

## Design and Characterization of Biomimetic Ca-P Coated 3D Printed Scaffolds for Bone Tissue Engineering

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**INTRODUCTION:** 3D printing technology, in the bone tissue engineering, holds promising perspective which permits to manipulate bone scaffolds to patients' specific needs. Among other important properties, osteoconductivity is also essential parameter for creating successful bone tissue scaffold to enhance the attachment and migration of osteoblasts and osteoprogenitor cells. Biomimetic coating, using simulated body fluid (SBF), is a simple alternative method to the harsh apatite coating processes. In the present study, we aimed to obtain bone-like apatite layer onto 3D printed polymeric scaffolds by means of using a biomimetic approach and to investigate the effects of different surface treatment and immersion conditions.

**METHODS:** 3D design of the scaffolds was created using SolidWorks® and imported into the printing software CURA as an STL. Scaffolds with dimensions of 1.6 x 1.6 x 0.6 cm have 3 distinct layers, that first layer stays in 90° angular to y axis, then second layer stays below it, with 0° angle to y axis. Third layer positioned at 45° angular position to y axis, then the sequence continues with first layer. They were fabricated from filaments of poly(L-lactide) and polycaprolactone blend (PLLA/PCL, 70/30 w/w) using a 3D printer (Ultimaker 2-Go, Ultimaker, Netherlands). Prior to biomimetic coating, the surfaces of the scaffolds were modified by dipping them into 1M NaOH, saturated CaCl<sub>2</sub> and saturated K<sub>2</sub>HPO<sub>4</sub> solutions, consecutively. After drying at 37°C, the 3D constructs were placed into falcon tubes containing 1x SBF solutions and incubated at 37°C for up to 21 days. To investigate the effect of immersion conditions, 3 groups were determined: samples with continuous agitation, solution renewed and still. The scaffolds were then removed from the solutions, rinsed with distilled water, dried, and characterized by different methods, including scanning electron microscopy (SEM), Electron Dispersive Spectroscopy (EDS) and X-ray Diffraction (XRD) analysis.

**RESULTS:** The SEM images revealed that scaffolds, pre-treated with 1M NaOH alkaline solution for 3 hours, and then followed by that 0.2M CaCl<sub>2</sub> and 0.2M K<sub>2</sub>HPO<sub>4</sub> solutions, were able to induce the formation of biomimetic Ca-P layer on scaffold surfaces.

Three distinct groups with different immersion conditions were tested for biomimetic Ca-P coating approach. Despite EDX results represented clear Ca-P layer formation for all groups, needle-like crystal formation was observed for the group with continuous agitation unlike the others. Regarding XRD results, both frequent solution renewals and 100 rpm agitation, resulted to decrease in diffraction peaks which correspond to apatite. Nonetheless, its characteristic peaks were apparent for all groups at the end of 21 days of immersion in SBF, indicating the successful biomimetic coating.

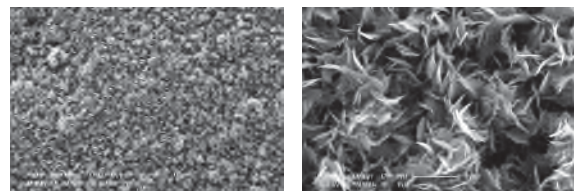


Fig. 1: SEM micrographs of PCL/PLA scaffold immersed in SBF with agitation for 21 days; x5000 (left) and x50000 (right).

**DISCUSSION & CONCLUSIONS:** By using 3D printing and biomimetic coating approach, bone-like apatite structure was successfully formed on printed PLLA/PCL scaffolds. These observations were confirmed by SEM and EDS and XRD analysis. Moreover, it was found that immersion conditions could affect the crystalline structure of formed apatite. The further investigations will be more focused on *in vitro* and *in vivo* biocompatibility of the produced structures to confirm their potential as a preferable alternative to existing bone tissue engineering scaffolds.