Biomimetic fibrinogen-hyaluronan conjugates for nucleus pulposus regeneration

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INTRODUCTION: Degenerative disc disease is one of the largest health problems faced worldwide. With age, the water content of the nucleus pulposus (NP) decreases and the disc gradually becomes less effective as a cushion. As a result, mechanical load on the annulus fibrosus (AF) leads to weakening of the AF and eventually to its cracking through which part of the gelatinous NP may prolapse. The IVD does not possess self-repair capacity. A novel nano-biopolymer conjugate: Hyaluronic acid (HA)-Fibrinogen (FBG) Protein Link (HPL) was developed to mimic native extracellular matrix for minimally invasive disc regeneration treatment. The present study aimed to evaluate different formulations of HPL for their ability to provide an optimal three dimensional environment for NP cell growth and differentiation.

METHODS: HPL at different FBG:HA ratios (2, 4) and HA molecular weights (B, C) were supplied by Procore Ltd, IL. P1 bovine NP cells (NPCs) were seeded into HPL gel beads at a density of 120,000 cells per bead. Each bead was prepared using 20 µL of HPL solution and 10 µL of thrombin (1U/mL final concentration) to cause gelation. Cell-gel constructs were cultured in DMEM + 10% FCS + 50 µg/mL ascorbic acid for 3, 7 or 14 days. Constructs of pure FBG and non-conjugated FBG-HA mixtures were cultured as controls.

Outcome measurements included Live/Dead staining, histology, DNA and glycosaminoglycan (GAG) content, and mRNA expression of collagen type I and II, aggrecan, Sox9, carbonic anhydrase 12 (CA12), keratin 19 (KRT19), and biglycan (BGN).

RESULTS: Live/Dead assay showed that more than 95% of the cells were viable at all time points. The DNA content of FBG and FBG-HA mixture gels decreased over time, compared with HPL gels, which demonstrated consistent DNA amounts, suggesting improved stability of the HPL constructs.

Toluidine Blue staining on all time points showed rounded cells in the HPL gels, FBG and FBG-HA mixture indicative of an NPC-like phenotype. In all materials, bNPCs accumulated more at the edges. The extracellular matrix accumulation was also more intense at the edges of gels.

There was more accumulation of GAG in HPL compared to FBG or FBG-HA mixtures. HPL B2 showed least degradation and retained the highest GAG by day 14 (Fig. 1).

![Fig. 1: GAG/DNA value in hydrogels after 14 days of culture. B2, B4, C4: HPL gels; MB2: FBG-HA mixture; F: FBG. Mean±SD, n=6.](http://www.ecmjournal.org)

There was a trend for highest gene expression of collagen II and transcription factor Sox9 in HPL B2 conjugates. A decrease in aggrecan and collagen II expression was observed during culture, while the NP markers carbonic anhydrase 12, keratin-19 and biglycan were maintained or up-regulated in all materials.

DISCUSSION & CONCLUSIONS: HPL provides the cells with a 3D environment made of HA as a major natural matrix component of the NP, and FBG, which facilitates gelation and provides stability. The present study indicates that HPL is capable of supporting NPCs retention and growth while retaining at least partially their differentiated phenotype. HPL may be suitable as injectable hydrogel for biological NP regeneration. A more extensive study will be required to establish whether there are significant differences in NPC activity between different HPL formulations.

ACKNOWLEDGEMENTS: Funded by the European Commission under the FP7 – NMP Project NPMimetic.