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DEVELOPMENT OF CONICAL SOLUBLE PHOSPHATE GLASS FIBRES FOR DIRECTIONAL GENERATION OF MICROCHANNELS IN DENSE COLLAGEN IMPLANTS.

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INTRODUCTION: Successful integration of the tissue engineered construct depends greatly on the ability of host tissues to innervate and vascularise the implant. To achieve this goal we proposed using dissoluble phosphate-based glass fibres to create microchannels in the plastic compressed collagen gel. To make the ingrowth dynamic we hypothesized that fibres should be conically shaped, so that after implantation the microchannel will open in the direction of increasing diametre. PC collagen is a novel technique for the rapid fabrication of dense collagen bio-mimetic tissues by rapid expulsion of the liquid from hyperhydrated collagen gel.[1] Dissolution of phosphate glass (PG) fibres compressed into collagen gels, produce microchannels[2] but products from fast dissolving glasses may be detrimental to the seeded cells.[3] In this study we tested the viability of Schwann cells (SC) and human bone marrow stromal cells (hBMSC) in the PCC-PGF system and possibility of fabrication of the conically shaped fibres

METHODS: Cells seeded PG-collagen constructs were prepared as previously described[2] (PG fibre diameter 30-40 µm, composition ratio: 0.5 (P_2O_5) : 0.25(CaO) : (Na_2 O); distilled water dissolution time 8-10 hrs). PG-constructs were fabricated with $1x10^6$ SC and cultured 3 days and $2x10^6$ hBMSC which were cultured for 6 days. Constructs were stained with both ethidium homodimer-1 and for p75 (SC marker antigen) and ethidium homodimer-1 and calcein AM (hBMSC seeded constructs) to determine live-dead cell ratio. Conical fibres were prepared by placing a strip of fibres into the 1% TRITON X-100 in Tris buffer (pH 7.4) solution and removing equal amounts of liquid every hour for 8 hours. Hydrogel build-up was removed by dehydrating in ascending alcohols. Loss in diametre was determined using scanning electron microscope.

RESULTS: Viability of cells immediately post compression of constructs with incorporated PGF was not significantly affected relative to controls without fibres for both cell types with small increase in cell death at the beginning of incubation period which coincides with the PGF dissolution and so glass products release. For both cell types alignment parallel to the direction of microchannels was noticed at the end of incubation period (SC-3d, hBMSC – 6d).

Gradual staged dissolution of PG fibres resulted in linear reduction in diameter along the fibre length (Fig1). The mean loss in diameter for over 20 fibres was 8.85 ± 2. 8 µm over 19.5 mm, giving a mean rate of change 0.5 mm/mm.

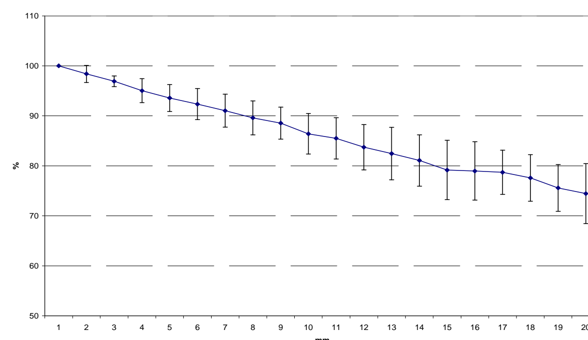

Figure 1 Mean diameter loss over the length of phosphate based glass fibres after treatment in percent ± standard deviation (n=20).

DISCUSSION & CONCLUSIONS: Physical compression has little effect on SC and hBMSC viability, whereas glass dissolution products have some negative influence on cells. This suggests that less dissoluble glass composition is needed for *in vivo* experiments. Reduction in diametre of the glass fibres makes it possible to create a construct with dynamically opening microchannels and procedure can be tailored to different glass compositions.

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