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Polymer-ceramic bone bricks for tissue engineering

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Abstract: This paper investigates the use of polymer-ceramic composite scaffolds for bone regeneration. Different ratios between Poly-ε-caprolactone (PCL) and Hydroxyapatite (HA) were considered. Scaffolds were produced using two different lay-down patterns (0/90° and 0/45°) and pore sizes (350µm, 500µm and 700µm). Compressive tests and cell proliferation tests are reported. Human adipose derived stem cells (hADSCs) were used for the biological characterization.

I. Introduction

As part of an EPSRC/GCRF (Engineering and Physical Sciences Research Council/Global Challenges Research Fund) we are developing a novel low cost osseointegrated modular prosthetic solution to treat large bone loss injuries to enable limb salvage [1]. The immediate application is to treat Syrian refugees who have been displaced to Turkey, but the knowledge can be applied to other conflict or natural disaster area. The project proposes to build on the current treatment of the external fixation but with the addition of an engineering internal prosthetic implant to improve patient outcomes, avoid painful limb lengthening and reduce recovery time. A patient specific prosthetic to fill the bone lost will be produced using biodegradable and biocompatible modular pieces (Bone Bricks-Figure 1), from a pallet of shapes and sizes that fit together. This paper investigates different polymer-ceramic compositions, topologies and lay-down pattern strategies. Structures were printed using additive manufacturing and were morphologically, mechanically and biologically characterized.

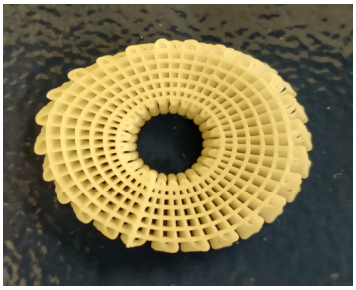

Figure 1: Example of bone brick produced using a 0/90º lay-dawn pattern and a pore size gradient (from 700µm to 300µm). The total porosity is 74%

II. Material and Methods

II.I Materials

PCL (CAPA 6500, M_w=50,000 Da) in the form of 3mm pallets was supplied by Perstorp Caprolactones (Cheshire, UK). It is a semi-crystalline linear aliphatic polymer, biocompatible and biodegradable, with low melting point and easy to print. HA (M_w=502.31 g/mol, MP=1100˚C) in the form of nanopowder (< 200nm particle size) was supplied by Sigma-Aldrich (Dorset, UK).

PCL/HA blends were prepared by melt blending considering four different concentrations (5, 10, 15 and 20 wt% of HA). PCL, in form of pallets, was melted at around 100°C, in a porcelain bowl, to guarantee the melting status of the polymer before adding the bioceramic material. The mixing of the material took approximately 1 hour to obtain a uniform mixture and after cooling the material was cut in small pallets.

II.II Scaffolds Fabrication

Scaffolds were produced using an extrusion based additive manufacturing machine (3D Discovery from RegenHU, Switzerland). Two lay-down patterns (0/90° and 0/45°) and three different pore sizes (350µm, 500µm and 700µm) were considered. Process parameters were: melting temperature of 90°C, screw rotation velocity of 12rpm and deposition velocity of 20mm/s. A needle diameter of 330µm was used.

II.III Scaffold Characterization

The morphological analysis was preformed using the HITACHI S-3000N (Hitachi, Japan) scanning electron microscopy (SEM). Compression tests were performed using the Instron 3344 (Instron®, USA). The scaffolds were placed in the centre of the machine and were compressed at 5mm/min rate and with a load of 2000N. The alamar blue assay was used to biologically characterize the scaffolds. Human adipose derived stem cells were used (STEMPRO, Invitrogen, USA). MesenPRO RS™ basal media, 2% (v/v) growth supplement, 1% (v/v) glutamine and 1% (v/v) penicillin/streptomycin (Invitrogen, USA) was used for cell culture.

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III. Results and Discussion

II.I Morphological Characterization

Produced scaffolds present a regular distribution of pores with uniform dimensions as shown in Figure 2. Results also show that the printed scaffolds presented pore sizes (366.17 ± 25.81μm, 522.60 ± 5.77μm and 721.56 ± 14.36μm) similar to the designed ones.

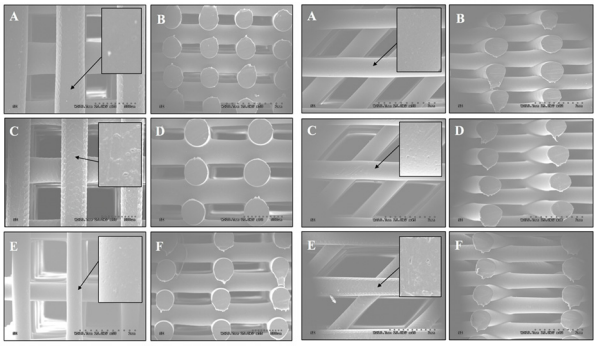

Figure 1: SEM images of (A) PCL/HA scaffolds with 350μm of pore size, (C) PCL/HA scaffolds with 500μm of pore size and (E) PCL/HA with 700μm of pore size. The left images correspond to scaffolds produced with a 0/90° lay-dawn pattern while the right images to scaffolds produced with a 0/45° lay-dawn pattern.

III. II. Mechanical Characterization

Compressive modules are presented in Figure 3. Results show that the compressive modulus decreases by increasing the pore size. For the same pore size and material composition the compressive modulus decreases by decreasing the angle between filaments (moving from 0/90º lay-down pattern to 0/45º). For the same lay-down pattern and pore size compressive modulus increases by increasing the HA concentration. Therefore, the highest value of 80.17MPa was achieved for a lay-down pattern of 0/90º, pore size 250μm and 20 wt% HA. The lowest value, 10.5MPa was obtained for the 0/45º lay-down pattern, 700 μm of pore size, PCL scaffolds without any HA reinforcement.

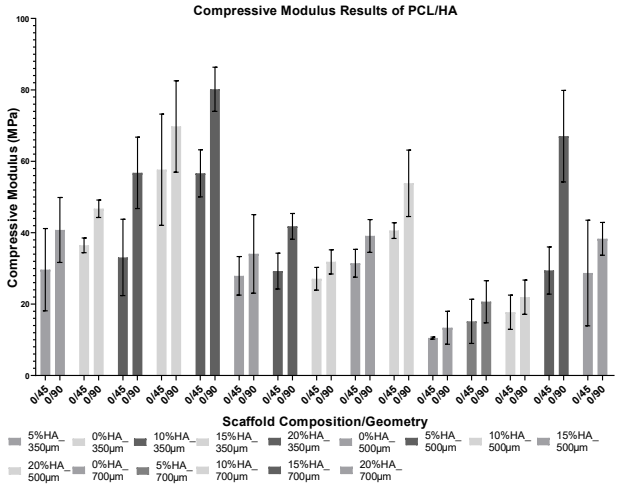

Figure 2: Compressive modulus as a function of scaffold architecture, pore size and material composition.

III. III. Biological Analysis

Alamar Blue results are presented in Figure 4 for days 1,7 and 14 of cell seeding. Results show that in all cases cells are attaching and proliferating. High cell activity is observed for scaffolds containing HA and produced using a lay-down pattern of 0/45º due to the high surface area provided by this configuration. No significant differences were observed in terms of pore sizes. During the presentation additional results for other topologies will be provided.

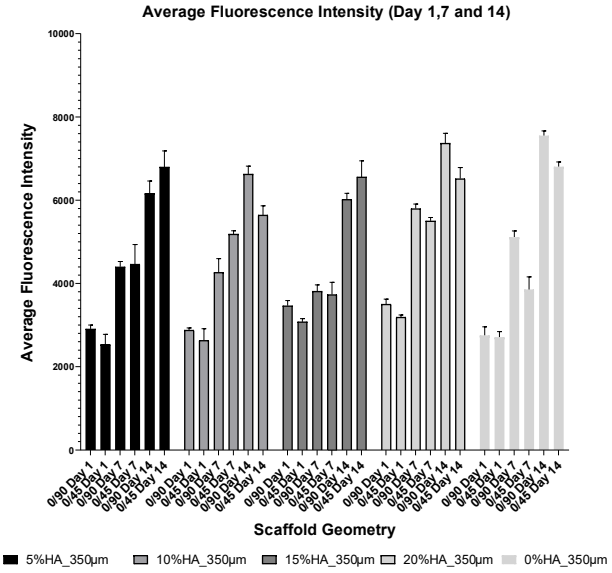

Figure 3: Average Fluorescence Intensity as a function of scaffold architecture, pore size and material composition for different days after cell seeding. All scaffolds have a pore size of 350μm

IV. Conclusions

Results show that scaffold topology and material composition play a key role on the mechanical and biological performance of scaffolds. In both cases, the best results were observed for scaffolds containing high levels of HA. However, an opposite trend was observed for the lay-down pattern. Reduced angles between filaments increase the surface area increasing cell attachment and proliferation but also decreases the mechanical performance. No toxicity effects were observed, being possible to conclude that the processing conditions did not induced any significant modifications on the materials.

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AUTHOR'S STATEMENT

Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study.

REFERENCES

[1] Bone Bricks: Low cost effective modular osseointegration prosthetics for large bone loss surgical procedures (EP/R01513/1). Funded by Engineering and Physical Sciences Research Council (EPSRC) of the UK, the Global Challenges Research Fund (GCRF) under the call Diagnostics, prosthetics and orthotics to tackle health challenges in developing countries.