Biomaterialization of marine-patterned C-scaffolds

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Patterned surfaces of marine-derived carbon scaffolds were subjected to a biomimetic approach to be covered with a calcium phosphate thin film. The process was based on Dulbecco's phosphate-buffered saline solution and investigated in different periods of immersion (from hours to days). A complete physicochemical characterization was performed to demonstrate the optimal calcium/phosphorus ratio, thickness and adherence to the substrate of these biomimetic calcium phosphate coatings, which still retained the naturally derived patterning. A chemical mechanism to explain the coating formation has been proposed and documented, based mainly on the presence of carboxylic groups on the C-scaffold surface, what promoted the anchorage of calcium ions at the first stage and the later binding of phosphate groups to calcium ions. The biological response of MC3T3-E1 preosteoblasts on the calcium phosphate-coated scaffolds was investigated to demonstrate the non-cytotoxicity, adequate morphology and spreading of cells after 7 d of culture, being this proliferation aligned, promoted by the patterning of the scaffold.

1. Introduction

Nowadays, the design of 3D scaffolds for the replacement, repair and regeneration of the different human tissues is one of the main pillars and challenges of regenerative medicine. In the case of bone tissue, it is important that its tissue engineering scaffolds present a determined surface chemistry, hierarchical porosity and surface topography that will promote cell attachment and differentiation.

One of the most successful strategies for improving the biocompatibility of the device is to provide it with a calcium phosphate coating resembling, thus, the mineral part of the human bone tissue (hydroxyapatite), which promotes an osteogenic, osteoconductive or osteoinductive activity. However, these bioactive ceramic coatings have also been cited as an efficient strategy to overcome the fibrous tissue encapsulation, a common problem for synthetic metallic and polymeric implants *in vivo*.¹

To produce bioceramic coatings, different techniques such as plasma spray, pulsed laser deposition or electron beam evaporation have

been intensively studied, but, trying to follow the main postulates that regenerative medicine promotes, the deposition of this calcium phosphate by means of a biomimetic process seems to be the main challenge. Thus, the biomimetic method was first developed by Kokubo² with the purpose of obtaining a calcium phosphate layer by using a simple process based on the immersion of the material (metal, polymer or ceramic) in a simulated body fluid (SBF) with ion concentrations similar to human blood plasma obtaining a bone-like apatite layer on various types of organic polymer and titanium substrates. However, traditional SBF solutions require of lengthy incubation times, typically more than 7 d, to get obtained a uniform calcium phosphate coating. Recent efforts to shorten the time needed for coating have focused on increasing ionic concentrations. Although still a matter of some controversy, the ability of implants to form a calcium phosphate in SBF is object of much interest, and it could give important information on *in vivo* behavior.^{3,4} The use of Dulbecco's phosphate-buffered saline solutions (DPBS; Sigma-Aldrich, Schnellendorf, Germany) instead of SBF has also been investigated as a biomimetic fluid. Results have been obtained

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with DPBS but with a different perspective since the titanium discs immersed in the DPBS already presented a calcium phosphate coating on them, for example, by electron beam evaporation.⁵ The idea in that case was to incorporate the calcium phosphate coating on other biological or ionic compounds added to the DPBS to increase functionality. The direct immersion of different materials in DPBS to obtain the calcium phosphate layer will be studied in this study.

On the other hand, the biodiversity that characterizes the marine environment represents an enormous potential for obtaining suitable 3D porous biostructures already patterned in nature.^{6–8} In recent years, several authors have used different marine species (coral skeletons, sea urchins and sponges) as 3D micro- and nano-scaled porous biomatrices where the results confirmed that the 3D topography, and the surface parameters of these materials helped to promote the cell differentiation.^{9–11} The sea rush *Juncus maritimus* Linnaeus has a vascular system uniformly distributed throughout its section that provides a hierarchical interconnected macro-, micro- and nanoporosity distributed along the entire plant. As an added value, this plant presents a double surface patterning in the upper epidermal layers aligned in the direction of the plant's growth. The supporting material proposed in this study to be immersed in DPBS will consist on a bioinspired carbon scaffold (C-scaffold) obtained from *J. maritimus*, with hierarchical porosity and surface topography maintained from the original plant that has already been proved to promote cell attachment and alignment as well as an important osteogenic activity.^{10–12}

The intrinsic properties of these marine C-scaffolds together with the strong biocompatibility of calcium phosphate coatings have inspired the design of the bioceramic presented in this study. Thus, the aim of this study was to obtain a calcium phosphate layer on the porous C-scaffold obtained from *J. maritimus* by a simple biomimetic method using DPBS solution. The physicochemical study, the fundamentals of the calcium phosphate anchorage on the surface scaffold and a preliminary biocompatibility evaluation of the calcium phosphate-coated scaffold by using preosteoblastic cells are presented.

2. Materials and methods

2.1 Preparation of C-scaffolds

The cylindrical scaffolds were obtained as follows: The sea rush *J. maritimus* was subjected to air drying for several days and then introduced in a pyrolysis furnace where the thermal decomposition was carried out by a gradual increase in temperature of 2°C/min up to 500°C. The furnace was then maintained at that temperature for 10 min, followed by a gradual decrease of 20°C/min down to room temperature. The obtained carbon samples were cut to obtain pieces of 15 mm in length and 2 mm in diameter. Finally, to remove the remaining marine salts (sodium chloride and potassium chloride) scaffolds were subjected to ultrasonic baths of 60 min with warm milli-Q water, renewing it every 15 min and dried at room temperature. More details on the scaffold fabrication can be found in previously published works.^{10,14}

2.2 Biomimetic process

Containers used for holding and immersing the samples were ultrasonically washed with milli-Q water followed by HCl 1N and, again, milli-Q water to, finally, let them dry at room temperature. On the other hand, the carbon samples were cleaned in ultrasound baths with milli-Q water during 60 min. Then, C-scaffolds were placed in the upright position in the containers and immersed with 50 ml of DPBS (Sigma Aldrich) at $60.0 \pm 0.5^\circ\text{C}$ for 6 h and 7 d, respectively. After each incubation time, samples were rinsed with warm milli-Q water during 40 min and air dried slowly at room temperature.

2.3 Physicochemical characterization

The morphology and semiquantitative elemental composition of the C-scaffold, before and after the biomimetic process were studied under a scanning electron microscope (SEM) Philips XL30 (Eindhoven, The Netherlands) equipped with an energy-dispersive X-ray spectroscopy (EDS; Eindhoven, The Netherlands). EDS was used to confirm the presence of calcium and phosphorous ions and to elicit information regarding the calcium/phosphorus ratio of the biomimetic coatings. The pore distribution was measured by mercury porosimetry with an Autopore IV 9500 Micromeritics, Norcross, USA (University of Santiago). The thickness of the calcium phosphate biomimetic coating was evaluated in transversal and longitudinal section by introducing them in resin, followed by polishing of the cylindrical sample and the evaluation of the interface between the C-scaffold and the calcium phosphate coating by using back scattering electron detector (BSE; Eindhoven, The Netherlands) and the software Image J (Java, National Institutes of Health, USA). Six measures were taken in different points of one section, at different sections and samples. The Fourier transform infrared (FTIR) spectra were collected using a Thermo Nicolet 6700 Fourier transformed infrared spectrometer (Madison, USA) in the range $4000\text{--}450\text{ cm}^{-1}$. The FTIR spectra were obtained in transmission mode by using pressed potassium bromide tablets. These tablets were prepared by grinding the sample (2 mg) with a completely dehydrated potassium bromide (200 mg) in an agate mortar (5–10 min). To complement the study, effusion measurements with a heating ramp of 2°C/min were carried out on the C-scaffold.

2.4 In vitro cell culture

A cell suspension of the preosteoblastic line MC3T3-E1 (ECACC, UK) of 1.7×10^5 cells/ml in 100 µl of MEM- α (Sigma-Aldrich, St. Louis, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen, USA) was added directly on the surface of the calcium phosphate-coated scaffolds, which were placed in 96-well tissue culture plates. Cells were cultured for 7 d in a humidified atmosphere with 5% carbon dioxide and at 37°C. The culture medium was renewed every 2–3 d.

Cell morphology was analyzed by SEM. Three replicates were fixed and dehydrated, as described elsewhere,¹³ to be analyzed using Philips XL30 and FEG JEOL JSM 6700F (Tokyo, Japan). The cellular cytoskeleton was observed by confocal laser scanning microscopy (CLSM). After 7 d of incubation time, cells were fixed with a solution of paraformaldehyde as described elsewhere.¹⁰ Alexa fluor 488 phalloidin (Life Technologies, California, USA) and propidium iodide (Sigma-Aldrich, St. Louis, USA) solutions were added to visualize the cellular cytoskeletal actin filaments and cell nuclei, respectively. Cells were observed with a confocal microscope Bio-Rad MRC 1024 (California, USA).

2.5 Statistical analysis

The data of the thicknesses of the calcium phosphate layers were obtained through scanning electron micrographs in six random fields and were averaged for each sample. Data are indicated as mean $\pm$ standard deviation. The significant differences were assessed with the probability associated with two-tailed Student's *t*-test.

3. Results and discussion

3.1 C-scaffolds' microstructure

Figure 1(a)–1(c) shows the microstructure of the marine plant *J. Maritimus* by SEM micrographs, where a highly porous structure can be observed distributed through the whole section of the plant even to the outer layers in an interconnected network. Figure 1(b) presents higher-magnified images of the pores of 20–25 μm , that correspond to the xylem, and of around 5 μm , the phloem, more clearly shown in Figure 1(c). Figure 1(d)–1(f) shows the microstructure of the bioinspired C-scaffold obtained from this marine plant where the preservation of the original porosity of the sea rush can be observed with hierarchical distribution of pore sizes disposed through the whole section of the carbon (Figure 1(d)), with pores of 20–25 μm (Figure 1(e)) and around 5 μm (Figure 1(e) and more clearly in Figure 1(f)) perfectly preserved.

Figure 1. Scanning electron micrographs in cross-section at different magnifications of natural *Juncus maritimus* (a–c) and carbon scaffolds (d–f). White star indicates xylem and black star phloem

Figure 2, previously reported in Ref. 10, presents the pores' distribution of these bioinspired C-scaffolds obtained by mercury porosimetry. A three-modal distribution is shown with macropores (80–150 μm in diameter), mesopores (1–10 μm) and nanopores (0.03–0.2 μm) with higher volume of pores of 3 and 8 μm followed by 0.15 μm and of around 140 μm . The per cent of total porosity resulted in a value of 63%. These results of porosity give this material a great potential as scaffold since that high porosity levels will support migration and proliferation of osteoblasts and mesenchymal cells, bone tissue ingrowth, vascular invasion, nutrient delivery and matrix deposition in empty spaces. In fact, the presence of macroporosity (size $>100 \mu\text{m}$) has a critical impact on osteogenic outcomes, promotion of vascularization and mass transportation of nutrients and waste products.¹⁵ Meso- and microporosity, size around 10 μm , favor capillary formation and, finally, nanoporosity allows diffusion of molecules for nutrition and signalling¹⁶, and it has been demonstrated to favor the adsorption of proteins for the anchorage of the cells.^{17,18}

Figure 3 presents the surface patterning of the marine plant and the C-scaffold by SEM micrographs. The macropatterning of the natural plant with ridges of around 100 μm in width oriented in the plant's growth direction (Figure 3(a)) containing a micropatterning with ridges of around 7 μm oriented in the same direction (Figure 3(b)) are shown. Arranged in rows, the stomata can be observed (in Figure 3(a)), which corresponds to oval-shaped pores used by the plant to regulate the gas exchange and water loss. The double macro- and micro-scaled patterning and the stomata appeared perfectly preserved or even enhanced on the C-scaffolds (Figure 3(c) and 3(d)). This surface patterning makes of this scaffold as a very promising structure to promote cell alignment (as previously referred in Ref. 10). At the same time, it has been demonstrated that the surface topography, at the micrometer and submicrometer level, affects the attachment, proliferation and differentiation of cells on biomaterials.^{19–21} An increase in surface porosity and roughness of biomaterials improves cellular attachment, proliferation, differentiation and bone-ingrowth^{17–22}; thus,

Figure 2. Pore size distribution obtained by mercury porosimetry of carbon scaffolds (data modified from Ref. 10; <http://dx.doi.org/10.1088/1748-6041/6/4/045012>; reproduced by permission of IOP Publishing. All rights reserved)

Figure 3. Scanning electron micrographs at different magnifications of the surface for natural *Juncus maritimus* (a–b) and its corresponding carbon scaffold (c–d)

the presence of the stomata in the scaffold can help the new formed tissue to better attach to the scaffold by this bone-ingrowth.

In order to deepen on the characterization of this C-scaffold, FTIR and effusion measurements were carried out. Figure 4 presents the FTIR spectrum of the scaffold where the main vibration modes were identified as follows:

- Hydrocarbons groups located between 3044 and 2760 cm^{-1} . The C-H groups exhibited sharp peaks located between 2800 and 2960 cm^{-1} associated to CH_2 symmetric stretching (2860–2875 cm^{-1}), CH_2 asymmetric stretching (2910–2930 cm^{-1}) and C-H₃ asymmetric stretching (2950–2960 cm^{-1}).^{23–25} Furthermore, the peak at 873 cm^{-1} attributed to C-H out of plane bending in benzene derivatives.²⁶ In fact, well-resolved peak emerged at 1380 attributed to C-H₃ methyl umbrella bending mode.²³
- Oxygen-containing functional groups²⁷ such as C=O (1700–1765 cm^{-1}) and C-O (1230–1100 cm^{-1}). The absorption band of OH bonds, found in the range of 3600–3400 cm^{-1} , is due to the adsorbed molecules of water.²⁸ At the same time, the absorption bands found at 2388 and 2278 cm^{-1} are attributed to the carbon dioxide adsorbed from the atmosphere.²⁹ On the other hand, the broad OH stretch band between 3080 and 2980 cm^{-1} could correspond to carboxylic acid present at the surface of the C-scaffold²³ as occurs with the weaker band at 1735 cm^{-1} associated to a C=O stretching mode and with the band at 1110 cm^{-1} attributed to C-O stretching mode. The band at 1430 cm^{-1} position could be attributed to OH in plane bending and the peak at 750 cm^{-1} is associated to carbon monoxide bending vibration in-plane.^{23,29} Furthermore, the peaks in the region of 1230 and 1210 cm^{-1} indicate the appearance of aromatic phenyl C-O.³⁰

Figure 4. Fourier transform infrared spectrum of carbon scaffold before being immersed in Dulbecco's phosphate-buffered saline solution

Concerning the effusion experiment (Figure 5), the decomposition temperature of the C-scaffold and the partial pressures obtained, allowed us to assess the nature of the groups on its surface. The main functional groups were thermally decomposed at different temperatures during the effusion experiment releasing first water vapor around 100°C, carbon monoxide and carbon dioxide at 400°C, again carbon monoxide, water vapor and little of carbon dioxide at around 550°C and, finally, around hydrogen at 650°C. Regarding the thermal decomposition studies, it has been already published³⁰ that the most of the oxygen complexes on the carbon's surface, decompose as carbon dioxide and carbon monoxide. At the same time, the amounts and temperatures at which these gases appeared are characteristic of the oxygen-containing surface groups, and it is proven that the carbon dioxide and carbon monoxide desorption profiles correspond to the decomposition of the carboxylic anhydride group.

3.2 Biomimetic calcium phosphate coating

Figure 6 shows the surface of the C-scaffolds after being immersed in DPBS solution at 60°C for 6 h (Figure 6(a)–6(c)) and 7 d (Figure 6(d)–6(f)) by SEM micrographs at three different magnifications. It can be observed as after 6 h of incubation a homogeneous calcium phosphate layer appeared covering the complete surface perfectly adapted to the patterning surface of these C-scaffolds (Figure 6(a)). Even the inside of a stoma seems to be filled (Figure 6(b)). The calcium phosphate layer was composed by random clusters of nanoflakes where the needle-like particles could be perfectly appreciated (Figure 6(c)). After 7 d, (Figure 6(d)–6(f)), the layer was again covering the entire surface of the scaffold, adapting to the macro- and micro-patterning and to the surface topography, preserving it. The stoma was completely filled and covered by the layer of calcium phosphate (Figure 6(e)). The incubation time did not affect the morphology of the calcium phosphate layer formed on the surface of the C-scaffolds, which presented, in both cases (6 h and 7 d), a morphology based on nanoclusters randomly organized (Figure 6(c) and 6(f)).

Figure 5. Partial pressures of carbon monoxide, carbon dioxide, hydrogen and water from effusion measurements of carbon scaffold before being immersed in Dulbecco's phosphate-buffered saline solution

Figure 6. Scanning electron micrographs of the surface of the carbon scaffolds immersed in Dulbecco's phosphate-buffered saline solution for 6 h (a–c) and 7 d (d–f) at three magnifications

Figure 7 presents the C-scaffold immersed in DPBS for 7 d in a detail of a longitudinal section by SEM micrographs (Figure 7(a) and 7(b)) and the EDS spectra of C-scaffolds after immersion in DPBS solution for 6 h (Figure 7(c) and 7(d)). Figure 7(a) shows the calcium phosphate coating, that was only distributed

Figure 7. Scanning electron micrographs of calcium phosphate C-scaffolds in longitudinal section after 7 d of immersion in Dulbecco's phosphate-buffered saline solution (a, b) and energy-dispersive X-ray spectroscopy spectra after 6 h (c) and after 7 d (d).

at the surface of the scaffold, maintaining intact the impressive interconnected and hierarchical porosity of the inside of the C-scaffold. In Figure 7(b), detail of the calcium phosphate coating is presented where an entire and compact layer can be clearly appreciated. To confirm the presence of calcium and phosphorous ions and to elicit information regarding the calcium/phosphorous ratio of the biomimetic coatings, EDS analysis was performed (Figure 7(c) and 7(d)). Figure 7(c) presents the spectra obtained for the surface of C-scaffold immersed in DPBS for 6 h and Figure 7(d) for 7 d. As it can be observed, both EDS spectra revealed the presence of calcium and phosphate elements on both coatings. The carbon signal came from the scaffold itself. Furthermore, EDS semiquantitative measurements established with the ratios between calcium and phosphorous intensities detected, showed approximate values for calcium/phosphorus ratio of 0.63 for 6 h coating and of 0.93 for 7 d.

The most searched calcium/phosphorus ratio found in literature for many years has been 1.67, what corresponds to stoichiometric hydroxyapatite. This material has been widely used as bone graft or as coatings on metal alloys to improve bone growth around orthopedic implants. The ratio of 1.5 corresponds to tricalcium phosphate, extensively used as drug delivery agents or injectable cements for filling bone defects. It has been recently published³¹ that an *in vitro* study of the calcium/phosphorus stoichiometry influence in different applications of calcium phosphate ceramics such as coatings or as bone cements for filling defects. Thus, different calcium/phosphorus ratios (0.5, 1.0, 1.5, 2.5) were tested, and the more appropriate response as a coating was found for calcium/phosphorus ratio 1.0 with higher viability of osteoblasts together with significant highest alkaline phosphatase activity values. Thus, the ratio of 0.93 obtained for the calcium phosphate layer on the C-scaffold at the present work after immersion in DPBS for 7 d corresponds perfectly with the intended application as a coating.

Figure 8. Fourier transform infrared spectrum of carbon scaffold after being immersed in Dulbecco's phosphate-buffered saline solution

At the same time, the thicknesses of the calcium phosphate coatings were estimated, as previously mentioned, by analyzing the resin-immersed coated C-scaffolds in cross-section with BSE, and then measurements were performed with the Image J software. Thus, the calcium phosphate layers thicknesses were of $0.8 \pm 0.2 \mu\text{m}$ and $0.9 \pm 0.2 \mu\text{m}$ after 6 h and 7 d of DPBS immersion, respectively, without a statistically significant difference between them. As a result of this, the authors concluded that the growth of the calcium phosphate coating, in a static process, was already stabilized after 6 h of immersion probably due to the depletion of ions from the DPBS solution.

Figure 8 presents the FTIR measurement carried out on the C-scaffold after immersion in DPBS to characterize the coating on the scaffold. Thus, as it can be observed, it was found that the typical stretching (at 1054 cm^{-1}) and bending (at 605 cm^{-1}) peaks attributed to phosphate groups. Moreover and in agreement with the results previously reported at Figure 4, the spectrum presented the bands already identified for the uncoated C-scaffold.

Finally, and in order to understand the mechanism of the calcium phosphate anchorage on the surface of the C-scaffold, the results of the FTIR and effusion experiments together with literature have been discussed as follows: Thus, C-scaffolds were obtained after a pyrolysis up to 500°C in oxygen atmosphere. This heat treatment in an oxygen atmosphere increased the concentration of the oxygen groups on the surface of carbon samples³⁰ and that temperature caused the decomposition of carboxyl groups of the surface.²⁹ After pyrolyzation, the C-scaffolds were subjected to a cleaning process in distilled water, which promoted the subsequent hydrolysis and the formation of the carboxylic acid groups (already shown in FTIR spectra at Figure 4) by means of the anhydride carboxylic groups. As it was previously published by Otake *et al.*,³⁰ those carboxylic acid groups present on the C-surface can be neutralized by divalent ions as, in that case, barium hydroxide. Moreover, and concerning with the formation mechanisms of a calcium phosphate coating through *in vitro* immersion in SBF proposed by Kokubo *et al.*,³² they demonstrated that functional groups with negative charge on the substrate will favor the nucleation and accumulation of ions from the SBF with positive charge promoting, at this way, the first bond to obtain

Figure 9. Scanning electron micrographs at three different magnifications of MC3T3-E1 *in vitro* test on C-scaffold after 7 d of phosphate-buffered saline incubation

Figure 10. Morphological evaluation by confocal laser scanning microscopy of MC3T3-E1 cells on C-scaffold after 7 d of incubation. (a) Maximum projection image and (b) an image obtained going in a certain depth in the z axis. Cell nuclei and F-actin filaments were stained in red and green, respectively

the bone-like apatite layer. In the case of carbon materials, the presence of carboxyl groups make the surface negatively charged, as it has been already proven³³ on the functionalized carbon nanotubes. Therefore, by combining these theories with FTIR spectroscopy results and effusion experiments, the authors have elucidated that the negatively charged surface of acidic oxygen of the C-scaffolds can be neutralized after immersion in DPBS solution by combining electrostatically with the dissolved Ca^{2+} ions at the DPBS solution. Later, while the concentration of Ca^{2+} ions at the C-scaffold surface increases, the neutral charge will become positive starting, then, the electrostatic attraction of negatively charged PO_4^{3-} ions, again from DPBS, to form the calcium phosphate layer bonded to the surface of the C-scaffold.

3.3 Coatings biocompatibility

In order to check the biocompatibility of these calcium phosphate-coated C-scaffolds, samples were incubated with the MC3T3-E1 preosteoblastic cell line for 7 d. Figure 9 presents the cells morphology on the surface after 7 d of incubation in three different magnifications. As it can be observed in Figure 9(a), almost the whole surface has been covered by a layer of cells that have proliferated by extending their filopodia mainly in the direction marked by the patterning of the surface of the C-scaffold (Figure 9(b)) and with the characteristic flat morphology of this osteoblast-like cells adapting perfectly to the surface topography (Figure 9(b) and 9(c)). It is clearly observed in Figure 9(c) that the calcium phosphate coating is still covering the whole surface after 7 d of incubation and how the cells still aligned in the pattern direction, as occurred in the uncoated scaffold described by López-Álvarez *et al.*¹⁰

The cytoskeleton of the cells after 7 d of incubation was analyzed by CLSM (Figure 10). The cell nuclei are seen in red and the actin filaments of the cytoskeleton in green. In Figure 10(a), the oriented actin fibers, as well as the nuclei, can be clearly observed. The completely covered layer of cells can be appreciated and

also, as their actin filaments are preferentially oriented in the direction of the patterning of the material (indicated by the white arrow) and in both the lower parts of the profile (grooves) and the higher ones (ridges). It is an interesting fact that the coating did not modify at all the surface patterning of these scaffolds so that they continue promoting the cell alignment as it did in the uncoated scaffold previously published.¹⁰ When going in depth in the z axis through the coating and at higher magnification, several images of isolated cells could be observed and again they were growing by following the direction of the patterning of the scaffold (Figure 10(b)).

4. Conclusions

The viability to obtain calcium phosphate coatings on C-scaffolds from marine sources using a biomimetic method was demonstrated. A homogeneous calcium phosphate layer, composed by random clusters of nanoflakes with needle-like particles, with around 1 μm of thickness was obtained in short periods of time (hours). The calcium/phosphorus ratio obtained after immersion in DPBS for 7 d was of 0.93, which is within the values recommended for a better osteoblastic behavior in coating applications. The mechanism of calcium phosphate engagement on the C-scaffolds surface has been concluded as follows: negatively charged surface of C-scaffolds are neutralized by combining electrostatically with Ca^{2+} ions from DPBS solution and while the concentration of Ca^{2+} ions at the C-scaffold surface increases, the neutral charge will become positive, initiating then the electrostatic attraction of negatively charged PO_4^{3-} ions, again from DPBS, to form the calcium phosphate coatings. Finally, their biocompatibility was demonstrated by preliminary *in vitro* studies where preosteoblastic cells (MC3T3-E1) that presented healthy morphology and proliferated by covering the entire scaffold. A good adherence of the calcium phosphate coatings during the 7 d of cell incubation was observed together with the cell alignment. This study demonstrated the viability to develop and assemble in a single device the excellent osteoconductive properties inherent in the C-scaffold with the osteoinductive properties provided by the surface patterning together with the calcium phosphate coating.

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