TISSUE ENGINEERING OF HYPERTROPHIC CARTILAGE FOR BONE REPAIR

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INTRODUCTION: An alternative approach to bone tissue engineering may be to find a suitable cell source to engineer a cartilage-based construct (e.g. hypertrophic cartilage) that can mineralise *in vitro* or *in vivo*. Cartilage has the advantage that it can survive in a relatively hypoxic environment, which may allow more time for vascularisation of the engineered graft to develop post-implantation. The aim of this research was to investigate the conditions that promote generation of a cartilage construct with characteristics of hypertrophic tissue

METHODS: Chondrocytes isolated from sternum, articular and nasal cartilage were expanded in monolayer and then cultured as pellets under chondrogenic (DMEM with 10% FCS, 1μg/ml insulin, 50 μg/ml L-ascorbic acid) and osteogenic (chondrogenic medium with 10mM B-glycerophosphate and 10^{-8}M dexamethasone) conditions for 28 days. The stage of their differentiation was evaluated by immunolocalisation of collagens, histochemical detection of alkaline phosphatase and biochemical analysis of glycosaminoglycans content. Development of collagen X assay is in progress.

RESULTS: Preliminary data shows that successful process of chondrocytes re-differentiation can occur using this culture system with cells of different passages (bovine nasal P_1 chondrocytes pellet, see Fig.1). Toluidine blue staining demonstrated the presence of sulfated glycosaminoglycans in the extracellular matrix. Immunohistochemical analysis of pellets revealed little staining for collagen type I and strong for collagen type II. Alkaline phosphatase activity detected in a limited proportion of the samples (mainly from cells at early passages, see Fig.1 D) was indicative of chondrocytes entering the hypertrophic stage. No calcium deposit was detected.

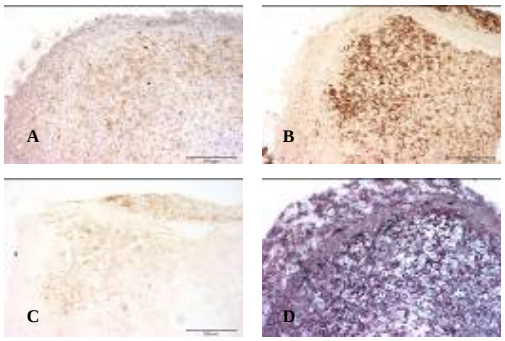

Fig. 1: Bovine nasal chondrocytes pellet, cultured under standard chondrogenic conditions; (A) collagen I; (B) collagen II; (C) alkaline phosphatase

DISCUSSION & CONCLUSIONS: In conclusion, chondrocytes can be induced to express alkaline phosphatase suggesting that they follow the process of hypertrophic differentiation and therefore show some potential for use in bone tissue repair. Ongoing work is directed at determining culture conditions to accelerate process of chondrogenic and osteogenic differentiation

REFERENCES: Kato Y, I.M., Koike T, Suzuki F, Takano Y (1988). "Terminal differentiation and calcification in rabbit chondrocyte cultures grown in centrifuge tubes: Regulation by transforming growth factor/8 and serum factors." Cell Biology 85: 9552-9556.

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