presence of a solvent [27] and others [28–31]. In this work we have adapted the method based on the use of templates for generating the porous structure. A bonded micro-sphere template was first built and then dissolved after the polymerization process of the forming material in the free spaces of the template. This technique allows controlling the interconnectivity between pores and their size. There are only a few examples in literature where the 3D architecture can be controlled in such a way, it could be mentioned scaffolds obtained by rapid prototyping technologies [2, 32, 33] and, recently, those fabricated using a technique similar to that developed by us here but focused on biodegrable materials [34–36].

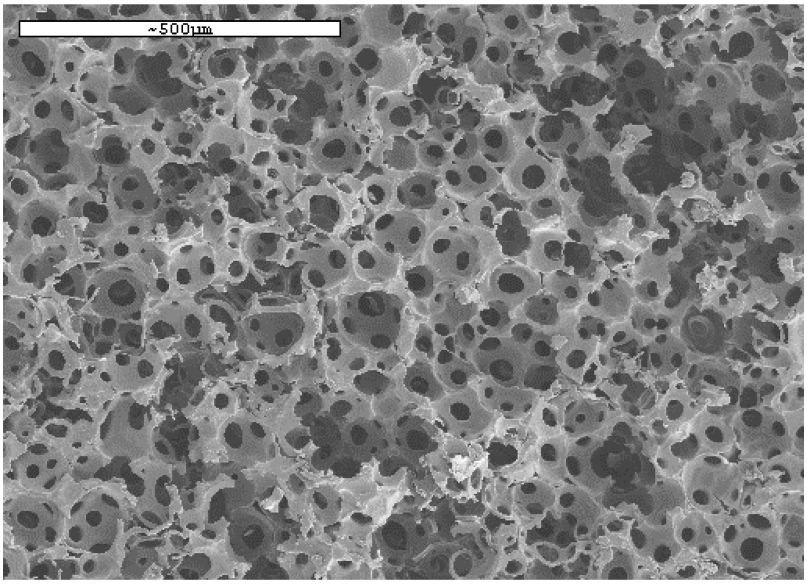
2. Materials and methods

2.1. Materials

Poly(methyl methacrylate) microspheres of known size, 90 ± 10 μm, (PMMA Colacryl dp 300) were used as porogen. Ethyl acrylate (EA) and hydroxyethyl methacrylate HEMA (Scharlau 98% pure) were used as monomers, benzoin (Scharlau 98% pure) as photoinitiator and ethyleneglycol dimethacrylate, EGDMA (Aldrich 99% pure) as crosslinking agent.

2.2. Preparation of the scaffolds

PMMA spheres were introduced between two plates and sintered by keeping temperature at 180 °C for one hour at a constant pressure. After cooling the template at room temperature, a monomer solution was introduced in the empty space between the PMMA spheres. A wide range of hydrophilic/hydrophobic materials were prepared by changing the percentage of EA and HEMA in the original solution; besides 1 wt% of benzoin and 2 wt% of EGDMA was always added to the corresponding monomer solution. The copolymerization was carried out up to limiting conversion under a UV radiation source at room temperature. Five monomer feed



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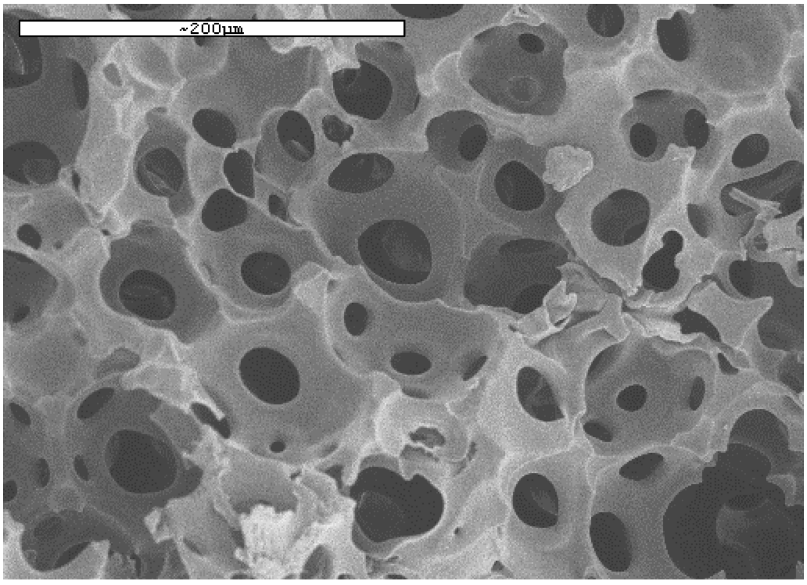


(b)

Figure 1 SEM micrographs of hydrophobic PEA scaffolds at different magnifications.

compositions were chosen, given by the weight fraction of HEMA in the original mixture of 1, 0.7, 0.5, 0.3 and 0 (EA). After polymerization took place, the PMMA matrix was removed by soxhlet extraction with ethyl acetate during 24 h. After this stage the PMMA template is completely removed. The porous sample is kept 24 h more in a soxhlet with ethanol in order to remove completely low molecular weight substances. Samples were dried in vacuo to constant weight before characterization.

2.3. Characterization of the scaffolds

The volume fraction of pores in the scaffold, porosity, was determined gravimetrically by swelling the sample in water using a vacuum accessory. The porosity P is defined as

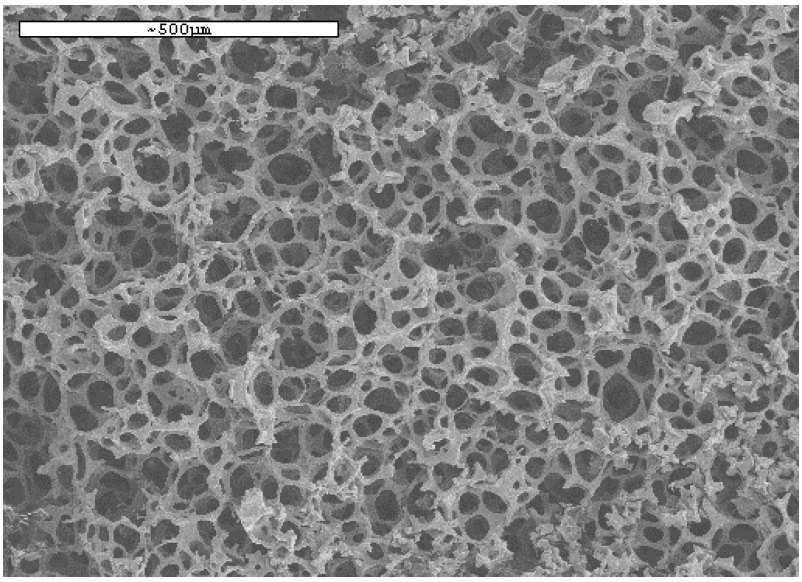
$$P = \frac{V_{\text{pore}}}{V_{\text{pore}} + V_{\text{polymer}}} \tag{1}$$

where V_{pore} is the part of the volume occupied by pores and V_{polymer} is the volume occupied by the polymer. Let m_{s}^{sw} be the mass of the scaffold swollen in water and m_{s}^{d} the mass of the dry scaffold. Water sorbed in the scaffold is distributed between two phases: water in pores and water sorbed in the polymer that forms the scaffold. Assuming that the equilibrium water content measured on dry basis (mass of water absorbed in equilibrium divided by the mass of dry polymer), w^*, of the material that constitutes the scaffold is the same as that of the bulk material of the same composition, the mass of water located in pores $m_{\text{w}}^{\text{pores}}$ is

$$m_{\text{w}}^{\text{pores}} = m_{\text{s}}^{\text{sw}} - m_{\text{s}}^{\text{d}} - m_{\text{w}}^{*}, \tag{2}$$

where m_{w}^{*} is the mass of water absorbed in the polymer that forms the scaffold, i.e.,

$$m_{\text{w}}^{*} = m_{\text{s}}^{\text{d}} \cdot w^{*} \tag{3}$$



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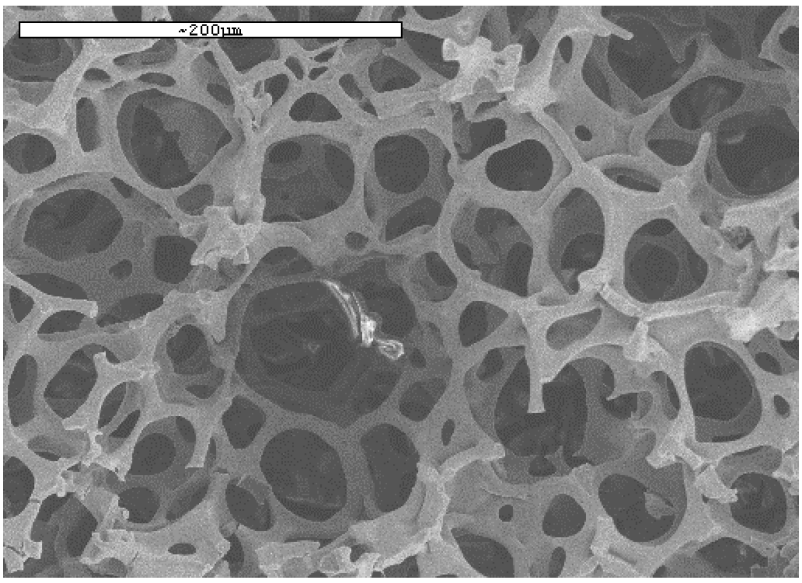


(b)

Figure 2 SEM micrographs of EA/HEMA copolymer scaffolds (30% HEMA) at different magnifications.

taking into account the density of water ρ_w, the amount of water located in pores gives their volume,

$$V_{pore} = \frac{m_s^{sw} - m_s^d(w^* + 1)}{\rho_w}. \quad (4)$$

The mass of water absorbed in equilibrium by the bulk polymer divided by the mass of the dry polymer, w^*, is given in Table I, as well as w_s^*, the mass of water (both pores and polymer) per mass of dry scaffold.

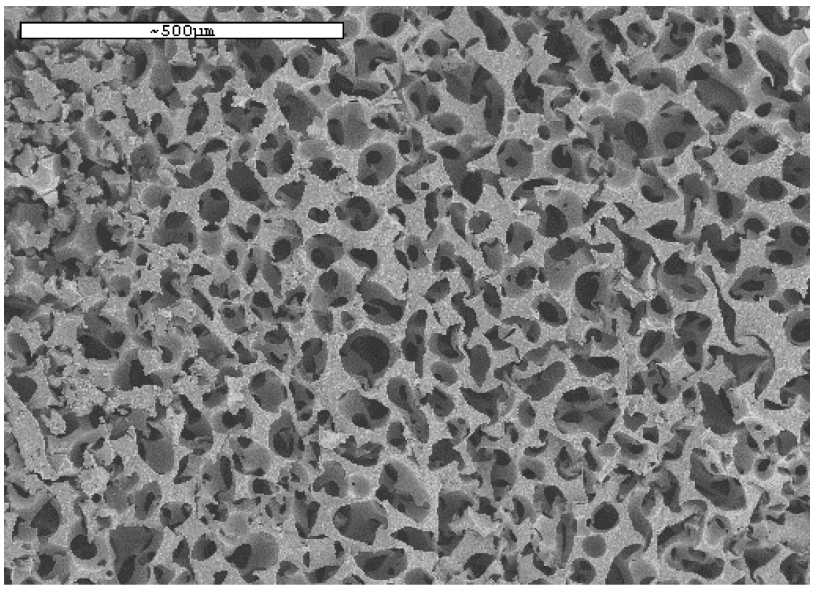
On the other hand, the volume of the scaffold occupied by the polymer can be obtained by measuring the density of the corresponding bulk material ρ_b

$$V_{polymer} = \frac{m_s^d}{\rho_b} \quad (5)$$

ρ_b was determined by weighing each one of the samples both in air and immersed in *n*-octane at 25 °C. A Mettler AE240 balance (sensitivity 0.01 mg) with density accessory Mettler ME3360 was employed. Porosity measurements were done at least in three different samples of each one of the compositions; porosity values were reproducible up to 3% and are shown in Table I.

TABLE I Density and equilibrium water content w^* (the mass of water absorbed in equilibrium by the polymer divided by the mass of the dry polymer) of the bulk-copolymerized systems and of the scaffolds w_s^*. The porosity of the scaffolds with different compositions is shown in the last column

Copolymer compositon	Bulk density (g/cm^3)	w^*	w_s^*	Porosity (%)
PEA	1.13	0.007	2.25	78 ± 2
30% HEMA	1.17	0.06	2.50	83 ± 2
50% HEMA	1.18	0.13	2.80	81 ± 1
70% HEMA	1.19	0.19	3.30	76 ± 3
PHEMA	1.21	0.35	4.50	75 ± 2



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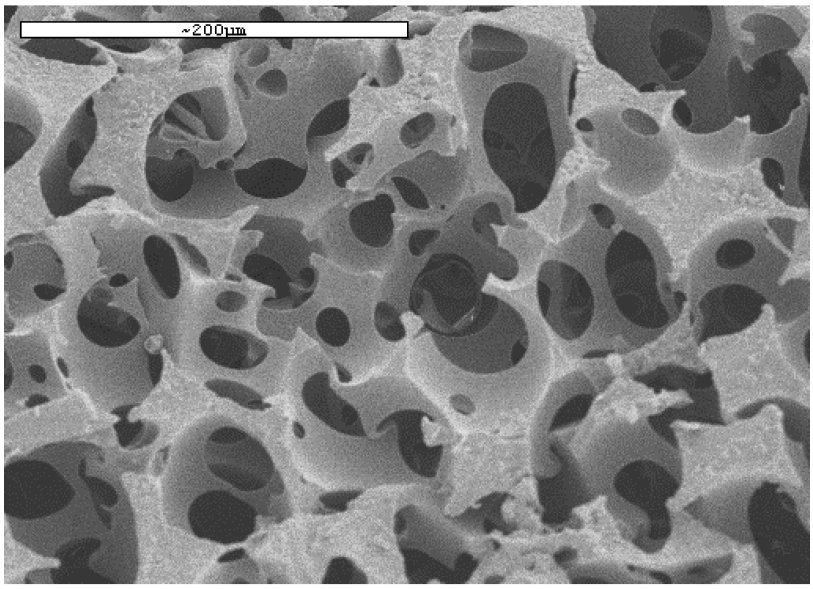


(b)

Figure 3 SEM micrographs of hydrophilic PHEMA scaffolds at different magnifications.

Tensile dynamic-mechanical spectroscopy, DMS, was performed at a heating rate of 1 °C/min in a Seiko DMS210 instrument from −150 to 200 °C at a frequency of 1 Hz. Samples for DMS experiments were rectangular approximately 15 × 3 × 1 mm^3.

Scanning electron micrographs SEM were performed in a Hitachi S-3200N device. Transversal and longitudinal slices of the dry samples were coated with gold before observation at 15 kV.

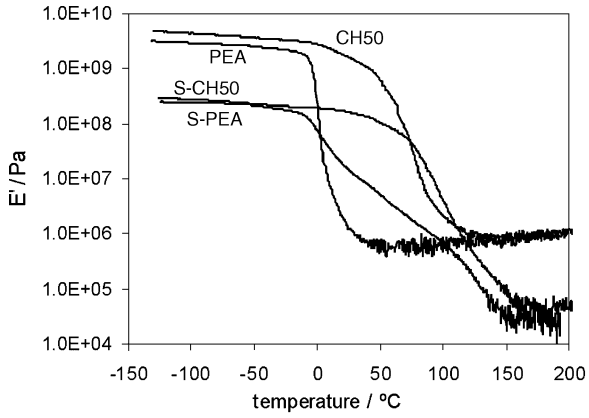
3. Results and discussion

The methodology employed in this work permitted to obtain a macroporous structure of interconnected spherical pores in a whole range of composition from pure hydrophilic PHEMA to pure hydrophobic PEA. PHEMA is a biocompatible material that has been used in a wide variety of biomedical applications such as ophthalmologic prostheses, vascular prostheses, drug delivery system and soft-tissue replacement [37]. When HEMA is polymerized the resulting material is hard and glassy with a glass transition temperature around 90 °C [38]. When swollen in water it becomes a soft and flexible rubber. The copolymerization with a hydrophobic component plays a double role. On the one hand mechanical reinforcement is obtained in the swollen state and, on the other hand, copolymerization allows to optimize the desired hydrophobicity of the material with a specific application in mind. The molecular structure of the system obtained after copolymerization of EA and HEMA has been previously studied in detail by us [38]; there it was concluded that the material, even though bulk copolymerized from the monomer mixture, consists of a distribution of nano-aggregates of alternating HEMA-rich and pure hydrophobic domains, a structure that, in principle, can favour both cell adhesion and diffusion of nutrients [39].

SEM micrographs of the resulting scaffolds (Figs. 1–3) show the 3D spherical interconnected porous network. The same geometrical structure can be obtained with a broad range of compositions, from pure hydrophobic PEA (Fig. 1) to pure hydrophilic PHEMA (Fig. 3); the average diameter of the pores, between 65–85 μm, does not depend on chemical composition of the material but only on the size of the PMMA spheres used as a template. This is the reason why the pore size and connectivity could be easily modified changing both the sphere diameter and the sintering process: changing the temperature and pressure the contact between the spheres can be regulated, what results in a controlled variation of the porous structure of the polymer scaffold (Fig. 2). This fact has already been proved with paraffin spheres [35].

Porosity of the scaffolds is shown in Table I. Volume fraction of pores is around 80% independently of the chemical composition of the material, i.e. keeping the same percentage of pores, the hydrophilic/hydrophobic ratio of the material of the scaffold can be changed in the whole range. Such a high porosity has a reflection in the lowering of the mechanical properties of the porous systems when compared with those of the same bulk material. Dynamic mechanical spectroscopy of the copolymers scaffolds compared with those obtained in the bulk copolymerized systems shows that the rubbery modulus is much lower in the porous systems (Fig. 4(a)). Besides, since the rubbery modulus depends very sensitively on the geometric architecture of the porous solid [40], the fact that the same reduction with respect to the bulk is obtained for different copolymer compositions of the scaffolds supports the hypothesis that the same pore distribution and interconnectivity is obtained for the different copolymer compositions.

From a more fundamental point of view it is noteworthy that the main relaxation of the material polymerized in presence of the PMMA template presents a broader relaxation than the corresponding bulk polymer. The effect is also independent of composition and takes place both in pure systems (PEA, PHEMA) and copolymers (Fig. 4). This broadening suggests a different molecular architecture of the porous material from that of the bulk polymer, and must be related to the much higher surface to volume ratio in the material polymerized in the free spaces of the template. In general the surface free energy contribution to the modulus is non-negligible in materials with high porosity. This alone can account for differences in the mechanical properties of porous and bulky samples of the same material composition. Besides that, in our case one should also take into account that the material in the immediate vicinity of the pore surfaces has been polymerized out of monomer units which might have penetrated to some extent the PMMA



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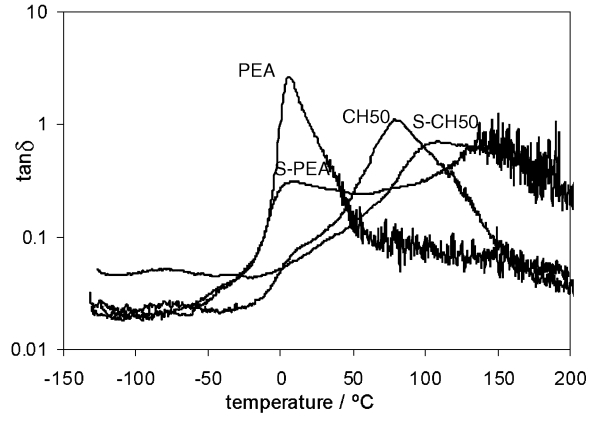

(b)

Figure 4 Dynamic-mechanical relaxation spectra of bulk (PEA) and porous (S-PEA) PEA and the copolymerized systems with 50% HEMA (bulk CH50, scaffold S-CH50) measured at 1 Hz. (a) E'. (b) Mechanical loss tangent.

spheres. Thus, the surface layer of the matrix network may have a different structure from the network in the bulk region of the material. These facts would broaden the dynamics of the system and the regions of the material able to undergo cooperative motions presents a broader distribution of sizes that results in a broader relaxation process.

4. Conclusions

We have applied a methodology that permits to obtain scaffolds with 3D interconnected pores and independently controlled pore size and hydrophilicity. Highly periodic and regular pore architectures can be obtained in this way. The mechanical behaviour of the porous samples is significantly different from that of the bulk material of the same composition: not only is the modulus lower, but also there are indications of a distinct relaxational behaviour due to the effect of the surface layer.

Acknowledgements

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