tiate along the chondrogenic lineage could be used to promote NP regeneration. Here we present an in vitro response of bone marrow-derived MSC and nasal chondrocytes (NC) to modified gellan gum-based hydrogels. Both ionic- (iGG-MA) and photo-crosslinked (phGG-MA) methacrylated gellan gum showed no cytotoxicity in extraction assays with MSC and NC. Furthermore, the materials did not induce pro-inflammatory responses in endothelial cells. MSC and NC attached and formed a monolayer on the hydrogel surface. Moreover, both cell types could be encapsulated into the hydrogels and remained viable for at least 2 weeks, as shown by live cell staining and histochemistry. Importantly, encapsulated MSC and NC showed an increased expression of chondrocytic markers in response to chondrogenic conditions. Altogether, the data confirm the potential of modified gellan gum-based materials in NP tissue engineering.

Acknowledgements: The funding was provided by EU FP7 project Disc Regeneration (Grant No. NMP3-LA-2008-213904).

05.P11
Evaluation of different formulations of gellan gum-based hydrogels for tissue engineering of intervertebral disc

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Gellan gum based-hydrogels present advantageous features for application as acellular and cellular nucleus pulposus (NP) substitutes due to the possibility of fine-tuning its physico-chemical and biological properties. In this study, ionic-crosslinked hydrogel discs were produced by means of mixing a raw and chemically modified material, i.e., high acyl gellan gum (HAGG) and methacrylated low acyl gellan gum (GG-MA), respectively. The hydrogel discs were characterized in terms of its mechanical properties and degradation/swelling ability. The biocompatibility of the different hydrogel formulations was assessed in vitro using NP rabbit cells isolated from the intervertebral disc. The biological performance of the developed gellan gum-based hydrogels formulations was evaluated by: (i) culturing of NP cells in the presence of the hydrogel leachables, and (ii) seeding or encapsulation of the NP cells within the hydrogels. The present work demonstrated that as HAGG content increases, the modulus of the hydrogels decreases. Moreover, the increase of the HAGG content induces a higher weight loss of the GG-MA/HA-GG formulation as compared to GG-MA hydrogel. The in vitro study revealed that hydrogels are non-cytotoxic and support the encapsulation of rabbit NP cells. The methacrylated gellan gum and formulations possessing high acyl gellan gum present tunable properties that may be interesting for application as NP substitutes.

05.P12
A novel injectable hydrogel for intervertebral disc regeneration

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Intervertebral disc (IVD) degeneration is a leading cause of lower back pain and major health problem worldwide. Current treatments do not enable disc repair and so there is interest in a tissue engineering therapy to generate functional fibrocartilage. Mesenchymal progenitor cells (MPCs) are prime candidates for such a therapy because they can be readily isolated, expanded in vitro and are able to differentiate into cartilage. However, use of MPCs will require an effective delivery system, as well as the correct physical and chemical cues to direct and support differentiation of these cells to generate new functional tissue. We have developed an injectable hydrogel system based on polyethylene glycol and hyaluronan and examined the effects of adding both bound and soluble pentosan polysulphate (PPS), a factor which has been shown to induce chondrogenesis in MPCs even in the absence of additional growth factors. We show that the gelation rate and mechanical strength of the resulting hydrogels can be tuned and have optimized the conditions required to produce gels with the desired properties for an IVD scaffold. We show that human immunoselected STRO-1+MPCs remain viable within the gels for long-term culture periods and show elevated expression of chondrogenic markers including Sox9 and Collagen-II, compared to monolayer MPCs, which is further enhanced in the presence of PPS. These data provide evidence that such a system may be of use for the treatment of IVD degeneration.

05.P13
Thermo-reversible hydrogel for nucleus pulposus replacement: feasibility under static loading in a mild papain-induced disc degeneration model

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Nucleus pulposus (NP) replacement by application of injectable hydrogels seems a straightforward approach for intervertebral disc (IVD) tissue engineering. We investigated a thermo-reversible hydrogel (TH-RHG), based on a modified polysaccharide with a thermo-reversible polyamide poly(N-isopropylacrylamide = pNIPAAM). The gel behaves like a liquid at room temperature and is gelling at >32 °C. Here we investigate the performance of the TH-RHG in situ in bovine IVD with preconditioning of human mesenchymal stem cells (hMSC) with GDF-5. IVDs with bony endplates were harvested from calf tails and cultured in vitro for 16 days (d) with induced 'cavity' in the NP using our papain disc degeneration model (PDDM). Primary hMSCs from bone marrow (ethically approved) were seeded at 4 M cells/ml in the TH-RHG and pre-conditioned with 100 ng/ml GDF-5 for 7 days. Then, the TH-RHG was injected into the IVD and kept under static loading of 0.1 MPa for 7 days. MRI was employed to image the cavity before and after loading at day 9 and day 16, and of hMSCs RT-PCR, cell viability, sulphated glycosaminoglycan synthesis and DNA content was measured at day 1, 8 and 15. MRI images confirmed a central positioning of the TH-RHG. A drop in volume across all groups in the NP region and consequently of disc height was observed between day 9 and day 16. hMSCs expressed a more 'discogenic' phenotype after exposure to the 3D organ culture system.

Acknowledgements: Funded by Hans Jörg Wyss Award #102646 from AOSpine International.