Studies on the Formation of Biomimetic Apatite Layers on 3D-Printed Biodegradable Polymeric Scaffolds: Effect of Different Dynamic Coating Routes

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Statement of Purpose: On what concerns to the fabrication of three-dimensional (3D) architectures for Tissue Engineering (TE) of hard tissues the appropriate surface chemistry for cell attachment, differentiation and proliferation is a key issue that always needs to be addressed. An attractive approach is to coat the scaffold with a bone-like apatite layer in order to induce a bioactive behavior. This can be achieved through a biomimetic route, in the presence of a simulated body fluid (SBF) [1,2]. However, due the incremented complexity, there are only few works reporting the coating of biodegradable porous 3D architectures. In fact problems can arise from substrate degradation and due to difficulties on coating the inner pores of the scaffolds without compromising its initial morphology. In order to overcome these problems a biomimetic coating methodology has been previously proposed [2]. It uses a sodium silicate gel as an alternative nucleating agent for the formation of the apatite layer. Based on this methodology we propose in the present study a novel dynamic biomimetic coating route on which the scaffolds can be evenly coated inside of a specially designed flow perfusion bioreactor. This condition is more close to the *in vivo* scenario where the flow of human blood plasma may have an effect on the formation of bone apatite.

Materials and Methods: A blend of starch/polycaprolactone (SPCL, 30/70 %wt) was used for the first time, in the production of scaffolds using a 3D printing technology (Bioplotter, EnvisionTec GmbH, Germany). When compared with other methods, rapid prototyping enables a superior control over scaffold morphology, allowing for the fabrication of scaffolds with designed porosity and interconnectivity. In this case alternated layers with a 0º/90º/0º/90º orientation period were generated. The selected biomaterial presents a rather good biological behaviour [3]. To produce the biomimetic coatings cubic samples (0,5 cm^3) were impregnated with a sodium silicate gel followed by immersion in a simulating body fluid (SBF, pH=7.4, 37 ºC) with an ion concentration nearly equal to the human blood plasma, for 7 days. The samples were then placed in contact with a renewed SBF solution under different conditions: static, agitation (80 U/min.) and circulating flow perfusion (Q=4mL/min; t_R=15s) for periods up to 14 days. In all the conditions the total volume of SBF available *per* sample was 50 mL (solid-to-liquid ratio of 1g/400mL/week). For the last condition a special bioreactor was designed. After the coating process the samples were washed in distilled water and dried at room temperature. The as-treated scaffolds and the evolution of the biomimetic apatite coatings were characterized by Scanning Electron Microscopy (SEM)/Electron Dispersive Spectroscopy (EDS), Fourrier Transformed Infra-Red (FTIR) and Thin-Film X-Ray Diffraction (TF-XRD). SEM and FTIR were performed specifically to the coatings in different parts of the scaffold: top, bottom and lateral sides and middle of the sample, in order to investigate any changes in the morphology or chemical structure of the scaffold. The cross-sections were also analyzed and the coating thickness measured. The pH of the solutions was monitored and the elemental composition was followed by Induced Coupled Plasma Spectroscopy (ICP).

Results and Discussion: SPCL scaffolds were successfully produced by a 3D printing technology (Fig. 1.a)). These scaffolds exhibited a controlled, with controlled morphology and porosity. Sodium silicate treatment followed by immersion in SBF solution resulted in the formation of a well defined bone-like apatite layer after the first week under static conditions (Fig.1.b) that exhibit the typical needle-like structure when observed by SEM at higher magnifications. This layer was well adhered to the surface and could also be observed inside of the scaffolds, clearly covering each fiber without compromising its initial morphology. A standard biomimetic treatment is never so effective on reaching the bulk of complex structures [1]. By FTIR and TF-XRD analysis it was possible to confirm that a poorly crystalline carbonated apatite was formed, being very similar to bone apatite.

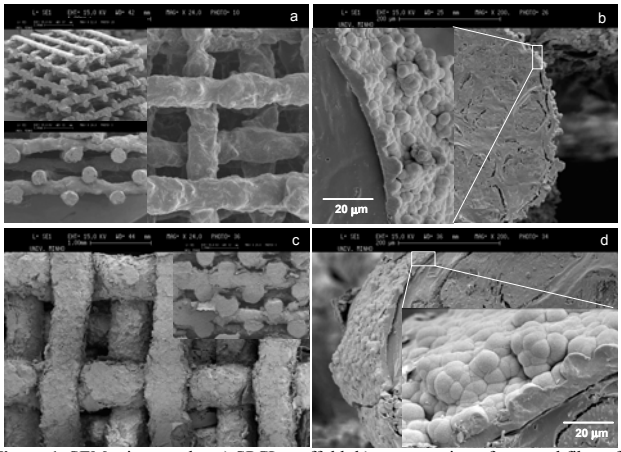

Figure 1. SEM micrographs: a) SPCL scaffold; b) cross-section of a coated fiber after 7 days in SBF; c) coated scaffold after 14 days in SBF from which the last 7 were under flowing conditions (side facing the flow) and d) correspondent cross-section.

After the second week, when comparing static, agitation and circulating flow perfusion (Fig.1.c) conditions, several differences were observed in the obtained coatings. For instances, the coating thickness (Fig. 1.d) was higher in the perfusion condition, while the adhesion of the apatite layer was poorer when agitation was present, due to a higher degradation of the substrate. By means of observing the cross-sections, it was possible to detect, in case of the static condition, that apatite tended to accumulate in the intersection of the fibers, probably due to a locally increase in Ca and P concentration in the SBF. No differences in the apatite morphology and chemical structure were detected from the inside to the outside of the scaffolds, with the exception of the perfusion condition for which a densification of the apatite structure was observed in the coating side facing the flow. Nevertheless, by FTIR no changes could be detected on coating chemical structure.

Conclusions: By using different biomimetic environments it was possible to effectively coat a bone-like apatite layer on newly developed SPCL scaffolds. Dynamic conditions, besides mimicking better the biological milieu, allowed to obtain more stable apatite layers that can better follow the contour of the scaffold complex morphology. The circulating flow perfusion condition has proved to be advantageous over agitation one, since in the later case a higher degradation of the substrate was observed.

Acknowledgements: Portuguese Foundation for Science and Technology (PhD grant to A. L. Oliveira SFRH/BD/10956/2002 under POCTI Program). This work was partially supported by FCT through POCTI and/or FEDER programmes. This work was also partially supported by the EU Project HIPPOCRATES (NMP3-CT-2003-505758) developed in the scope of EU Project EXPERTISSUES (NMP-CT-2004-500283).

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