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# Bioactivity assessment of additively manufactured doped-HA composite scaffolds for bone tissue engineering

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*Abstract: Composites are promising candidates for treating bone defects, but manufacturing of composite scaffolds is challenging. This study aimed to fabricate composite scaffolds based on polycaprolactone (PCL) and doped Hydroxyapatite (HA) via a single step melt extrusion additive manufacturing technique. Starting from the raw powder forms, the printed scaffolds were produced and then characterized for morphology, mechanical behavior and in vitro mineralization. MicroCT revealed the dispersion of ceramic particles in the PCL matrix. Also, SEM showed the ceramic particles on the surfaces of printed scaffolds. Furthermore, bioactivity assays confirmed the enhanced apatite deposit formation on composite scaffolds compared to PCL alone.*



## I. Introduction

Natural bone is a composite of organic (collagen) and inorganic (calcium phosphate) materials that in combination provide bone with flexibility and load bearing properties. The use of composites with adequate mechanical properties and bone forming ability (i.e. bioactivity) are needed for treating bone defects. PCL has been widely investigated for bone tissue applications as it is biocompatible, biodegradable and for its good processability. However, PCL is hydrophobic and has low interaction with surrounding tissues, including low bioactivity. Consequently, to enhance the bioactivity of this material the combination with calcium phosphates (i.e. HA and its doped forms) has been of interest for the research community [1]. Doping of HA with Sr & Zn enhances its osteogenic potential, Zn also adds anti-bacterial properties [2,3].

However, the manufacturing of composites is still a challenging task. Particularly, solvent dissolution and/or filament production are two key steps in the majority of biomanufacturing methods, including 3D printing. PCL is usually dissolved in a solvent and the ceramic particles are suspended to create a composite paste, which is then printed, whereas in the powder form polymers and ceramics are mixed and subsequently extruded to create material coils, before further processing [4]. In this work, a solvent free and single-step melt-extrusion process was used to manufacture PCL composite scaffolds with tailorable bioactive properties and mechanical characteristics.

## II. Materials and methods

Cylindrical porous composite scaffolds, see Fig 1a, with 7mm diameter and 0.8 mm inter strand spacing were additively manufactured using an EnvisionTEC BioPlotter via melt extrusion of raw materials. The composite powders containing 10% and 20% w/w of SrHA and ZnHA in PCL (Polyscience Europe Ltd) were processed following the protocol reported in [1] and 3D scaffolds produced according to the conditions reported in Table 1.

*Table 1: Compositions and 3D printing processing conditions*

| ***Sample*** | ***Printing Temperature (°C)*** | ***Printing Pressure (bar)*** | ***Printing speed (mm/s)*** |
|---|---|---|---|
| ***PCL*** | 130 | 6.0 | 0.6 |
| ***PCL/10SrHA*** | 140 | 6.2 | 0.6 |
| ***PCL/20SrHA*** | 140 | 6.5 | 0.3 |
| ***PCL/10ZnHA*** | 140 | 6.2 | 0.6 |
| ***PCL/20ZnHA*** | 140 | 6.5 | 0.3 |

The bioactive potential of the scaffolds was assessed via the in vitro mineralization assay in simulated body fluid (SBF), according to Kukubo protocol for 28 days [5]. All the scaffolds were individually soaked in 5ml SBF solution and stored at 37°C. The solution was changed twice a week and the scaffolds collected at each time point were dried overnight before further characterization. Surface morphology was evaluated by scanning electron microscopy (SEM), Hitachi FE-SEM SU5000 microscope equipped with energy dispersive X-ray (EDX). The internal microstructure of 3D printed scaffolds was characterized by Bruker Skyscan1275 micro computed tomography (MicroCT) and mechanical behaviour (n=5) via compressive tests using an Instron 5500S, equipped with a 500 N load cell and a constant loading rate of 0.5 mm/min.

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## III. Results and discussion

The manufacturing process was a single-step approach and did not require any post processing. An initial visual inspection of the scaffolds was conducted to verify the suitability of the printing conditions. MicroCT analysis confirmed the microstructure of the additively manufactured scaffolds, based on the designed digital file and reproducibility of the printing process. The scaffold showed a highly inter-connected porous structure as reported in the cross-sectional microCT view (see Fig 1b), which in addition evidenced the consistent distribution of the ceramic particles along each strand (see Fig 1c). Thus, proving the effectiveness of the melt extrusion process for additively manufacturing PCL composite tissue-like substitutes.

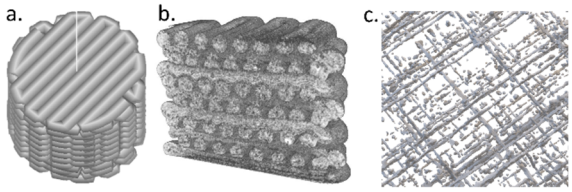


*Figure 1: Structure of scaffolds. a) CAD model of scaffold. b) Cross-sectional view of internal microstructure of composite scaffold showing distribution of 10SrHA in PCL polymeric matrix. c) Distribution of 10SrHA within the 3D structure.*

The SEM images revealed the presence of the doped HA particles on the surfaces of the composites' scaffolds at day 0 (see Fig 2), whereas a smoother surface characterized the pure PCL scaffolds. Also, the formation of new apatite deposits increased on the surface of composite scaffolds after soaking in SBF solution for 28 days (Fig 2) in comparison to PCL. EDX analysis confirmed the chemical composition of the deposits, based primarily on calcium and phosphorus. Amongst all the scaffolds the largest apatite deposits were observed on PCL/20SrHA scaffolds, implying that the addition of 20% w/w of SrHA to PCL greatly improved its bioactivity. This is in line with the enhanced apatite formation with increasing SrHA content observed by previous studies [4]. In relation to the mechanical properties, these were found in the range of human cancellous bone, even after 28 days in SBF immersion, as reported in Table 2.

## IV. Conclusions

In this study a solvent free manufacturing technique was successfully used for the fabrication of PCL composite scaffolds with high reproducibility and interconnected architecture. All the produced scaffold showed suitable mechanical properties for bone tissue applications; although, the composite combinations increased the bioactive potential of PCL scaffolds by attracting more apatite deposition, and with 20SrHA showing most profound effect. Further biological evaluation would be conducted with the composite scaffolds to confirm their bioactive potential *in vitro* as well as anti-bacterial properties. Hence, this manufacturing approach could be further investigated to produce patient-specific implants with tailorable properties.

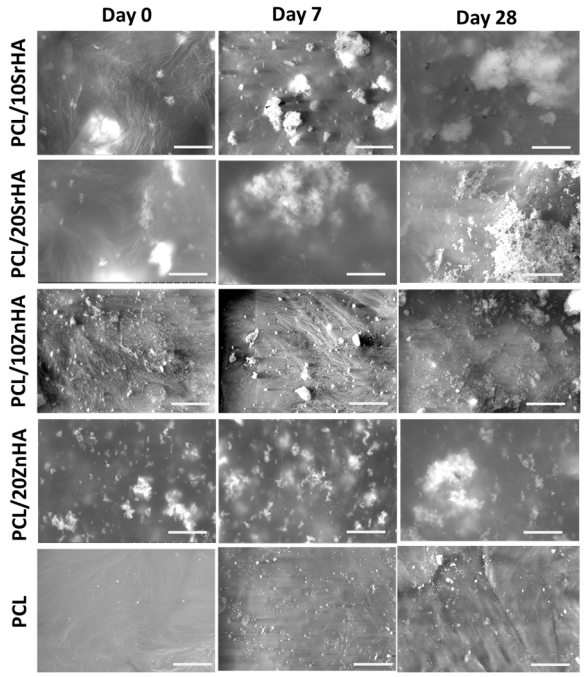


*Figure 2: SEM images of composite scaffolds at different time points depicting apatite crystal formation on different compositions. Scale bars 3µm*

*Table 2: Mechanical properties of 3D scaffolds*

| ***Sample*** | ***Compressive Modulus - Day 0*** | ***Compressive Modulus - Day 28*** |
|---|---|---|
| ***PCL*** | 47.9±2.44 MPa | 47.2±3.06 MPa |
| ***PCL/10SrHA*** | 44.2±0.85 MPa | 39.3±0.52 MPa |
| ***PCL/10ZnHA*** | 52.2±3.0 MPa | 44.4±0.94 MPa |

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### AUTHOR'S STATEMENT

Authors state no conflict of interest.

### REFERENCES

[1] D Pierantozzi. 3D printed Sr-containing composite scaffolds: Effect of structural design and material formulation towards new strategies for bone tissue engineering. Compos Sci Technol, vol 191, pp.108069. 2020.

[2] X Cui. Strontium modulates osteogenic activity of bone cement composed of bioactive borosilicate glass particles by activating Wnt/β-catenin signaling pathway. Bioact Mater, vol 5 no 2, pp. 334–347. 2020.

[3] YS Cho. Evaluation of the Antibacterial Activity and Cell Response for 3D-Printed Polycaprolactone/Nanohydroxyapatite Scaffold with Zinc Oxide Coating. Polymers, vol 12, no 10, pp. 2193. 2020.

[4] A Zimmerling. 3D printing PCL/nHA bone scaffolds: exploring the influence of material synthesis techniques. Biomat Res, vol 25 no 1, pp.1-12. 2021.

[5] T Kokubo. How useful is SBF in predicting in vivo bone bioactivity? Biomat, vol 27 no 15, pp.2907-2915. 2006

[6] G Turnbull. 3D bioactive composite scaffolds for bone tissue engineering. Bioact mat, vol 3 no 3, pp.278-314. 2018.