
A new route to produce starch-based fiber mesh scaffolds by wet spinning and subsequent surface modification as a way to improve cell attachment and proliferation

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Abstract: This study proposes a new route for producing fiber mesh scaffolds from a starch-polycaprolactone (SPCL) blend. It was demonstrated that the scaffolds with 77% porosity could be obtained by a simple wet-spinning technique based on solution/precipitation of a polymeric blend. To enhance the cell attachment and proliferation, Ar plasma treatment was applied to the scaffolds. It was observed that the surface morphology and chemical composition were significantly changed because of the etching and functionalization of the fiber surfaces. XPS analyses showed an increase of the oxygen content of the fiber surfaces after plasma treatment (untreated scaffolds O/

C:0.32 and plasma-treated scaffolds O/C:0.41). Both untreated and treated scaffolds were examined using a SaOs-2 human osteoblast-like cell line during 2 weeks of culture. The cell seeded on wet-spun SPCL fiber mesh scaffolds showed high viability and alkaline phosphatase enzyme activity, with those values being even higher for the cells seeded on the plasma-treated scaffolds. © 2009 Wiley Periodicals, Inc. *J Biomed Mater Res* 92A: 369–377, 2010

Key words: biodegradable fiber; bone tissue engineering; starch biomaterials; wet spinning; plasma treatment

INTRODUCTION

Tissue engineering offers a promising new approach to create biological alternatives for regeneration of different tissues. It involves the use of tissue-specific cells seeded in a scaffold, which can guide the cell growth and tissue formation in three dimensions. To bring about the desired biological response, a scaffold should possess a number of

characteristics such as three-dimensional highly porous and interconnective structure, large surface area, adequate pore size, suitable surface chemistry and mechanical properties, and so forth.¹

Biodegradable polymeric fiber structures can provide a large surface area and a relatively large porosity which can be optimized for specific applications. Besides these, many tissues, such as nerve, muscle, tendon, ligament, blood vessel, bone, and teeth, have tubular or fibrous bundle architectures and anisotropic properties. Therefore, fiber-based structures find a number of applications in tissue engineering, including soft tissue repair,² vascular prostheses,³ bone,^{4,5} and cartilage⁶ scaffolds, among others.

There are three main techniques to produce fibers to be used in biomedical applications: melt spinning, dry spinning, and wet spinning. Melt spinning is based on the extrusion of polymeric melt, whereas the spinning process starts from polymeric solution in the case of dry and wet spinning. Most of the synthetic polyester fibers used in tissue engineering are produced by a conventional melt spinning tech-

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nique.^{7,8} However, excessively high processing temperatures may result in monomer formation during extrusion process. The excess monomer can catalyze the hydrolysis of the material as well as cause cytotoxic effect *in vivo*. The wet-spinning technique is an alternative way to produce biodegradable polyester fibers for the use in tissue engineering.^{9–12} The fiber properties can be tailored depending on the spinning rate, the concentration of the polymer solution and coagulation bath. A highly viscous solution and high spinning rate allow fiber precipitation at the bottom of the coagulation bath. This approach could be used to form scaffolds during the processing.

In design of a tissue engineering scaffold, surface physicochemistry is one of the most important issues to be considered. The physicochemical properties of the surface directly influence the scaffold performance by affecting the cellular response and ultimately new tissue formation.¹³ To improve the cell affinity, the surface hydrophilicity, surface energy, surface roughness, and surface charge can be modified by different methods, including mechanical, wet chemical, and plasma treatments. Chemical treatments have been widely used to modify the biomaterial surfaces.^{14,15} However, the main problem of these treatments is the influence of the treatment on the bulk properties of the material. The surface of the materials can be modified by plasma treatment without altering the bulk properties of the material.^{16,17} By this technique, it is possible to introduce or graft desired functional groups and polymer chains onto the surface. The surface roughness which plays an important role for cell attachment can be also changed by plasma treatment. Therefore, plasma is a valuable method for improve cell affinity to the tissue engineering scaffolds.

Starch-based scaffolds produced by different methodology have been proposed for use in different tissue engineering applications, including bone and cartilage regeneration.^{5,18–20} The melt-spun fiber meshes based on a starch/polycaprolactone blend have shown capability for growth and differentiation of rat bone marrow stromal cells.²¹

In the present study, we describe a new route for the production of starch-based fiber mesh scaffolds which allows forming of scaffolds during spinning. This new route also avoids polymer degradation which is the main problem of melt-based systems. The surface properties of the produced scaffolds are tailored by plasma treatment in order to enhance cell attachment and proliferation.

MATERIALS AND METHODS

Materials

A blend of starch/polycaprolactone (SPCL) (30/70 wt %) was used to produce fiber mesh scaffolds. All the

reagents used were analytical grade unless specified otherwise.

Wet-spinning process

Starch-based fiber mesh scaffolds were originally produced by wet spinning. To obtain a polymer solution with a proper viscosity, SPCL was dissolved in chloroform at a concentration of 40% (w/v). It must be noted that it was possible to obtain a homogenous suspension of starch particles into the solution even though the only the synthetic part of the blend was soluble in the chloroform. Methanol was used as a coagulant. The polymer solution was loaded into a syringe and placed in a syringe pump (World Precision Instruments, UK). A certain amount of polymer solution was subsequently extruded into a coagulation bath. The fiber mesh structure was formed during the processing by the random movement of the coagulation bath. The formed scaffolds were then dried at room temperature overnight in order to remove any remaining solvents.

After spinning, the internal architecture of the produced fiber meshes was characterized by microcomputed tomography (μ -CT).²² The surface morphology of the fibers was analyzed under a scanning electron microscope (SEM, Leica Cambridge S360 microscope). Specifically, three specimens were scanned in air using a μ -CT imaging system (μ -CT40, Scanco Medical AG, Bassersdorf, Switzerland) with a nominal resolution of 12 μ m. The reconstructed images were filtered using a constrained 3D Gaussian filter to partially suppress noise in the volumes ($\mu = 1.2$ voxel, support = 1 voxel) and binarized using a global threshold. Standard 3D morphometry as developed for trabecular bone was used to assess structural parameters for the scaffolds. These parameters included porosity and scaffold surface-to-volume ratio and were derived from a triangulated mesh allowing for computation of surface and volume.²³ In addition, pore size and fiber thickness were determined using the distance transformation method.²⁴ In this method, each pore (fiber) is filled with a nonredundant set of maximal spheres. Mean pore (fiber) size was then calculated as the volume-averaged diameter of all spheres making up the pore (fiber).

Plasma surface treatment

To improve cell attachment and proliferation, a plasma treatment was performed using a plasma reactor PlasmaPrep₅ (Gala Instrument GmbH, Germany) with a chamber size of 15 cm diameter and 31 cm length (5 L) and with a fully automated process control. To maximize the surface area to be exposed to the plasma, the samples were wired (necklace like) with a distance of 1 cm between them. The wire was fixed to a metal support which was placed into the chamber. The chamber was flushed with argon five times prior to treatment. A radio frequency source was used and a power of 30 W was applied for 15 min. Argon was used as a working gas and the pressure in the reactor was controlled (0.18 mbar) by adjusting its flow rate. The samples were kept in air after being removed from the reactor.

X-ray photoelectron spectroscopy

X-ray photoelectron spectroscopy (XPS) was used to quantitatively determine the surface composition of the scaffolds. The XPS analysis was performed using a 250 iXL ESCA instrument (VG Scientific) equipped with two X-ray sources: a source with Al K α 1,2 monochromatized radiation at 1486.92 eV and another one (dual) equipped with two anodes: Mg and Al.

The nonconductive nature of the samples required the use of an electron flood gun to minimize surface charging. Charge compensation at the surface was performed by using both a low energy flood gun (electrons in the range 0–14 eV) and an electrically grounded stain steel screen placed directly on the sample surface.

The first attempt to measure the samples was the use of the monochromatic X-ray source. However, this was impossible because of the high roughness of the samples which caused problems to build up the charge. Generally, charge neutralization for 3D surfaces is more difficult because of the “holes” on the surface. The best choice for these cases is Mg source which helps to neutralize the surface charge. Therefore, the measurements were carried out using nonmonochromatic Mg K α radiation ($h\nu = 1253.6$ eV). Photoelectrons were collected from a take off angle of 90° relative to the sample surface. The measurements were performed in the constant analyzer energy mode with a 100 eV pass energy for survey spectra and 20 eV pass energy for high-resolution spectra. C1s high-resolution scans were taken before and after experiments to check out the efficiency of charge compensation. Charge referencing was done by setting the lower binding energy C1s hydrocarbon (CH $_x$) peak at 285.0 eV.

The atomic composition of the samples was determined from the survey spectra using the standard Scofield photoemission cross sections. The chemical functional group identification was obtained from the high-resolution peak analysis of C1s envelopes. The spectra fitting were performed using the “chi-squared” algorithm to determine the goodness of a peak fit.

Cell culture studies

A human osteoblast-like cell line (SaOs-2) was used to test the cell attachment and proliferation on the scaffolds. A 1 mL of cell suspension containing 3×10^5 cells in a culture medium (low-glucose DMEM supplemented with 10% fetal bovine serum and 1% antibiotics/antimicrobics) were dropped onto scaffolds ($n = 3$). The cells on scaffolds were then allowed to grow for 2 weeks at 37°C in humidified atmosphere containing 5% CO $_2$ with medium changes every 2–3 days.

Morphological analysis

For the morphological examination, cell/scaffold constructs were rinsed with phosphate-buffered saline (PBS; Sigma, USA) and then fixed in 2.5% glutaraldehyde. The samples were dehydrated through graded series of ethanol

and dried before mounting onto brass stubs and sputter coated with gold. Finally, the samples were analyzed under a SEM at an acceleration voltage of 15 kV.

Cell proliferation-DNA assay

Cell proliferation was evaluated by quantifying DNA content using the PicoGreen dsDNA kit (Molecular Probes, USA). PicoGreen dsDNA quantitation reagent is an ultrasensitive fluorescent nucleic acid stain for quantitative analysis of double-stranded DNA (dsDNA) in solution.

For the assay, the scaffold/cell constructs ($n = 3$) were rinsed with PBS and incubated with sterile ultra pure water at 37°C for 1 h before putting at –80°C. The samples were then thawed and put in an ultrasonic bath for 15 min. An aliquot of each sample was transferred to the 96-well plate. A certain ratio of Tris-EDTA buffer and PicoGreen reagent prepared in the same buffer was added to the each well. The fluorescence was read at 485 and 528 nm excitation and emission, respectively. The DNA amount of each samples was then calculated using a standard curve.

Alkaline phosphatase activity

Alkaline phosphatase (ALP) activity from the scaffolds/cells constructs ($n = 3$) was quantified by the specific conversion of *p*-nitrophenyl phosphate (pNPP) into *p*-nitrophenol (pNP). To perform the assay, the seeded scaffolds were rinsed with PBS and transferred individually into eppendorf containing sterile ultra pure water. They were then frozen at –80°C and defrosted at room temperature before starting the assay. A buffer solution containing 0.2% (w/v) *p*-nitrophenyl phosphate was added to the samples in a ratio of 1:3. The enzyme reaction was carried out at 37°C for 1 h and then stopped by a solution containing 2M NaOH and 0.2 mM EDTA in distilled water. The absorbance of *p*-nitrophenol formed was determined at 405 nm with a reference filter at 620 nm. A standard curve was made using pNP values ranging from 0 to 600 $\mu\text{mol/mL}$. The results were expressed as μmol of pNP produced/ mL/h .

Statistical analysis

All the quantitative results were obtained from triplicate samples. Data were expressed as a mean \pm SD. For statistical analysis, a two-tailed Student's *t* test was used. Differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

Morphology of the scaffolds

Figure 1(a,b) presents respectively the μ -CT and SEM images of SPCL fiber mesh scaffolds produced by wet spinning. It can be clearly seen that the SPCL

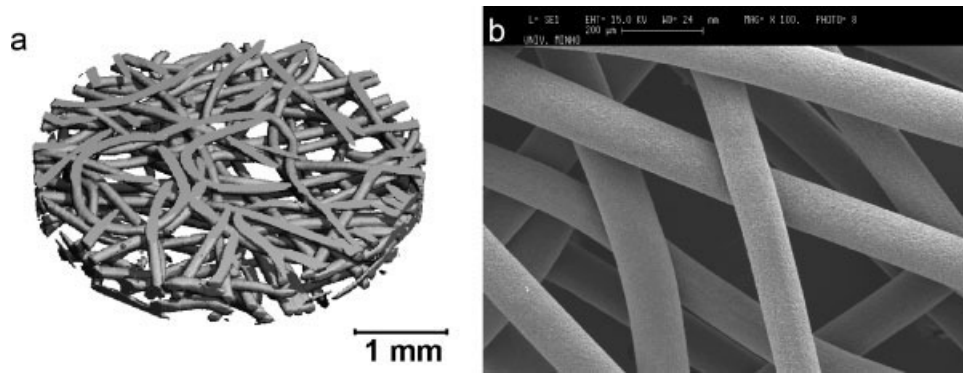


Figure 1. Morphology of the wet-spun SPCL fiber mesh scaffolds analyzed by (a) μ CT, showing thin section of sample, and (b) SEM.

fibers were randomly formed into a 3D structure. The porosity of the scaffolds, determined by μ -CT, was 77%. μ -CT morphometry showed that fiber diameter was 100 μm ; mean pore size was 250 μm , which is in the range of ideal pore size for bone regeneration.²⁵ The scaffolds have a high surface to volume ratio: the specific scaffold surface was 29 mm^2/mm^3 , similar to that of native bone.²⁴ Moreover, μ -CT showed that wet spinning was a very reproducible procedure; the coefficient of variation (CV) for fiber thickness was 4.2%, while for the other parameters the CV was 1.2% or below.

Previous studies have reported that 2D polymer surfaces could be easily modified by different plasma techniques.^{26–28} However, it becomes more complicated in the case of 3D porous polymeric structure because of the pore structures and low interconnectivity which do not allow the penetration of plasma exposition in the interior part of the structure because of the shadow effect. In the present study, the wet-spun scaffolds produced presented a highly porous and interconnected structure that can overcome this problem of plasma surface modification. In the case of wet-spun scaffolds, the treatment is very similar to treatment of a single fiber. As a

result, the surface exposed to the plasma is much higher, which allows for a more uniform and effective surface treatment.

Plasma treatment

Because of phase inversion during precipitation, the surface of the fibers exhibited a nonsmooth morphology [Fig. 2(a)]. After plasma treatment, a noticeable difference on the fiber surfaces was observed [Fig. 2(b)]. It is well known that the surface roughness typically tends to increase after a plasma treatment because of the so-called etching process.²⁹ When the plasma is composed of an inorganic gas, such as argon, helium, hydrogen, nitrogen, and oxygen, it leads to the etching reactions on the topmost layer of the polymeric surface.³⁰ This etching is a result of stripping off the topmost layer of the polymer because of the weight loss of the polymer during the plasma exposure. However, the SPCL fiber surfaces showed a denser and smaller nanosize roughness after plasma treatment. Since SPCL has a very low glass transition temperature (-60°C), it can be explained by melting and etching that consecu-

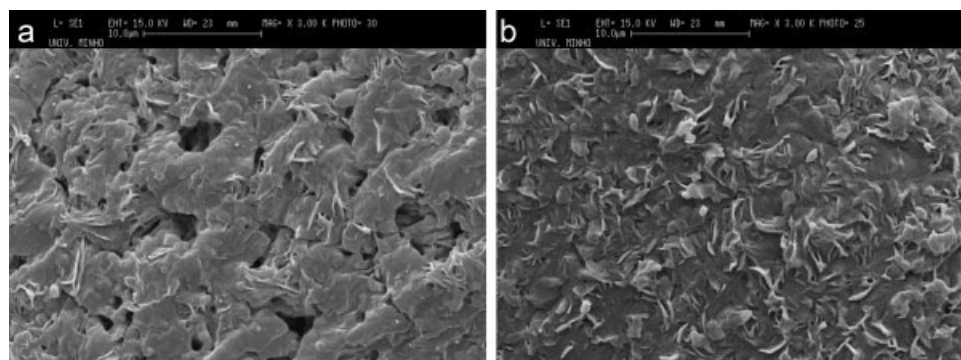


Figure 2. The surface of the SPCL fiber mesh scaffolds (a) before ($\times 3000$) and (b) after Ar plasma treatment ($\times 3000$).

TABLE I
XPS Data for Wet-Spun SPCL Scaffolds
Before and After Modification

	C%	O%	O:C/C:O Ratio
Theoretical PCL	75.0	25.0	0.33/3.00
Theoretical SPCL	68.9	31.1	0.45/2.20
Fiber mesh, untreated	75.9	24.1	0.32/3.15
Fiber mesh, modified	71.0	29.0	0.41/2.45

tively occurred on the surface during the plasma process.

XPS analysis was performed to obtain more detailed information for the chemical structures and groups presented on the surface before and after the performed modification. The results obtained are summarized in Table I.

As it can be seen from the table the measured oxygen content on the surface is lower than the theoretical one calculated for PCL. The difference between the bulk and surface composition of the material is not a surprise. It has been reported^{31,32} that in polymeric systems, the composition near to the surface can be markedly different from the bulk. In fact, because of the high complexity of blend chemistry, different components may predominate at the surface, depending on the blend composition, crystallinity of the components, complexity and degree of miscibility of the system, processing conditions, and also the nature of surrounding environment.^{31,33,34} However, after plasma modification the oxygen content on the surface increase and an O/C ratio of 0.41 was measured (compared to 0.32 for the untreated material). Oxidation is not the only process which is taking place on the surface during the plasma treat-

ment. As can be seen from the table the percentage of carbon was also changed after the treatment; it reduced from 75.9 to 71.0 confirming ongoing etching process.

More detailed analysis of C1s core level spectra of SPCL fiber mesh samples (Fig. 3) showed that they contain three main peaks at 285 eV, which was assigned to the main carbon backbone, at 286.4 eV for —C—O— and at about 288.8 eV for carboxyl bonded carbons. This is not unexpected because the main component of the blend poly(ϵ -caprolactone) has all of those bonds present in its structure.

The intensity of the peaks at 286.4 and 288.8 eV increased after treatment as it can be seen from Figure 3. The created active species on the surface by Ar plasma are very reactive and can recombine with the oxygen from the air. As a result surface functionalization with different oxygen containing groups occurs on the surface and this is the reason for the observed differences in the C1s core level spectra.

Cell culture

Morphological analysis

The morphology and the shape of the osteoblast seeded onto both untreated and plasma-treated fiber meshes showed a clear difference after different time of culturing (Figs. 4–6). It was observed that the cells were able to grow as a film on the treated surface while they were in spindle-like shape with cytoplasmic extension on the untreated surface after 3 days of culture. This was more evident after 7 days of cul-

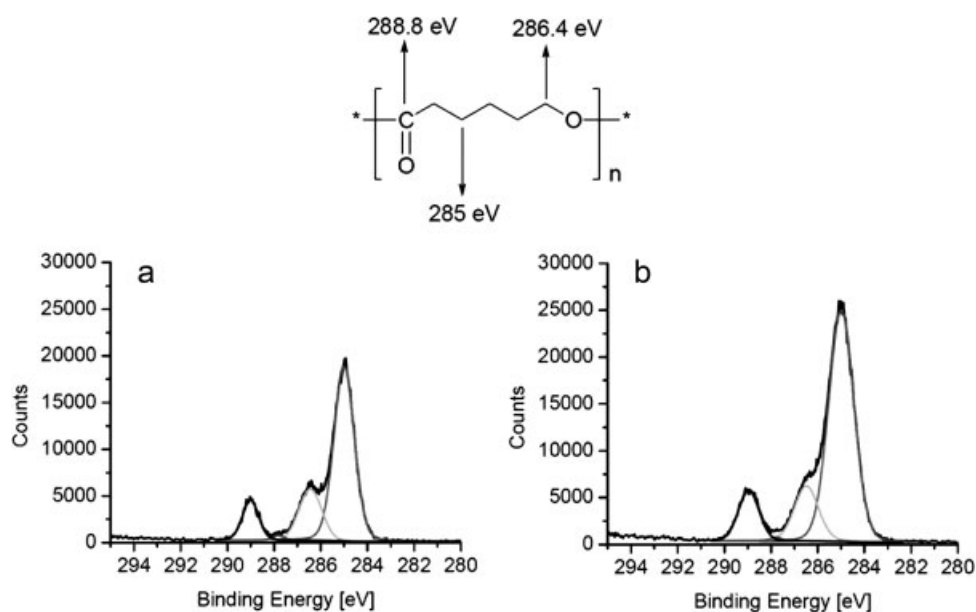


Figure 3. C1s core level spectra for wet-spun SPCL fiber meshes (a) before and (b) after Ar plasma treatment.

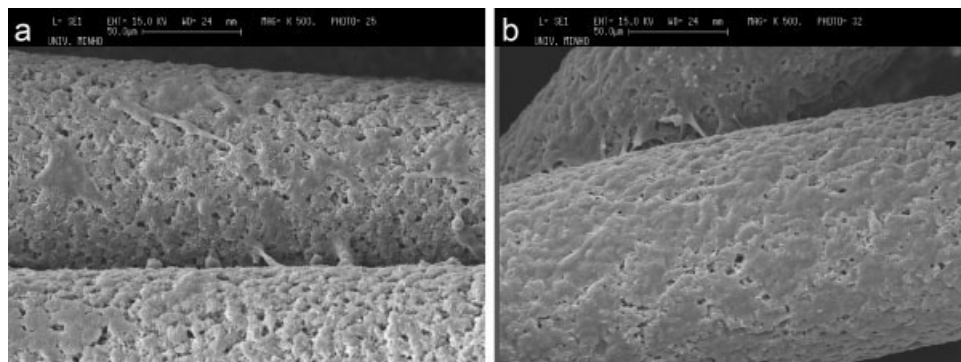


Figure 4. SEM micrographs of osteoblast-like cells seeded on SPCL fiber mesh scaffolds after 3 days of culture: (a) untreated and (b) Ar plasma-treated scaffold.

ture [Fig. 5(a–d)]. Both scaffolds were completely covered by cells, indicating the ability of the scaffolds for osteoblast cell attachment and proliferation. However, the osteoblasts seeded on the treated fiber meshes were able to bridge between the fibers after 14 days of culture (Fig. 6).

Cell viability

Besides the cell morphology, changing the surface chemistry and topography also influenced the metabolic activity of the cells. As it was discussed earlier, the oxygen content of the fiber surfaces significantly increased after plasma treatment because of degrada-

tion and functionalization of the fiber surfaces. It is known that osteoblasts are attachment-dependent cell.³⁵ They can only produce and mineralize their extracellular matrix if they attach in a proper surface that allows specific cell-surface interaction. They also can recognize the changes in the surface topography if it is above 0.5 μm .³⁶ Therefore, DNA amount was significantly different between untreated and treated scaffolds after 3 and 7 days of culture ($p = 0.045$ and $p = 0.015$ for 3 and 7 days, respectively), while it was not significant after 14 days (Fig. 7). This can be explained by the effect of plasma treatment in the initial stage of culture. The surface properties dictate the very first events occurring when the material becomes in a contact with the biological environ-

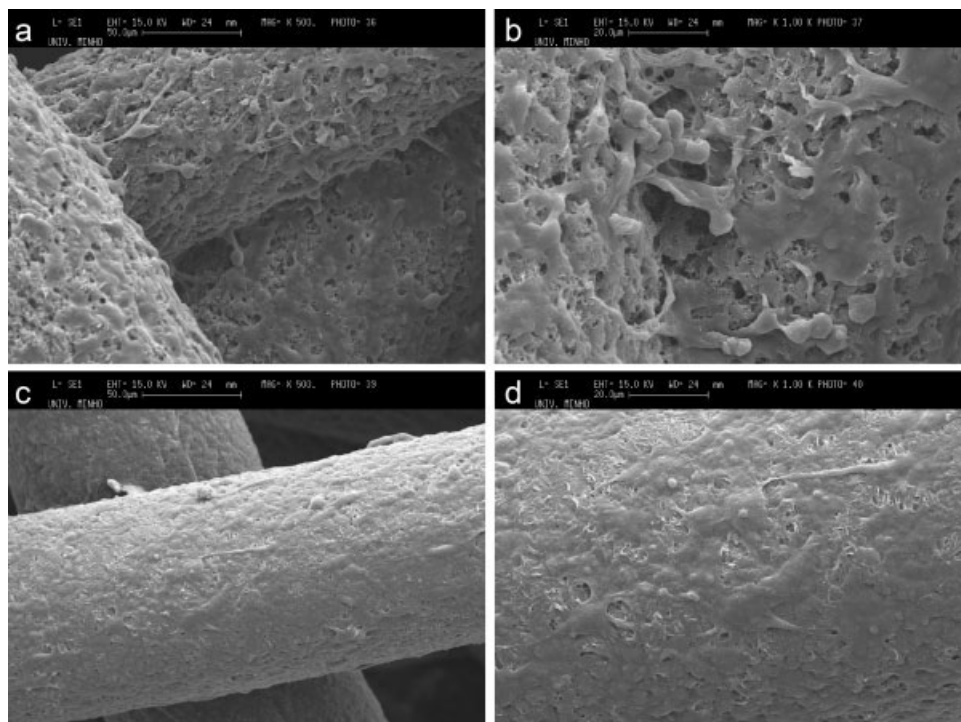


Figure 5. SEM micrographs of osteoblast-like cells seeded on (a, b) untreated and (c, d) Ar plasma-treated SPCL fiber mesh scaffolds after 7 days of culture.

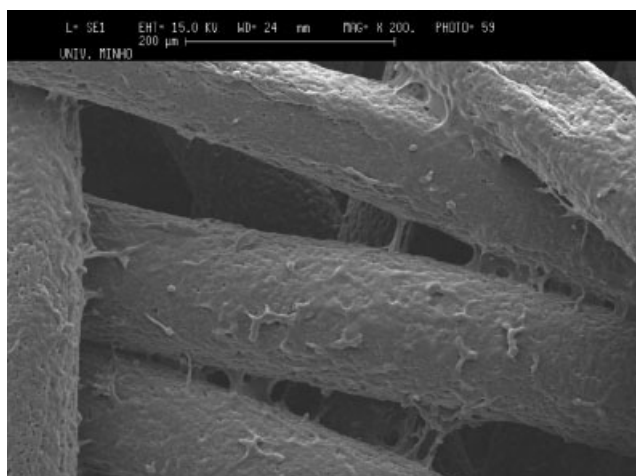


Figure 6. SEM micrographs of osteoblast-like cells seeded on Ar plasma-treated SPCL fiber mesh scaffolds after 14 days of culture.

ments. Thus, at latter time stage *in vitro* performance of the material is also affected by other parameters imposed by the presence of cells at the surface. Once they reached confluency, the rate of cellular death and proliferation becomes equal and hence the proliferation stops. This fact can explain the decrease in the difference between untreated and treated samples after 14 days of culture.

ALP activity

ALP is an enzyme which is used as an early marker for osteoblast differentiation. Although its exact role is not very clear, it is known that it plays an important role in bone matrix mineralization process.³⁵ Regarding the present experiments, ALP ac-

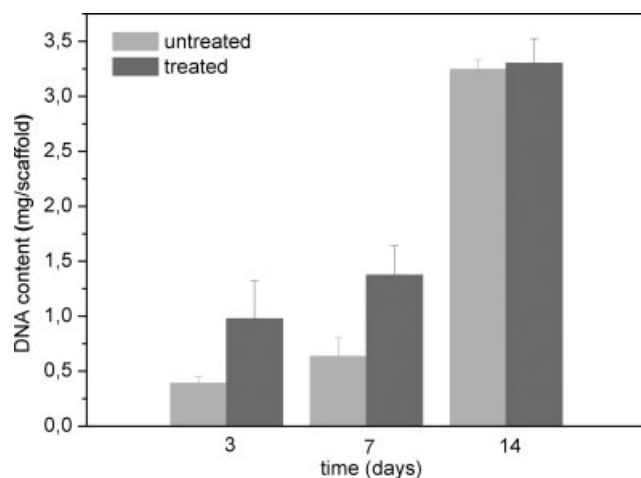


Figure 7. Cell viability and proliferation of human osteoblast-like cells determined by DNA. Error bars represent means \pm SD for $n = 3$.

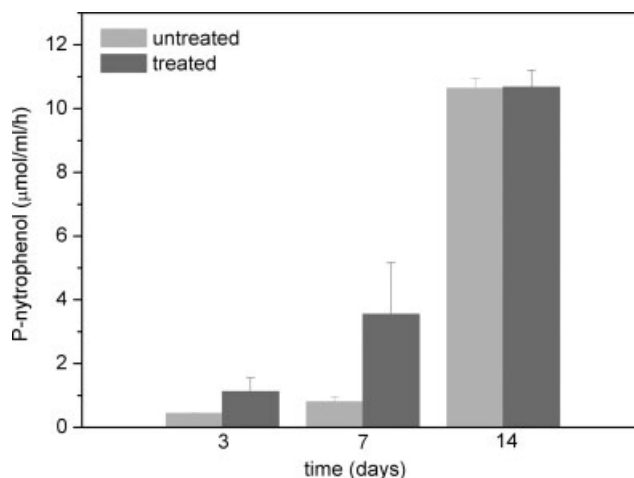


Figure 8. The ALP activity of human osteoblast-like cells seeded on untreated and Ar plasma-treated wet-spun SPCL fiber meshes. Error bars represent means \pm SD for $n = 3$.

tivity of the cells cultured onto the SPCL fiber meshes increased with the culture period (Fig. 8). The enzyme activity of the cells was found to be even higher if the scaffolds were treated by plasma. On the other hand, the differences in the enzymatic activity between the cells seeded on untreated and treated scaffolds decreased after 14 days of culture. It has been reported that the deposition of extracellular matrix occurs before the mineralization starts.³⁷ This latest stage of cell differentiation precedes mineralization and thereby ALP enzyme activity which is associated with calcification tends to decrease.^{38,39} This phenomenon can explain the ALP results from the present study, indicating that the cells cultured on untreated and treated scaffolds might be in different stage of differentiation.

CONCLUSIONS

A new route was described to produce starch-based fiber mesh scaffolds. The results of this study indicated that a simple wet-spinning technique could be used to obtain highly porous fiber mesh scaffolds from a biodegradable SPCL blend. The scaffolds could be formed, in a very reproducible manner, during the process by means of optimizing the processing conditions. Furthermore, the developed scaffolds were able to support osteoblast-like cell attachment and proliferation. As a second step, the scaffolds surfaces were modified by using Ar plasma. After plasma treatment, the surface morphology and surface chemical composition of the fibers were significantly changed. Osteoblast-like cells were able to recognize these physical and chemical changes on

the surface and they showed higher cell viability and ALP enzyme activity on the treated scaffolds.

As a final conclusion, these results suggested that starch-based fiber mesh scaffolds can be easily processed by a wet-spinning technique into adequate scaffolds. Subjecting these scaffolds to Ar plasma can enhance their suitability for tissue engineering applications.

References

- Hutmacher DW. Scaffold design and fabrication technologies for engineering tissues—State of the art and future perspectives. *J Biomater Sci Polym Ed* 2001;12:107–124.
- Chen GP, Sato T, Ohgushi H, Ushida T, Tateishi T, Tanaka J. Culturing of skin fibroblasts in a thin PLGA-collagen hybrid mesh. *Biomaterials* 2005;26:2559–2566.
- Gafni Y, Zilberman Y, Ophir Z, Abramovitch R, Jaffe M, Gazit Z, Domb A, Gazit D. Design of a filamentous polymeric scaffold for in vivo guided angiogenesis. *Tissue Eng* 2006;12:3021–3034.
- Gomes ME, Holtorf HL, Reis RL, Mikos AG. Influence of the porosity of starch-based fiber mesh scaffolds on the proliferation and osteogenic differentiation of bone marrow stromal cells cultured in a flow perfusion bioreactor. *Tissue Eng* 2006;12:801–809.
- Tuzlakoglu K, Bolgen N, Salgado AJ, Gomes ME, Piskin E, Reis RL. Nano- and micro-fiber combined scaffolds: A new architecture for bone tissue engineering. *J Mater Sci Mater Med* 2005;16:1099–1104.
- Woodfield TBF, Malda J, de Wijn J, Peters F, Riesle J, van Blitterswijk CA. Design of porous scaffolds for cartilage tissue engineering using a three-dimensional fiber-deposition technique. *Biomaterials* 2004;25:4149–4161.
- Schmack G, Jehnichen D, Vogel R, Tandler B. Biodegradable fibers of poly(3-hydroxybutyrate) produced by high-speed melt spinning and spin drawing. *J Polym Sci Part B: Polym Phys* 2000;38:2841–2850.
- Yuan XY, Mak A. F. T. Kwok, K. W. Yung BKO, Yao KD. Characterization of poly(L-lactic acid) fibers produced by melt spinning. *J Appl Polym Sci* 2001;81:251–260.
- Williamson MR, Coombes AGA. Gravity spinning of polycaprolactone fibres for applications in tissue engineering. *Biomaterials* 2004;25:459–465.
- Nelson KD, Romero A, Waggoner P, Crow B, Borneman A, Smith GM. Technique paper for wet-spinning poly(L-lactic acid) and poly(DL-lactide-co-glycolide) monofilament fibers. *Tissue Eng* 2003;9:1323–1330.
- Williamson MR, Chang HI, Coombes AGA. Gravity spun polycaprolactone fibres: Controlling release of a hydrophilic macromolecule (ovalbumin) and a lipophilic drug (progesterone). *Biomaterials* 2004;25:5053–5060.
- Williamson MR, Black R, Kielty C. PCL-PU composite vascular scaffold production for vascular tissue engineering: Attachment, proliferation and bioactivity of human vascular endothelial cells. *Biomaterials* 2006;27:3608–3616.
- Mwale F, Wang HT, Nelea V, Luo L, Antoniou J, Wertheimer MR. The effect of glow discharge plasma surface modification of polymers on the osteogenic differentiation of committed human mesenchymal stem cells. *Biomaterials* 2006;27:2258–2264.
- Pashkuleva I, Reis RL. Surface activation and modification—A way for improving the biocompatibility of degradable biomaterials. In: Reis RL, San Roman J, editors. *Biodegradable Systems in Tissue Engineering and Regenerative Medicine*. Boca Raton, Florida: CRC Press; 2004. p 429–454.
- Garbassi F, Morra M, Occhiello E. *Polymer Surfaces: From Physics to Technology*. Chichester: Wiley; 1994. p 241–274.
- Huang FL, Wei QF, Wang XQ, Xu WZ. Dynamic contact angles and morphology of PP fibres treated with plasma. *Polym Test* 2006;25:22–27.
- Tsal PP. Surface modification of fabrics using a one-atmosphere glow discharge plasma to improve fabric wettability. *Text Res J* 1997;67:359–369.
- Santos MI, Fuchs S, Gomes ME, Unger RE, Reis RL, Kirkpatrick CJ. Response of micro- and macrovascular endothelial cells to starch-based fiber meshes for bone tissue engineering. *Biomaterials* 2007;28:240–248.
- Oliveira JT, Crawford A, Mundy JM, Moreira AR, Gomes ME, Hatton PV, Reis RL. A cartilage tissue engineering approach combining starch-polycaprolactone fibre mesh scaffolds with bovine articular chondrocytes. *J Mater Sci Mater Med* 2007;18:295–302.
- Salgado AJ, Coutinho OP, Reis RL, Davies JE. In vivo response to starch-based scaffolds designed for bone tissue engineering applications. *J Biomed Mater Res A* 2007;80:983–989.
- Gomes ME, Sikavitsas VI, Behravesh E, Reis RL, Mikos AG. 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* 2003;67:87–95.
- Rueggsegger P, Koller B, Muller R. A microtomographic system for the nondestructive evaluation of bone architecture. *Calcif Tissue Int* 1996;58:24–29.
- Muller R, Hildebrand T, Rueggsegger P. Noninvasive bone-biopsy—A new method to analyze and display the 3-dimensional structure of trabecular bone. *Phys Med Biol* 1994;39:145–164.
- Hildebrand T, Laib A, Muller R, Dequeker J, Rueggsegger P. Direct three-dimensional morphometric analysis of human cancellous bone: Microstructural data from spine, femur, iliac crest, and calcaneus. *J Bone Miner Res* 1999;14:1167–1174.
- Maquet V, Jerome R. Design of macroporous biodegradable polymer scaffolds for cell transplantation. *Porous Mater Tissue Eng* 1997;250:15–42.
- Pashkuleva I, Marques AP, Vaz F, Reis RL. Surface modification of starch based biomaterials by oxygen plasma or UV-radiation. *J Mater Sci Mater Med*. Submitted for publication.
- Khang G, Jeon JH, Lee JW, Cho SC, Lee HB. Cell and platelet adhesions on plasma glow discharge-treated poly(lactide-co-glycolide). *Biomed Mater Eng* 1997;7:357–368.
- Gugala Z, Gogolewski S. Attachment, growth, and activity of rat osteoblasts on polylactide membranes treated with various low temperature radiofrequency plasmas. *J Biomed Mater Res A* 2006;76:288–299.
- Tahara M, Cuong NK, Nakashima Y. Improvement in adhesion of polyethylene by glow-discharge plasma. *Surf Coat Technol* 2003;174:826–830.
- Inagaki N. *Plasma Surface Modification and Plasma Polymerization*. Pennsylvania: Technomic Publishing Company; 1996. p 22–28.
- Cho K, Lee J, Xing PX. Enzymatic degradation of blends of poly(epsilon-caprolactone) and poly(styrene-co-acrylonitrile) by *Pseudomonas* lipase. *J Appl Polym Sci* 2002;83:868–879.
- Tang YW, Labow RS, Revenko I, Santerre JP. Influence of surface morphology and chemistry on the enzyme catalyzed biodegradation of polycarbonate-urethanes. *J Biomater Sci Polym Ed* 2002;13:463–483.
- Vikman M, Hulleman SHD, Van der Zee M, Myllarinen P, Feil H. Morphology and enzymatic degradation of thermo-

- plastic starch-polycaprolactone blends. *J Appl Polym Sci* 1999;74:2594–2604.
34. Duguay DG, Labow RS, Santerre JP, Mclean DD. Development of a mathematical-model describing the enzymatic degradation of biomedical polyurethanes. I. Background, rationale and model formulation. *Polym Degrad Stab* 1995;47:229–249.
 35. Boyan BD, Lohmann CH, Dean DD, Sylvia VL, Cochran DL, Schwartz Z. Mechanisms involved in osteoblast response to implant surface morphology. *Annu Rev Mater Res* 2001;31:357–371.
 36. Anselme K. Osteoblast adhesion on biomaterials. *Biomaterials* 2000;21:667–681.
 37. Chim H, Ong JL, Schantz JT, Hutmacher DW, Agrawal CM. Efficacy of glow discharge gas plasma treatment as a surface modification process for three-dimensional poly (D,L-lactide) scaffolds. *J Biomed Mater Res A* 2003;65:327–335.
 38. Salgado AJ, Gomes ME, Chou A, Coutinho OP, Reis RL, Hutmacher DW. Preliminary study on the adhesion and proliferation of human osteoblasts on starch-based scaffolds. *Mater Sci Eng C: Biomimetic Supramol Syst* 2002;20:27–33.
 39. Holy CE, Shoichet MS, Davies JE. Engineering three-dimensional bone tissue in vitro using biodegradable scaffolds: Investigating initial cell-seeding density and culture period. *J Biomed Mater Res* 2000;51:376–382.