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RESEARCH ARTICLE

Starch–poly(ε-caprolactone) and starch–poly(lactic acid) fibre-mesh scaffolds for bone tissue engineering applications: structure, mechanical properties and degradation behaviour

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Abstract

In scaffold-based tissue engineering strategies, the successful regeneration of tissues from matrix-producing connective tissue cells or anchorage-dependent cells (e.g. osteoblasts) relies on the use of a suitable scaffold. This study describes the development and characterization of SPCL (starch with ε-polycaprolactone, 30 : 70%) and SPLA [starch with poly(lactic acid), 30 : 70%] fibre-meshes, aimed at application in bone tissue-engineering strategies. Scaffolds based on SPCL and SPLA were prepared from fibres obtained by melt-spinning by a fibre-bonding process. The porosity of the scaffolds was characterized by microcomputerized tomography (μCT) and scanning electron microscopy (SEM). Scaffold degradation behaviour was assessed in solutions containing hydrolytic enzymes (α-amylase and lipase) in physiological concentrations, in order to simulate *in vivo* conditions. Mechanical properties were also evaluated in compression tests. The results show that these scaffolds exhibit adequate porosity and mechanical properties to support cell adhesion and proliferation and also tissue ingrowth upon implantation of the construct. The results of the degradation studies showed that these starch-based scaffolds are susceptible to enzymatic degradation, as detected by increased weight loss (within 2 weeks, weight loss in the SPCL samples reached 20%). With increasing degradation time, the diameter of the SPCL and SPLA fibres decreases significantly, increasing the porosity and consequently the available space for cells and tissue ingrowth during implantation time. These results, in combination with previous cell culture studies showing the ability of these scaffolds to induce cell adhesion and proliferation, clearly demonstrate the potential of these scaffolds to be used in tissue engineering strategies to regenerate bone tissue defects. **Copyright © 2008 John Wiley & Sons, Ltd.**

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1. Introduction

Most of the tissue engineering strategies developed for the creation of hard tissue (such as bone and cartilage) substitutes relies on the use of a temporary three-dimensional (3D) scaffold material within which cells are seeded and cultured *in vitro* prior to implantation. In this type of strategy, the formation of new tissue is deeply influenced by the 3D environment provided by the scaffolds, i.e. its composition, porous architecture and, of course, its biological response to implanted cells and/or surrounding tissues. In order to meet all the necessary requirements, scaffold materials must be fabricated from

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polymers with adequate properties, but many of the scaffolds' features are also dictated by the processing methodology used to fabricate them. Biocompatibility is the first obvious demand, but an ideal tissue engineering scaffold should exhibit appropriate mechanical properties (Thomson *et al.*, 1995; Freed, 1998; Kim and Mooney, 1998; Vacanti and Bonassar, 1999; Chapekar, 2000; Middleton and Tipton, 2000; Atala, 2007; Hutmacher *et al.*, 2007) and a suitable degradation rate (Thompson *et al.*, 1995; Thomson *et al.*, 1995; Kim and Mooney, 1998; Chapekar, 2000; Hutmacher, 2000; Middleton and Tipton, 2000; Hutmacher *et al.*, 2007). Furthermore, the scaffold must possess adequate porosity, interconnectivity and permeability to allow the ingress of cells and nutrients (Thomson *et al.*, 1995; Kim and Mooney, 1998; Chapekar, 2000; Hutmacher, 2000; Hutmacher *et al.*, 2007) as well as the appropriate surface chemistry for enhanced cell attachment and proliferation (Freed *et al.*, 1994; Kim and Mooney, 1998; Langer, 1999; Chapekar, 2000; Atala, 2007; Hutmacher *et al.*, 2007).

The recent development of several different polymers and processing methodologies enabled the creation of scaffolds with a wide range of structures and geometries, as well as mechanical properties and degradation profiles, among other characteristics. Fibre-bonding methodologies produce fibre-mesh scaffolds that consist of individual fibres, either woven or knitted, into 3D patterns of variable pore size (Thompson *et al.*, 1995, 1997; Lu and Mikos, 1996; Maquet and Jerome, 1997; Langer, 1999). Fibre-meshes usually exhibit a large surface area for cell attachment which also enables rapid diffusion of nutrients enhancing cell survival and growth (Thompson *et al.*, 1995, 1997; Lu and Mikos, 1996; Maquet and Jerome, 1997; Langer, 1999). This, of course, results from a high interconnectivity among pores, which contrasts with the difficulty in controlling accurately the porosity (Thompson *et al.*, 1995; Lu and Mikos 1996; Maquet and Jerome 1997; Thompson *et al.*, 1997). These features make this a very attractive method for the production of scaffolds for tissue engineering.

Fibre-bonding methods include a great variety of processing methods that involve the knitting or physical bonding (by means of casting or compression procedures) of fibres prefabricated by wet or dry spinning from polymeric solutions or by melt spinning. Fibre-meshes may also be obtained in single-step methods, such as electrospinning.

Several studies demonstrate that scaffolds obtained by fibre-bonding processes have adequate structure for use in tissue-engineering strategies that use bioreactor cultures, probably because they provide highly interconnected porosity that enables hydrodynamic microenvironments to be created, with minimal diffusion constraints, that closely resemble natural interstitial fluid conditions *in vivo*, allowing large and well-organized cell communities to be achieved. In contrast, most of the porous structures obtained with other methodologies exhibit lower interconnectivity, which is very likely to generate complex fluid flow pathways through the scaffolds and does not allow for the distribution of cells throughout the whole construct.

In this work we report the development and characterization of the porous structure, degradation profile and mechanical properties of fibre-meshes based on SPCL (a blend of starch with polycaprolactone, 30 : 70% w/w) and SPLA (a blend of starch with polylactic acid, 30 : 70% w/w), obtained by a fibre-bonding process. In previous studies, these scaffolds have shown great potential for applications involving the regeneration of bone and cartilage using *in vitro* tissue-engineering strategies.

2. Materials and methods

2.1. Production of the scaffolds

Polymeric fibres based on SPCL [a 30 : 70% w/w blend of starch with poly(ε-caprolactone)] and on SPLA [a 30 : 70% w/w blend of starch with poly(lactic acid)] fibres were processed by melt spinning. An extruder equipped with a 12 mm diameter screw was used with a 0.5 mm monofilament die. Die temperature was 150 °C and the screw speed 1 r.p.m. Extrusion was performed in the ambient environment. Hot fibre was driven into a cooling bath (water 13 °C) and cold-drawn after the bath, using a caterpillar with a speed of 21 m/min and a winding unit with a speed of 28 m/min. Fibres were produced in the range 120–500 m diameter (SPCL) and ~150 m (SPLA).

Fibre-meshes scaffolds were prepared by a fibre-bonding process consisting of cutting and sintering the fibres previously obtained by the above-described melt-spinning method. Briefly, a selected amount of fibres was placed in a glass mould and heated in an oven at 150 °C (SPCL) or 190 °C (SPLA); immediately after removing the moulds from the oven, the fibres are slightly compressed by a Teflon cylinder (which runs within the mould) and then cooled at −15 °C. All samples were cut into discs of approximately 8 mm diameter and 1.5–2 mm height and sterilized using ethylene oxide.

2.2. Materials characterization

2.2.1. Porosity and porous structure

The porosity of the scaffolds was determined by microcomputerized tomography (CT; ScanCo Medical CT 80, Bassersdorf, Switzerland) at a resolution of 10 m, using at least three samples/group (of different porosity). The morphology of the porous structure was further characterized using a scanning electron microscope (Leica Cambridge S360; Leica Cambridge, UK), after sputter-coating the samples with gold (Jeol JFC 1100; Jeol, USA).

The SEM analysis allowed the morphology, size and distribution of the pores and the interconnectivity between the pores to be evaluated.

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2.2.2. Mechanical properties

The materials were mechanically tested by compression experiments in an Instron 4505 universal mechanical testing machine, using a load cell of 50 kN. Compression testing was carried out at a crosshead speed of 2 mm/min (4.7×10^{-5} m/s), until obtaining a maximum reduction in samples height of 60%. The materials were tested in the dry and wet states after 3 days of immersion in PBS. A minimum of six samples (of about 7 mm diameter and 5 mm height) of each type/condition was tested.

2.3. Degradation behaviour

The enzymatic degradation behaviour of SPCL and SPLA scaffolds was assessed by individually immersing the pre-weighed scaffolds in 10 ml phosphate-buffered saline (PBS, 0.01 M, pH 7.4) solution containing 115 U/L lipase (EC 3.1.1.3, from *Pseudomonas* sp., Sigma) and/or α-amylase (EC 3.2.1.1, from *Bacillus* sp., Sigma) at concentration of 145 U/l and incubated at 37 °C with constant shaking at 60 r.p.m. for different periods of time. A control in PBS alone was also performed. The degradation conditions used in the degradation studies are summarized in Table 1. All the prepared solutions were sterilized using a 0.2 m syringe filter and kept at 4 °C until further usage.

For each condition, a minimum of five samples was tested. All solutions were changed weekly and the solution pH was monitored. At the end of each degradation period, the samples were removed from the solution, rinsed with distilled water and weighted, to determine the water uptake, according to equation (1); one batch of samples was then dried to exhaustion (3 days at 37 °C) in order to determine the dry weight loss, using equation (2):

$$\%\ \text{Water uptake} = \frac{\text{final wet weight} - \text{initial weight}}{\text{initial weight}} \times 100\% \quad (1)$$

$$\%\ \text{Weight loss} = \frac{\text{initial weight} - \text{final dry weight}}{\text{initial weight}} \times 100\% \quad (2)$$

2.3.1. Characterization of the degraded samples and degradation products

Scanning electron microscopy (SEM). The surface morphology of the samples after degradation was analysed by SEM as described in Section 2.2.1.

Fourier transformed infrared spectroscopy with attenuated total reflectance (FTIR–ATR). The enzymatic degradation of SPCL and SPLA scaffolds was also studied by using FTIR–ATR to detect possible changes in the chemical composition of the scaffolds during degradation.

All spectra were recorded using at least 64 scans and 2 cm^{-1} resolution in a FTIR spectrophotometer (Perkin-Elmer, 1600 Series, USA) with a single reflection ATR system (MKII Golden Gate™, Specac, UK). Results were compared to samples analysed prior to degradation studies. The residues resulting from the degradation of SPCL samples after immersion in PBS with lipase and PBS with α-amylase and lipase solutions were analysed in KBr pellet, after being collected and dried in a lyophilizer.

3. Results and discussion

3.1. Characterization of the porosity and porous structure of the scaffolds based on SPCL e SPLA

The two types of scaffolds produced for this study exhibited a typical fibre-mesh structure, with a fibre diameter of roughly 180 m for SPCL and 210 m for SPLA, with highly interconnected pores and a porosity of approximately 75%, as determined by CT analysis. In Figure 1 it is also possible to observe that the surface of the fibres of both polymers is smooth and uniform.

3.2. Mechanical properties

The mechanical properties (tested on compression tests) of the produced scaffolds (based on SPLA and SPCL) in the dry and wet state are presented in Table 2. In general, scaffolds based on SPCL and SPLA present better mechanical properties than most scaffolds obtained from other biodegradable polymers aimed at tissue engineering applications (Zhang and Ma 1999; Nam *et al*., 2000), which demonstrates that these scaffolds exhibit appropriate mechanical properties to support cell adhesion and growth towards the interior of the structures, which has already been demonstrated in several *in vitro* studies (Gomes *et al*., 2003, 2006a, 2006b; Mendes *et al*., 2003; Oliveira *et al*., 2007; Santos *et al*., 2007).

SPLA-based scaffolds present a higher modulus than SPCL scaffolds. However, the modulus of SPLA scaffolds

Table 1. Summary of the degradation solutions and conditions studied

Material	Degradation solution	Degradation period
SPCL and SPLA	PBS PBS + α-amylase PBS + α-amylase + lipase	0, 2, 4, 6, 8, 10 and 12 weeks
SPCL	PBS+ lipase	

Table 2. Mechanical properties of the fibre-mesh scaffolds obtained by fibre-bonding

Polymer	Sample condition	Compression modulus (MPa)
SPCL	Dry	2.11 ± 0.40
	Wet	1.82 ± 0.40
SPLA	Dry	9.61 ± 7.40
	Wet	3.47 ± 3.12

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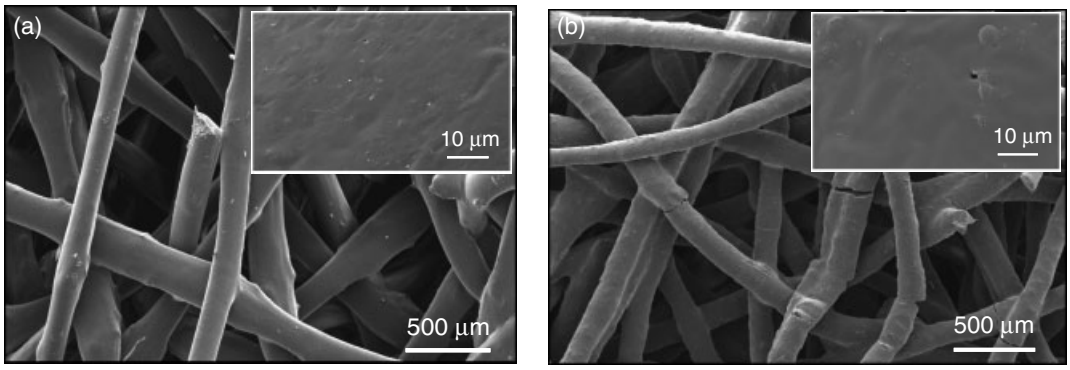

Figure 1. SEM micrographs of the porous fibre-meshes obtained by fibre-bonding: (a) based on SPCL; (b) based on SPLA. The micrographs embedded in the right corner of each picture correspond to higher magnifications of the same pictures, showing the surface morphology of the polymeric fibres in detail

decreases significantly after 3 days of immersion in PBS. This decrease of the modulus in the wet state is attributed to the plasticization effect due to the absorbed water. In contrast, the modulus of SPCL scaffolds is only slightly affected. This behaviour is probably associated with the different hydrophilic/hydrophobic characters of the two polymers, as SPCL is known to exhibit a higher hydrophobic character than SPLA. As a result, SPLA fibres take up more water and therefore become more 'flexible', showing lower compressive modulus after immersion in an aqueous solution.

3.3. Degradation behaviour

PCL and PLA are biodegradable aliphatic polyesters with important applications in the biomedical area (De Jong *et al.*, 2005; Williams *et al.*, 2005; Chastain *et al.*, 2006) and several studies have been performed on the degradation behaviour of these biomaterials (Li, 1999; Catiker *et al.*, 2000; Liu *et al.*, 2000; Chawla and Amiji, 2002; Xiao *et al.*, 2003; Tsuji, 2005; Pena *et al.*, 2006). It was found that PCL hydrolysis may be catalysed by lipase (Liu *et al.*, 2000; Chawla and Amiji, 2002; Pei *et al.*, 2002; Tsuji *et al.*, 2006), according with scheme presented in Figure 2a. The natural function of lipases is the hydrolysis of triglycerides to partial glycerides and fatty acids. Serum lipase is mainly derived from the pancreatic acinar cells but other sources of lipase in the human body are the digestive tract, adipose tissue, lung, milk and leukocytes (Tietz and Shuey, 1993). The serum lipase concentration in healthy adults is in the range 30–190 U/l (Tietz and Shuey, 1993). On the other hand, α-amylase enzyme is able to catalyse the hydrolysis of α-1,4-glycosidic linkages of starch, reducing the molecular size of starch and producing maltose and dextrins (Figure 2b). In humans, the enzyme occurs in a variety of tissues, but the highest concentrations are in the pancreas and in salivary glands. Low amylase activities are normally detected in the serum (46–244 U/l) (Junge *et al.*, 1989) of healthy subjects.

The selection of these two enzymes for the degradation experiments carried out in this study was therefore based on the catalytic activities of lipase and α-amylase enzymes in the degradation of SPCL and SPLA scaffolds.

3.3.1. Water uptake

Upon implantation, the biomaterials interact with the surrounding fluids, initially by uptaking them, which starts the degradation process. The water uptake makes the materials more flexible and promotes changes in the dimensions of the implant material. Simultaneously, a higher water uptake enhances the hydrolysis process

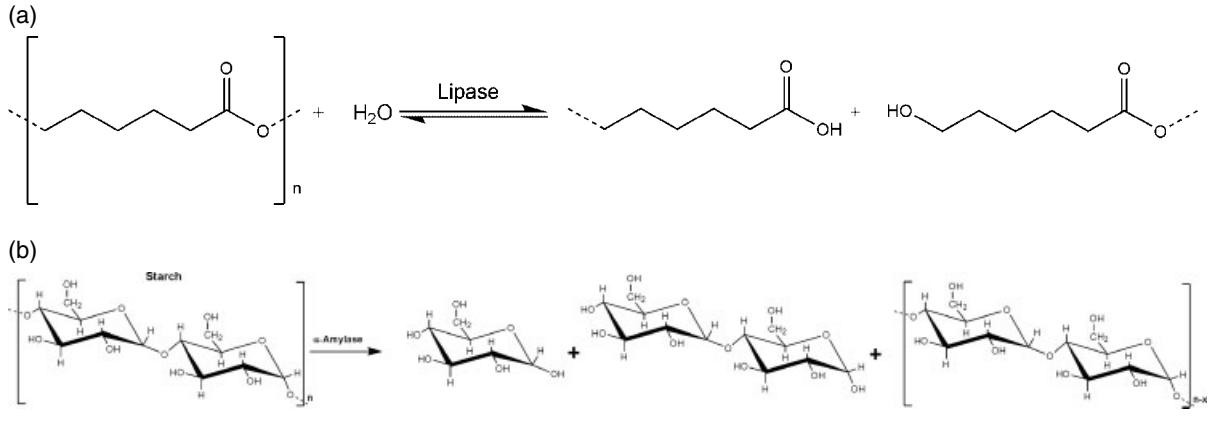

Figure 2. Enzymatic degradation of PCL by lipase (adapted from Xiao *et al.*, 2003) (a) and of starch by α-amylase (b)

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(Azevedo *et al.*, 2003). It is therefore very important to study the water uptake of biodegradable polymers, as this indicates the hydrophilic/hydrophobic character of the materials and therefore, their susceptibility to degradation by hydrolysis processes (Azevedo and Reis, 2005). Figure 3a, b shows the percentage of water uptake for SPCL and SPLA scaffolds, under the different conditions studied (i.e. different degradation media) for different periods of immersion (up to 12 weeks).

The data on water uptake for a given material are usually obtained when its weight in solution reaches equilibrium. However, in some cases, this equilibrium is never reached because of the simultaneous degradation of the materials, which leads to an increased water uptake of the materials over time as a consequence of the increased permeability due to degradation (Azevedo and Reis, 2005). This behaviour was observed for both materials under study and for all tested conditions, as it can be observed from Figure 3a, b.

The results show that SPCL fibre-meshes exhibit a lower water uptake ability (about 70% after 12 weeks of immersion in PBS) than SPLA fibre-meshes, which is related to the higher hydrophilic character of the PLA polymer, as already mentioned. Nevertheless, the high percentage of water uptake registered for the scaffolds studied is not responsible for significant changes in the dimensions of the material (swelling), which means that it also possible that the solution is also retained in the porous structure and not all absorbed by the polymeric fibres. This increased water uptake is mainly attributed to the high surface : volume ratio of the fibre-mesh scaffolds when compared with compact samples. In fact, previous water uptake experiments (Azevedo *et al.*, 2003; Correlo, 2003), performed with compact materials obtained from SPLA and SPCL, revealed much lower results for the same conditions, about 16% and 12.5%, respectively.

3.3.2. Weight loss

During degradation, the weight of the material may undergo significant changes, which can be assessed by comparing the weight registered after different periods of immersion in the degradation media to the initial weight (Azevedo and Reis, 2005). The weight loss for SPCL and SPLA scaffolds is represented graphically in Figure 4a and b, respectively, which show that the SPCL fibre-meshes, despite their lower water uptake than SPLA, presented a higher weight loss, i.e. a higher degradation rate.

In fact, after 12 weeks of immersion in PBS with lipase solution, the SPCL scaffolds were completely degraded, it being possible to identify only small fragments of material in the bottom of the tubes. In the presence of α-amylase, a weight loss of 1.5% for SPCL scaffolds was observed, while SPLA scaffolds presented a weight loss of 22% under the same conditions. This difference was probably related to the hydrophobic character of PCL, which impedes the diffusion of water and enzymes into the polymeric structure. When the two enzymes were present in the solution, a general increase in the weight

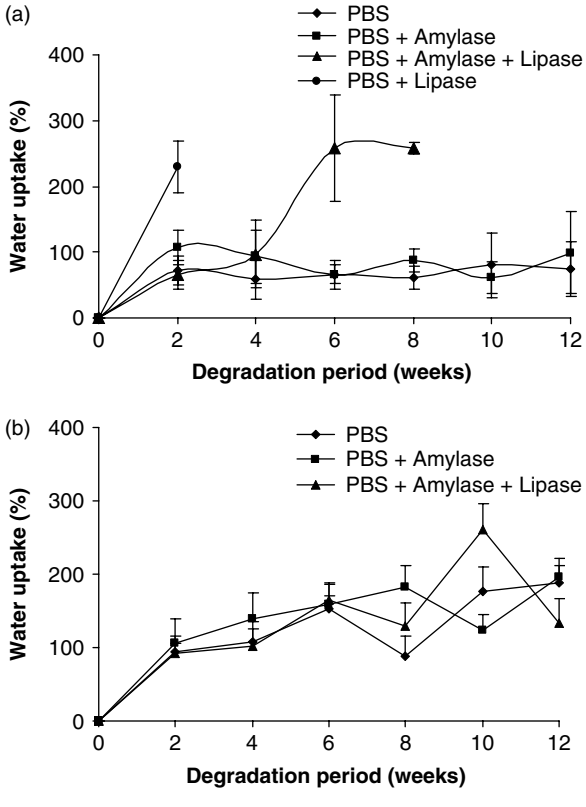

Figure 3. Water uptake in function of degradation time after immersion in different solutions (pH 7.4, 37 °C) of: (a) SPCL-based scaffolds; (b) SPLA-based scaffolds

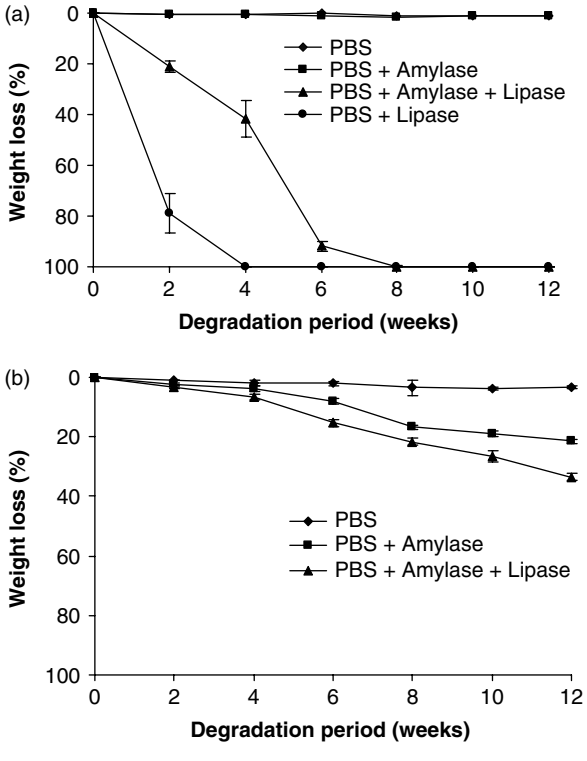

Figure 4. Weight loss in function of degradation time after immersion in different solutions (pH 7.4, 37 °C) of: (a) of SPCL-based scaffolds; (b) SPLA-based scaffolds

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loss was observed, about 33.5% for SPLA and 100% for SPCL. When the samples were simply immersed in PBS, the weight loss was about 1%, corresponding most probably to the 'release' of plasticizers and other additives used during processing of the polymer granules.

The difference observed for both polymer scaffolds in the presence of lipase may be explained by the affinity of this enzyme for the substrate. In fact, several studies (Gan *et al.*, 1997, 1999; He *et al.*, 2003) demonstrated the susceptibility of PCL to enzymatic hydrolysis in the presence of the lipase extracted from *Pseudomonas* sp. However, the same was not observed for PLA.

Observing the results shown in Figure 4, it should also be highlighted that, in spite of the 100% weight loss registered for SPCL fibre-meshes in PBS with lipase and in PBS with α-amylase and lipase, the latter condition leads to a lower degradation rate. This could be due to the adsorption of α-amylase on PCL substrate, reducing the surface area available for the action of lipase.

Therefore, from the obtained results, it becomes clear that the presence of enzymes in the degradation environment contributes, to different degrees, to the faster degradation rate of the materials under study, this effect being more significant in the presence of lipase than α-amylase.

Comparing the results obtained for the two different polymers, it can be concluded that SPLA is more susceptible to degradation by α-amylase, while the hydrolytic action of lipase is considerably higher in the degradation of SPCL.

3.3.3. Morphological characterization of the degraded scaffold samples

Figure 5 and 6 show images obtained by SEM of the surface of SPCL and SPLA fibre-meshes after different immersion periods in different degradation solutions.

In general, the degradation of the studied scaffold materials resulted in modification of the surface topography, i.e. the surface roughness, of the polymeric fibres. The samples which were immersed in PBS (Figures 5, 6) did not show significant changes in their surface morphology, which is in agreement with previous statements regarding the low weight loss, and therefore reduced degradation

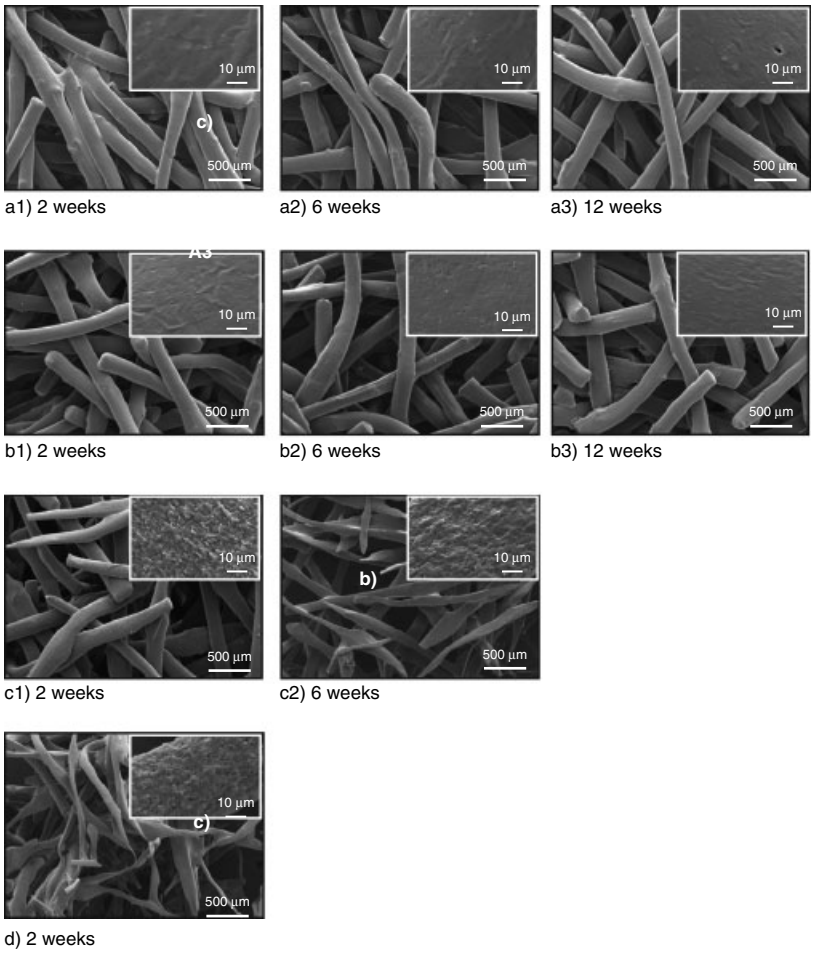

Figure 5. SEM micrographs of SPCL fiber meshes after degradation tests for different periods of time in a)1 to a3); PBS b1) to b3) PBS + α-amylase solution; c1) e c2) PBS + α-amylase and lipase solution and d) PBS + lipase solution. Inserts correspond to magnifications of the respective pictures showing in detail the surface morphology of the degraded fibres. Note that samples immersed in PBS + α-amylase and lipase solution for 12 weeks and samples immersed in PBS + lipase solution for more than 6 weeks were completely degraded and therefore, no pictures correspondent to those conditions could be shown

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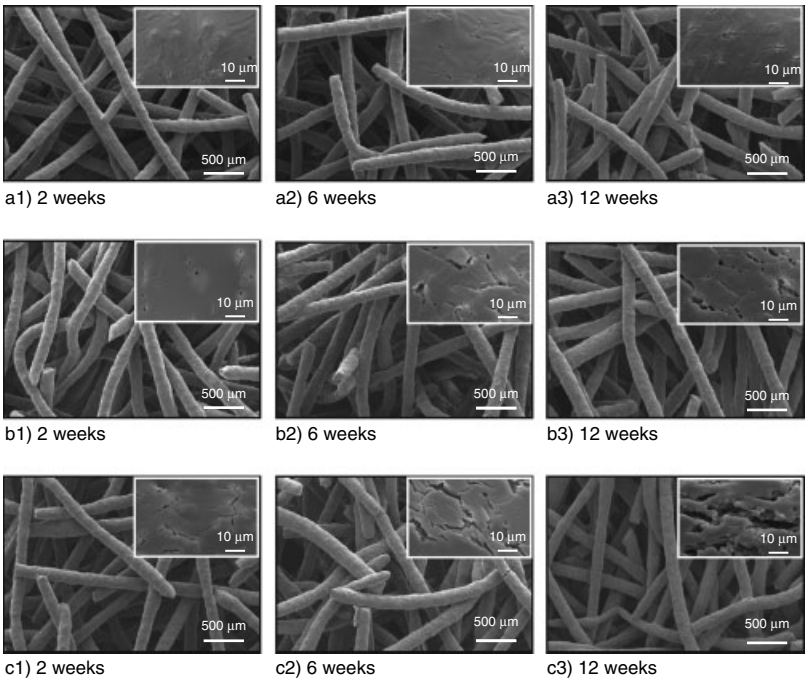

Figure 6. SEM micrographs of SPLA fiber meshes after degradation tests for different periods of time: a1) to a3) in PBS; b1) to b3) in PBS + α-amylase solution; c1) to c2) in PBS + α-amylase and lipase solution. Inserts correspond to magnifications of the respective pictures showing in detail the surface morphology of the degraded fibres

of the materials in these solutions during the time period studied.

The SPCL scaffolds that were immersed in PBS with α-amylase (Figure 5b), also did not exhibit evident changes in morphology. This can be explained by the possible absence of starch on the surface of the fibres, since this is a minor component in the polymeric blend. In contrast, the SPLA fibres started to present small surface fissures after only 2 weeks of immersion in PBS with α-amylase and lipase, which increased with the degradation time, i.e. as a result of the continuous action of the enzymes on the surface (particularly of the starch) of the fibres.

When immersed in the degradation solution containing lipase (Figure 5c, d) it was possible to observe an increased surface roughness of the SPCL fibres with time, probably associated to the breakdown of polymeric chains as a direct consequence of the enzymatic hydrolysis. Similarly to the observations registered for the SPCL fibre-meshes immersed in PBS with α-amylase, the formation of small fissures on the surface of the SPLA fibres was also observed (Figure 6b), which also increased with immersion time. As expected, these surface modifications were more pronounced in the presence of lipase, since this enzyme specifically catalyses the hydrolysis of the ester bonds present in the polyesters that compose about 70% of the polymeric blends used to produce the scaffolds studied.

In addition to these surface changes observed after degradation, SEM analysis of the SPCL fibres after degradation in the presence of lipase (Figure 5c, d) also revealed a remarkable decrease in the diameter of the fibres with immersion time. This is a very important finding, considering the final application and role of the scaffold during tissue regeneration. In fact, upon implantation of the TE construct, a gradual degradation of the scaffold material was expected, leaving enough space for the new tissue ingrowth and facilitating the overall objective of such strategies, which is the substitution of the temporary construct by the native tissue.

It is important to stress that the concentrations of amylase and lipase in the degradation solutions that were used in this study were in the range of the physiological concentrations of these enzymes found in human blood. The results obtained show the dramatic influence of these enzymes in the degradation rate of the starch-based scaffolds. Nevertheless, *in vivo*, these materials will face a different environment, much more complex and probably more aggressive and obviously very difficult to simulate *in vitro*, and therefore one cannot directly correlate the degradation profiles obtained from these *in vitro* tests with the *in vivo* degradation that the scaffolds will undergo in the specific conditions and sites where they will be implanted.

3.3.4. Characterization of the degraded samples and degradation products by FTIR–ATR

The FTIR spectra of PCL and PLA polymers and their blends with starch, SPCL and SPLA, respectively, are shown in Figure 7. The characteristics bands of PCL were located at 1720 cm^{-1}, corresponding to the C═O stretch ester carbonyl group. The peaks at 1500–1150 cm^{-1} were related to the asymmetrical stretch of –COO– and the stretch of –C–O bonding at the main polymer chain. The

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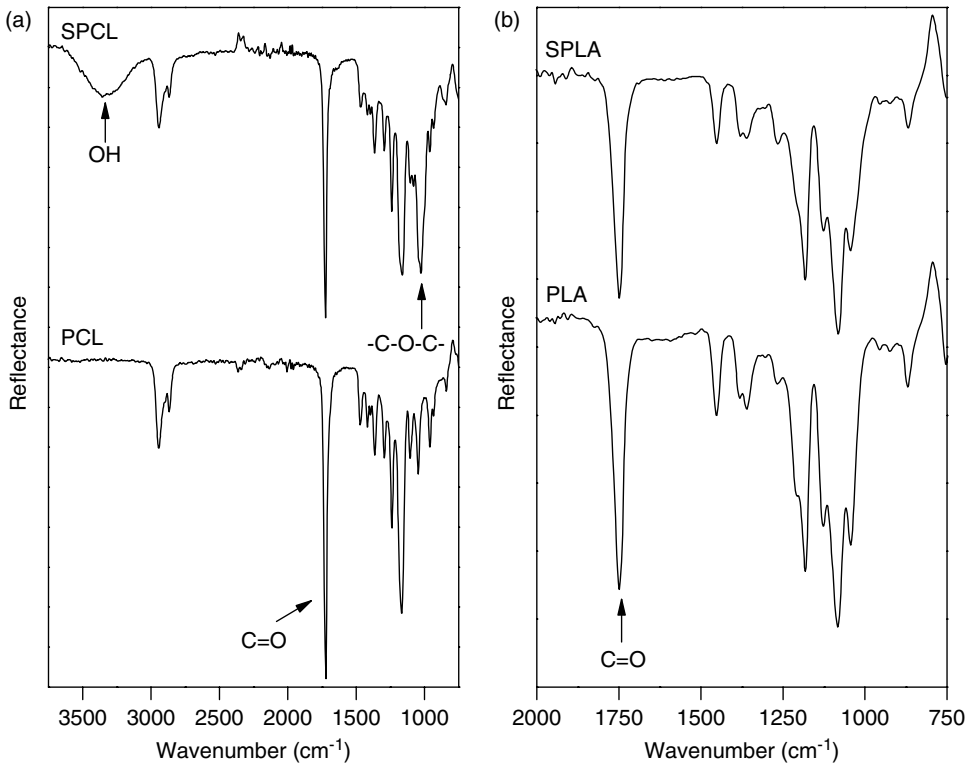

Figure 7. FTIR–ATR spectra of: (a) polycaprolactone (PCL) and PCL with corn starch (SPCL); (b) poly(lactic acid) and PLA with corn starch (SPLA)

SPCL spectrum exhibited the same characteristic bands of PCL but also bands from starch, viz. the bands related to OH group and –C–O–C–of glycosidic bonds typically from starch (Figure 7a).

The main characteristic bands of PLA polymer were located at 1188, 1120, 1088 and 1040 cm^{-1} and the carbonyl band around 1760 cm^{-1} (Catiker *et al.*, 2000; Figure 7b). Comparing the PLA and SPLA spectra, no visible differences could be observed between them, which may indicate that starch was not present at the surface and consequently was not being detected.

Figure 8a shows the FTIR spectra of SPCL before and after degradation in different solutions. As degradation proceeded, the intensity of the band corresponding to the ester bond decreased when the SPCL scaffolds were incubated in the presence of lipase. At the same time, the band of the OH group became more intense, due to the formation of an alcohol during the hydrolysis of ester bonds (Pena *et al.*, 2006) catalysed by lipase (Figure 2). The effect of α-amylase was expected to be observed by a decrease in the intensity of the band around 1050 cm^{-1}, indicating the action of α-amylase in cleaving the glycosidic linkages of starch. However, this was not case. The band corresponding to –C–O–C– remained unchanged after 12 weeks in the presence of α-amylase when compared with non-degraded samples (control). These results were in agreement with weight loss results where no significant weight loss was observed when the SPCL scaffolds were incubated with α-amylase (1%, Figure 4). The same behaviour was observed when the scaffolds were incubated in PBS without enzymes.

Contrary to SPCL scaffolds, the IR spectra of SPLA did not show visible changes after degradation in the presence of lipase (Figure 8b). In fact, it was demonstrated by Vert and co-authors (Liu *et al.*, 2000) that *Pseudomonas* lipase can degrade PCL but cannot degrade poly(L-lactide) (PLLA). The same was observed for the other conditions. Despite this absence of chemical changes, the weight loss results showed that after 12 weeks of degradation in the presence of α-amylase, the SPLA scaffolds registered a weight loss of 22% (Figure 4)

4. Conclusions

The present study focused on the challenge of producing and characterizing adequate 3D constructs from two different biodegradable starch-based polymers, as these materials present an outstanding potential for providing the necessary support to new and developing tissue-engineering strategies.

In the authors' view, it is clear that scaffolds obtained from SPCL and SPLA, obtained by bonding fibres of these two polymers previously obtained by melt-spinning, which have been described and discussed in detail, present an outstanding potential for providing the adequate porous structure and mechanical properties to be used in a range of tissue-engineering strategies. Most importantly, it was shown that the degradation behaviour of the scaffolds studied may be dramatically different according to the composition of the surrounding degradation environment. In particular, it was demonstrated that the degradation

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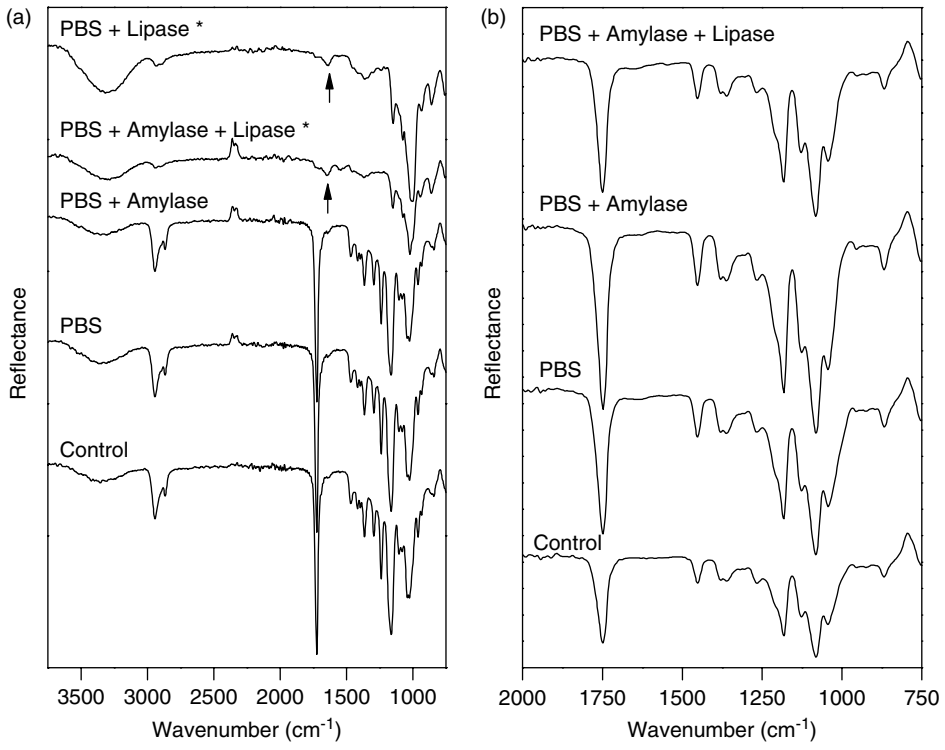

Figure 8. FTIR–ATR spectra of: (a) SPCL and (b) SPLA-based scaffolds after degradation in different solutions at 37 °C, for a period of 12 weeks. The spectra marked with (*) correspond to residues of the samples that were deposited in the bottom of the tubes

profile of SPCL- and SPLA-based fibre-meshes is strongly affected by the presence of enzymes.

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References

Atala A. 2007; Engineering tissues, organs and cells. *J Tissue Eng Regen Med* **1**(2): 83–96.

Azevedo HS, Gama FM, *et al*. 2003; *In vitro* assessment of the enzymatic degradation of several starch based biomaterials. *Biomacromolecules* **4**(6): 1703–1712.

Azevedo HS, Reis RL. 2005; Understanding the enzymatic degradation of biodegradable polymers and strategies to control their degradation rate. In *Biodegradable Systems in Tissue Engineering and Regenerative Medicine*, Reis RL, San Roman J (eds). CRC Press: Boca Raton, FL; 177–201.

Catiker E, Gumusderelioglu M, *et al*. 2000; Degradation of PLA, PLGA homo- and copolymers in the presence of serum albumin: a spectroscopic investigation. *Polymer Int* **49**(7): 728–734.

Chapekar M. 2000; Tissue engineering: challenges and opportunities. *J Biomed Mater Res Appl Biomater* **53**: 617–620.

Chastain SR, Kundu AK, *et al*. 2006; Adhesion of mesenchymal stem cells to polymer scaffolds occurs via distinct ECM ligands and controls their osteogenic differentiation. *J Biomed Mater Res A* **78A**(1): 73–85.

Chawla JS, Amiji MM. 2002; Biodegradable poly(ε-caprolactone) nanoparticles for tumor-targeted delivery of tamoxifen. *Int J Pharmaceut* **249**(1–2): 127–138.

Correlo VM, Gomes ME, *et al*. 2007; Tissue Engineering Using Natural Polymers, In *Biomedical Polymers*, Jenkins M (ed), Woodhead publishing Ltd, Cambridge, 197–217.

De Jong WH, Bergsma JE, *et al*. 2005; Tissue response to partially *in vitro* predegraded poly-L-lactide implants. *Biomaterials* **26**(14): 1781–1791.

Freed LE, Vunjak-Novakovic G. 1998; Culture of organized cell communities. *Adv drug Delivery* **33**(1–2): 15–30.

Freed LE, Vunjak-Novakovic G, *et al*. 1994; Biodegradable polymer scaffolds for tissue engineering. *Biotechnology* **12**(7): 689–693.

Gan ZH, Liang QZ, *et al*. 1997; Enzymatic degradation of poly(ε-caprolactone) film in phosphate buffer solution containing lipases. *Polym Degrad Stabil* **56**(2): 209–213.

Gan ZH, Yu DH, *et al*. 1999; Enzymatic degradation of poly(ε-caprolactone)/poly(DL-lactide) blends in phosphate buffer solution. *Polymer* **40**(10): 2859–2862.

Gomes ME, Bossano CM, *et al*. 2006a; *In vitro* localization of bone growth factors in constructs of biodegradable scaffolds seeded with marrow stromal cells and cultured in a flow perfusion bioreactor. *Tissue Eng* **12**(1): 177–188.

Gomes ME, Holtorf HL, *et al*. 2006b; Influence of the porosity of starch-based fibre-mesh scaffolds on the proliferation and osteogenic differentiation of bone marrow stromal cells cultured in a flow perfusion bioreactor. *Tissue Eng* **12**(4): 801–809.

Gomes ME, Sikavitsas VI, *et al*. 2003; Effect of flow perfusion on the osteogenic differentiation of bone marrow stromal cells cultured on starch-based three-dimensional scaffolds. *J Biomed Mater Res A* **67A**(1): 87–95.

He F, Li SM, *et al*. 2003; Enzyme-catalyzed polymerization and degradation of copolymers prepared from ε-caprolactone and poly(ethylene glycol). *Polymer* **44**(18): 5145–5151.

Hutmacher DW. 2000; Scaffolds in tissue engineering bone and cartilage. *Biomaterials* **21**(24): 2529–2543.

Hutmacher DW, Schantz JT, *et al*. 2007; State of the art and future directions of scaffold-based bone engineering from a biomaterials perspective. *J Tissue Eng Regen Med* **1**(4): 245–260.

Junge W, Troge B, *et al*. 1989; Evaluation of a new assay for pancreatic amylase – performance characteristics and estimation of reference intervals. *Clin Biochem* **22**(2): 109–114.

Kim BS, Mooney DJ. 1998; Development of biocompatible synthetic extracellular matrices for tissue engineering. *Trends Biotechnol* **16**(5): 224–230.

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Langer R. 1999; Selected advances in drug delivery and tissue engineering. *J Control Release* **62**(1–2): 7–11.
Li SM. 1999; Hydrolytic degradation characteristics of aliphatic polyesters derived from lactic and glycolic acids. *J Biomed Mater Res* **48**(3): 342–353.
Liu L, Li S, *et al*. 2000; Selective enzymatic degradations of poly(L-lactide) and poly(ε-caprolactone) blend films. *Biomacromolecules* **1**(3): 350–359.
Lu L, Mikos AG. 1996; The importance of new processing techniques in tissue engineering. *MRS Bull Mater Res Soc* **21**(11): 28–32.
Maquet V, Jerome R. 1997; Design of macroporous biodegradable polymer scaffolds for cell transplantation. *Porous Mater Tissue Eng* **250**: 15–42.
Mendes SC, Bezemer J, *et al*. 2003; Evaluation of two biodegradable polymeric systems as substrates for bone tissue engineering. *Tissue Eng* **9**: (suppl 1): S91–101.
Middleton JC, Tipton AJ. 2000; Synthetic biodegradable polymers as orthopedic devices. *Biomaterials* **21**(23): 2335–2346.
Nam YS, Yoon JJ, *et al*. 2000; A novel fabrication method of macroporous biodegradable polymer scaffolds using gas foaming salt as a porogen additive. *J Biomed Mater Res* **53**(1): 1–7.
Oliveira JT, Crawford A, *et al*. 2007; A cartilage tissue engineering approach combining starch–polycaprolactone fibre-mesh scaffolds with bovine articular chondrocytes. *J Mater Sci Mater Med* **18**: 295–302.
Pei M, Solchaga LA, *et al*. 2002; Bioreactors mediate the effectiveness of tissue engineering scaffolds. *FASEB J* **16**(12): 1691–1694.
Pena J, Corrales T, *et al*. 2006; Long-term degradation of poly(ε-caprolactone) films in biologically related fluids. *Polym Degrad Stabil* **91**(7): 1424–1432.
Santos MI, Fuchs S, *et al*. 2007; Response of micro- and macrovascular endothelial cells to starch-based fibre-meshes for bone tissue engineering. *Biomaterials* **28**(2): 240–248.
Thompson R, Wake MC, *et al*. 1995; Biodegradable polymer scaffolds to regenerate organs. *Adv Polym Sci* **122**: 247–274.
Thompson R, Yaszemski M, *et al*. 1997; *Polymer Scaffold Processing. Principles of Tissue Engineering*, Lanza R, Langer R, Chick W (eds). Academic Press: New York; 263–272.
Thomson RC, Yaszemski MJ, *et al*. 1995; Fabrication of biodegradable polymer scaffolds to engineer trabecular bone. *J Biomater Sci Polym Ed* **7**(1): 23.
Tietz NW, Shuey DF. 1993; Lipase in serum – the elusive enzyme – an overview. *Clin Chem* **39**(5): 746–756.
Tsuji H. 2005; Poly(lactide) stereocomplexes: formation, structure, properties, degradation, and applications. *Macromol Biosci* **5**(7): 569–597.
Tsuji H, Kidokoro Y, *et al*. 2006; Enzymatic degradation of biodegradable polyester composites of poly(L-lactic acid) and poly(ε-caprolactone). *Macromol Mater Eng* **291**(10): 1245–1254.
Vacanti CA, Bonassar LJ. 1999; An overview of tissue engineered bone. *Clin Orthop* **367**: S375–381.
Williams JM, Adewunmi A, *et al*. 2005; Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. *Biomaterials* **26**(23): 4817–4827.
Xiao Y, Qian H, *et al*. 2003; Tissue engineering for bone regeneration using differentiated alveolar bone cells in collagen scaffolds. *Tissue Eng* **9**(6): 1167–1177.
Zhang RY, Ma PX. 1999; Poly(α-hydroxyl acids) hydroxyapatite porous composites for bone-tissue engineering. I. Preparation and morphology. *J Biomed Mater Res* **44**(4): 446–455.

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