Injectable Hydrogel as a Reservoir System for Nucleus Pulposus Regeneration
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Introduction
Degeneration of the intervertebral disc (IVD) is the main cause of neck and low back pain¹. During this irreversible process the nucleus pulposus (NP) loses its gel-like consistency and becomes more fibrotic². The hypothesis of this work is that a supplementation of the degenerated IVDs with cell seeded composite hydrogels which mimic the native environment may provide the ideal environment to conduct the regeneration of the NP tissue. The objective of this study was to develop an optimally stabilised type II collagen hydrogel with 4S-StarPEG and enriched with varying hyaluronic acid concentrations acting as a reservoir system of cells for NP regeneration.

Materials and Methods
Different molar ratios of type II collagen (Symatese, France) and HA (CPN, Czech Republic) were tested in this study (9:0, 9:1, 9:4.5, 9:9). After neutralisation, NP cells which were extracted from 5-month old bovine caudal discs (2x10⁶) were added. Hydrogels stabilisation was performed using poly(ethylene glycol) ether tetrasuccinimidyl glutarate (4S-StarPEG) (JenKem Technology, USA). Hydrogels were incubated for one hour at 37°C before the surface addition of DMEM 10% FBS, 1% P/S. Hydrogel cellular compatibility and cell distribution were observed by live/dead assay. Levels of type I collagen, type II collagen and aggrecan expressions were quantified using real-time PCR. Rabbit adipose tissue derived stem cells (ADSCs) were seeded within the hydrogels and incubated for 14 days. Cell viability and proliferation were quantified to evaluate the cyto-compatibility of the hydrogels.

Results
After one hour, stable hydrogels cross-linked with 4S-StarPEG were obtained. Stability in culture was observed and confirmed by absence of mass loss after 14 days in culture (Fig.1 (A)). Levels of type II collagen mRNA expression was noted after 7 days in culture independent of the concentrations of HA used (Fig. 1 (B)). However, NP cell proliferation was seen after 14 days in culture in absence of HA. Hydrogels have been shown to support adipose derived stem cells growth and proliferation after 14 days.

Discussion and Conclusions
Type II collagen/hyaluronan hydrogel has shown good stability in culture and is able to support cell growth. Its injectability makes it a promising candidate as a reservoir of cells for intervertebral disc regeneration.

References

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