Study of New Bioactive Glass 13-93 Fibre-Based Scaffolds Tissue Engineering of Bone in Experimental Bone Defects

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Introduction

Restoration of large bone defects or construction of new bone structures during reconstructive surgery requires the use of autologous bone transfer or the use of bone-supporting bioactive material. In this study we investigate bioactive glass (BaG) 13-93, poly-L/DL-lactide (PLDLA) 70/30 and their combinations for their suitability as artificial bone in craniofacial plastic surgery.

Methods

In vitro analysis: Rabbit osteoblast-like cells (OBs) were obtained trypsin-collagenase treatment of bone explants, expanded in vitro using DMEM supplemented with 10% FCS, induced for 1-2 weeks with dexamethasone, glycerophosphate and vitamin C and characterized by determination of alkaline phosphatase (AP) activity. Human osteogenic sarcoma cell line SaOS2 was transduced with a retrovirus expressing green fluorescent protein (GFP) to monitor cell growth and density within three-dimensional scaffolds. Cells were grown and monitored by microscopic inspection.

In vivo analysis: Biopsies were taken from the iliac crest of a 6 to 8 week female rabbits. The harvested bone samples were digested using collagenase/trypsin and cells expanded in vitro for about 3 weeks. After characterisation of the expanded cells using a commercial AP kit, and AP staining, OBs were transferred onto the scaffolds and incubated for 48 h prior to implantation. A critical sized bone defect of 1 cm² diameter was made in the calvaria of the cell donor on both sides of the head. Group 1 was treated with BaG 13-93 fibrous scaffold attached to thin PLDLA 70/30 plate. Group 2 received BaG 13-93+PLDLA70/30 (as group 1) plus cultured OBCs. In Group 3 the defect was left untreated. The experimental setting comprised 9 animals, 3 in each group. The observation period was 3 wks and the healing process was assessed using serial weekly radiography and histology.

Results

In vitro cultivated cells derived from rabbit iliac crest were found to be AP positive (Fig.1). Rabbit as well as human sarcoma cells grew on three dimensional scaffolds to high cellular densities (Fig. 2) indicating that BaG 13-93-derived material supports the growth of OBCs in vitro.
Fig. 1: In vitro cultivated bone-derived rabbit cells stained for AP activity (400x).

Fig. 2: Left: GFP expressing SaOS2 cells grown on BaG 13-93 fibrous scaffolds (100x). Right: 400x magnification of DNA stained SaOS2 cells.

In contrast, when the three-dimensional scaffolds were implanted into rabbits to cover a large bone defect, they induced a strong inflammatory response (Fig. 3).

Fig. 3: Bone explant showing the dura mater covering the operation field. (A) 2-D PLDLA 70/30 plate, (B) 3-D BaG 13-93 fibrous scaffold with bulging white mass.

This response was not caused by the pretreatment regimen of the 3-D scaffolds as also observed when scaffolds were pre-incubated with phosphate buffered saline. The 2-D PLDA 70/30 plates did not initiate an inflammatory response. (Fig. 2A).

Discussion and Conclusions
BaG 13-93-derived 3-D scaffolds support the growth of OBs in vitro but induce a strong inflammatory response in vivo. In addition, due probably to, the rapid degradation of bioactive material in vivo.

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