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Production, Characterisation, and in vitro Degradation and Biocompatibility of a Solvent-Free Polylactic-Acid/Hydroxyapatite Composite for 3D-Printed Maxillofacial Bone-Regeneration Implants

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Abstract : The current gold-standard for maxillofacial reconstruction surgery (MRS) utilizes auto-grafted cancellous bone as a filler. This study was aimed towards developing a polylactic-acid/hydroxyapatite (PLA-HA) composite suitable for fused-deposition 3D printing. Functionalization of the polymer through the addition of HA was directed to promoting bone-regeneration properties so that the material can rival the performance of cancellous bone grafts in terms of bone-lesion repair. This kind of composite enables the production of MRS implants based off 3D-reconstructions from image studies – namely computed tomography – for anatomically-correct fitting. The present study encompassed in-vitro degradation and in-vitro biocompatibility profiling for 3D-printed PLA and PLA-HA composites. PLA filament (Verbatim Co.) and Captal S hydroxyapatite micro-scale HA powder (Plasma Biotal Ltd) were used to produce PLA-HA composites at 5, 10, and 20%-by-weight HA concentration. These were extruded into 3D-printing filament, and processed in a BFB-3000 3D-Printer (3D Systems Co.) into tensile specimens, and were mechanically challenged as per ASTM D638-03. Furthermore, tensile specimens were subjected to accelerated degradation in phosphate-buffered saline solution at 70°C for 23 days, as per ISO-10993-13-2010. This included monitoring of mass loss (through dry-weighing), crystallinity (through thermogravimetric analysis/differential thermal analysis), molecular weight (through gel-permeation chromatography), and tensile strength. In-vitro biocompatibility analysis included cell-viability and extracellular matrix deposition, which were performed both on flat surfaces and on 3D-constructs – both produced through 3D-printing. Discs of 1 cm in diameter and cubic 3D-meshes of 1 cm3 were 3D printed in PLA and PLA-HA composites (n = 6). The samples were seeded with 5000 MG-63 osteosarcoma-like cells, with cell viability extrapolated throughout 21 days via resazurin reduction assays. As evidence of osteogenicity, collagen and calcium deposition were indirectly estimated through Sirius Red staining and Alizarin Red staining respectively. Results have shown that 3D printed PLA loses structural integrity as early as the first day of accelerated degradation, which was significantly faster than the literature suggests. This was reflected in the loss of tensile strength down to untestable brittleness. During degradation, mass loss, molecular weight, and crystallinity behaved similarly to results found in similar studies for PLA. All composite versions and pure PLA were found to perform equivalent to tissue-culture plastic (TCP) in supporting the seeded-cell population. Significant differences ($p = 0.05$) were found on collagen deposition for higher HA concentrations, with composite samples performing better than pure PLA and TCP. Additionally, per-cell-calcium deposition on the 3D-meshes was significantly lower when comparing 3D-meshes to discs of the same material ($p = 0.05$). These results support the idea that 3D-printable PLA-HA composites are a viable resorbable material for artificial grafts for bone-regeneration. Degradation data suggests that 3D-printing of these materials – as opposed to other manufacturing methods – might result in faster resorption than currently-used PLA implants.

Keywords : bone regeneration implants, 3D-printing, in vitro testing, biocompatibility, polymer degradation, polymer-ceramic composites
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