Low temperature 3D printing of β-TCP-based scaffolds

INTRODUCTION

β-TCP-based ceramics are widely used in orthopaedic surgery for treatment of bone defects or bone fusion (e.g. spondylodesis). Since organic molecules such as antibiotics or growth factors can be incorporated in the bioceramic, low-temperature bioceramic processing offers advantages over existing methods require a high-temperature step to produce a bioceramic (1). Finding a method by which these factors can be released “on demand” would offer an extra advantage. In this study we report a new direct process for low temperature 3D-plotting of a β-TCP-based bioceramic in which PolyPulse™ polymer particles are incorporated for “on demand” release of organic molecules.

METHODS

The major components of the bioceramic are beta-tricalcium phosphate (β-TCP), distilled water, sodium citrate, polyethylene glycol (PEG 200, 400 and 1000), pyrophosphoric acid and orthophosphoric acid. For the preparation of the Granules β-TCP (Merck) was milled in a wet suspension. The grain size was measured using laser granulometry. Pastes were extruded using a disposable mixing extruder, mounted on a xyz-table. Hardening of the pastes was initiated by pyrophosphoric acid peri- or post-printing at room temperature. The microstructure of different implants was characterized by using a scanning electron microscope. Samples were used for measuring compressive strength and bending strength. Mandelic acid (MAC) was incorporated with the BMA polymer at a ratio of 1:2.5. Eighty percent of the polymer particles were in the selected range of 15-20 to 200 µm. These particles were mixed with the β-TCP and finally the scaffolds contain 10% (w/w) of MAC-PolyPulse™ polymer. Super Paramagnetic Iron Oxide Nanoparticles (SPION) incorporated in the polymer were triggered by an oscillating magnetic field to release organic molecules. Plugs from the scaffold material were tested in cell culture (ATDC5 cells) (2) for cell toxicity, and the possibility to release MAC. After washing the scaffolds, the pUV-VIS spectrophotometer at 220 nm was used to monitor MAC release after a short magnetic trigger. Both scaffolds without (Bioceramic A, n=6) and with (Bioceramic B, n=6) PolyPulse particles were implanted into a 17 mm in diameter critical size defect of the iliac wing of sheep. As controls an empty defect (n=6) and defects filled with autologous bone (n=6) were included. All procedures were approved by the Graubünden Animal Commission and performed in an approved facility in accordance with the Swiss Animal Protection Law. After 12 weeks animals were euthanized, samples were taken for post-mortem micro-CT and histological analysis.

RESULTS

This β-TCP-based bioceramic hardens in a cold, acid initiated process and had a reproducible outcome. Rheological properties of the paste were favorable for use in the 3D-plotting process. 3D-plotting of the paste was achieved by printing of layered cubic structures. Constructs were hardened peri- or post-printing and incorporation of PolyPulse™ particles was successful. The compressive strength was 1.66 MPa and the bending strength 1.82 MPa. Incorporation of the PolyPulse polymer did not affect the mechanical properties of the material. SEM images of the scaffold shows the microsurface of the β-TCP-crystals are in the 10-20 µm range. The pore-size of the printed structures was 500 µm and the estimated pore-interconnectivity 98%. The optimal β-TCP-grain-size for 3D-plotting was between 5-25 µm.

The scaffold did not have a toxic effect on the proliferation and endochondral ossification of ATDC5 cells. Presence of the bioceramic in the culture medium slightly delayed cell proliferation under proliferation and differentiation conditions (Fig 2). An external oscillating magnetic field showed an increased release of MAC.

After implantation all sheep recovered well. Macroscopically there was a lateral incorporation of both A and B bioceramics. From micro CT analysis, the Bone Volume (BV) of the negative control groups was significantly lower than all three other treatments (p ≤ 0.013). There was no significant difference of BV between defects treated with Bioceramic A or B or autologous bone. Histology confirmed lateral bone incorporation into the scaffolds and no signs of necrosis or other adverse reactions were found.

DISCUSSION

Unlike classic fabrication of bioceramics in which the biomaterial reaches high temperatures (3), Gbureck et al. have described a successful method to directly 3D powder print a bioceramic (1). In contrast to their method the currently described method uses a paste for simultaneous control of geometry and incorporation/triggered release of organic molecules. Until now paste based low temperature bioceramic techniques were indirect whereby slurries of calcium phosphate cement are impregnated into a negative rapid-prototyped pattern of a low-melting-temperature material such as wax (4). Both for treatment of infections or release of growth factors an “on demand” release may have several advantages over relatively uncontrolled diffusion of growth factors/antibiotics from a bioceramic. In vivo results show a good biocompatibility and integration of the bioceramic. Further research is needed to uncover the full potential of the PolyPulse™ technique to release drugs and other biologicals from the bioceramic.

Figure 1: Micro CT images of: defect treated with autologous bone (first horizontal lane), empty defect, (second horizontal lane), defect treated with bioceramic A (third horizontal lane).

Figure 2: Volume of material in the defect, note that both bioceramics and positive control have a significant higher bone volume compared to the empty defect.

REFERENCES

(1) Gbureck et al., Adv Mater. 2007, 19, 795-800.

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