Development of a "Bioengineered block graft" for Maxillofacial Reconstruction

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Introduction

Bone grafting is now a common surgical procedure, particularly in the reconstruction of severe alveolar ridge resorption or sinus augmentation to enable dental implant placement. Tissue engineering aims to create replacement tissues in situations where the body no longer has the potential to do so. In order to create a functional bone construct, the scaffold design has to facilitate cellular migration and growth and guide tissue regeneration three dimensionally. Scaffolds can also function as carriers, incorporating biological signalling molecules (GFs) known to promote bone regeneration. GFs are potent molecules that regulate cellular events, and as such, are being exploited for dental applications. In this study, a porous β-metacalcium phosphate scaffold material was developed and fully characterised. An *in vitro* coculture model with osteoprogenitor cells and endothelial cells was used to exploit the potential of forming a 3D prevascular network in order to facilitate and enhance bone regeneration.

Aim

To evaluate both *in vitro* and *in vivo* cell and tissue response of the novel bone tissue engineering construct. The growth factor incorporated to enhance biological potential was Bone Morphogenetic Protein 7 (BMP-7). Bone formation, vascularisation potential and also biodegradable properties of the graft was evaluated.

Materials and Methods

1. Sample preparation

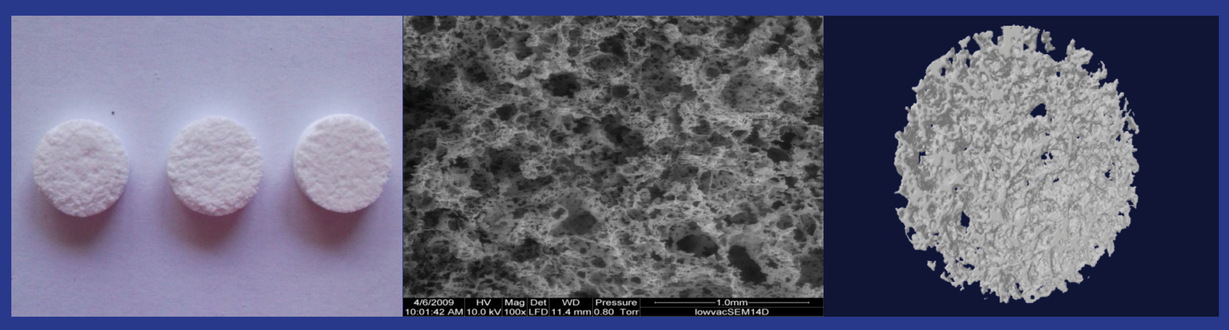

Figure 1. Porous calcium phosphate scaffolds were prepared by sintering a mixture of (4:1 weight) mono calcium hydrogen phosphate ($Ca(H_2PO_4)_2 \cdot H_2O$) (MCP) and poly-vinyl alcohol (PVA), with PVA as the porogen. γ-radiation was used to sterilise the porous scaffolds prior to biological evaluation.

2. Cells, Culture conditons and scaffold seeding

- Primary human bone marrow stem cells (hBMSC), Alveolar osteoblast cells (aHOB) and human umbilical vein endothelial cells (HUVECs) were employed to investigate biocompatibility and biofuntionality of scaffold. Cells were seeded in 12-multiwell plates at a density of $5x10^5$ per scaffold. Freshly lyophilised BMP-7 400ng/0.5g of CMP was adsorbed onto scaffold proir cell seeding
- Response of coculture of HOB and HUVEC on scaffold in the presence of BMP-7 and VEGF using collagen as a carrier.
- *In vitro* prevascularisation inside scaffold was evaluated using confocal microscopy

3. In vivo experiments

- Fourteen adult (>7 months) female New Zealand white rabbit were used in the study. Bilateral edentulous areas between incisor and molars of the maxilla were used as experimental sites. A 5 mm in diameter and 3 mm deep defect was created. The defects were filled with scaffold with or without 0.5 µg of BMP-7
- The animals were sacrificed after a healing period of 2,4 and 8 weeks. The experimental area then retrieved and X-ray microtomography (µCT) immediately after retrieval before tissue processing for histology study

Results

Cell adhesion and morphology study

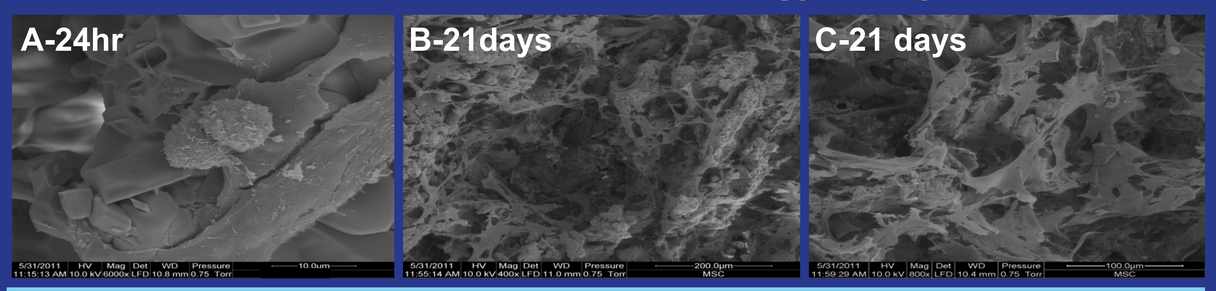

Figure 2. SEM of primary HOB cells and MSCs on CMP, (A) A large number of proliferating HOB cells was observed inside the pores and MSCs were observed migrating both on the surface and within the pores of the scaffolds (B,C).

In vitro prevascularised network using coculture model

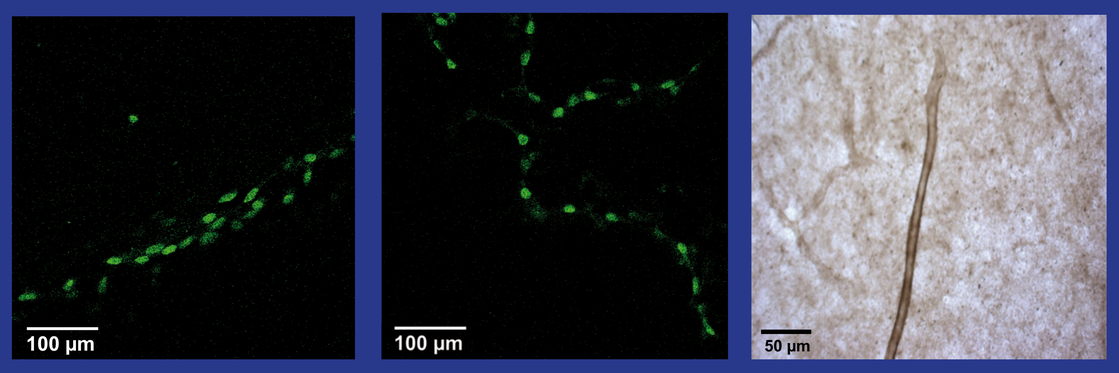

Figure 3. Confocal microscopy: CD31 immunofluorescent staining was used to observe tubular formations inside the scaffolds after 14 days in the presence of BMP-7 and VEGF.

Histology and Histomorphometry

At two weeks the healing process was observed with minimal inflammatory response. The defect area, although still mainly occupied with residual CMP ceramic was filled with granulation tissue. At four weeks osteoclasts were observed around the spaces caused by resorption of graft material. At eight weeks the CMP graft was completely resorbed.

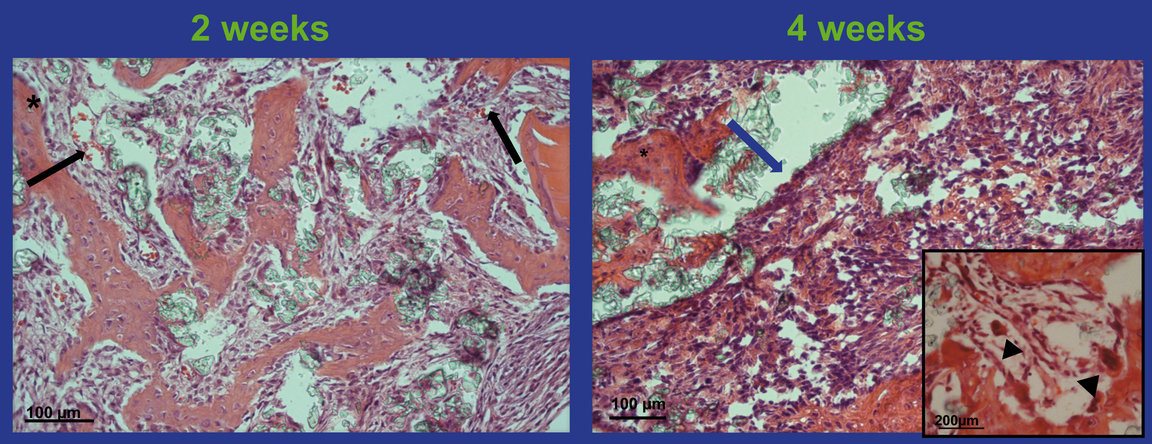

Figure 4. At two weeks, microvessels (<100µm) were seen in soft tissue (black arrows). By four weeks, larger areas of newly formed bone were observed and consisted of woven bone lined with an osteoblastic seam (blue arrow). Note resorption of graft materials by osteoclast-like cells (arrow heads).

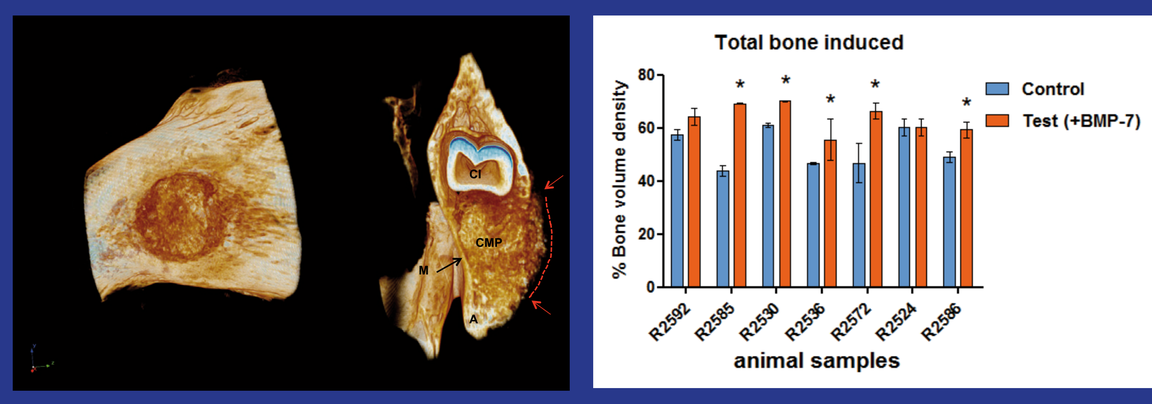

Figure 5. µCT analysis : The defect area was reconstructed and region of interest (ROI) was generated. Percent bone volume (BV/TV) was significantly increased in BMP loaded scaffold (P<0.05).

Methods	Control	Test (with BMP)	P value*
Axiosoft4.5	49.4±7.7	57.1±5.6	0.018
Grid intercept	50.3±7.9	58.3±4.8	0.018

Histomorphometric analysis indicated an approximate 15% increase in bone with the BMP-7 graft versus control. Of significance, was the very good concurrence between % bone area (2D) and % bone volume density (BV/TV) (3D) measured using the µCT instruments when compared to the histological image analysis.

Conclusion

This study confirmed good biocompatibility and osteoconductive property of a novel scaffold. It was observed to support cell attachment, proliferation, and differentiation. The loading of BMP- 7 resulted in an enhanced bone formation. Ongoing work is focussing on autologous stem cell seeded scaffolds and growth factor functionalization to enhance bone regeneration.

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