stress to convert to suspension culture. The cell-matrix complex immediately starts active contraction to form the three dimensional TEC. Two most cranial tail discs of nine rats were denucleated and/or treated with TEC (controls were denucleation only), giving three discs per group per time point. At 2, 8 and 12 weeks after implantation, the animals were euthanized and discs were evaluated. At the 8- and 12-week time point, untreated group experienced severe disruption of the anulus fibrosus and end plate, whereas such degenerative changes were significantly alleviated with TEC implantation. This study showed that TEC could prevent postnucleotomy disc degeneration.

05.P07
A novel nano-biopolymer for intervertebral disc regeneration: In vitro study on nucleus pulposus cells

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A novel nano-biopolymer, based on Hyaluronic acid (HA)-Fibrinogen (FBG) conjugate, was developed to mimic native extracellular matrix for minimally invasive intervertebral disc regenerative treatment. This study aimed to evaluate different formulations of HA-FBG conjugates for their ability to provide an optimal 3D environment for nucleus pulposus (NP) cell growth and differentiation. Bovine NP cells were seeded into conjugates of different HA molecular weights (B, C) and FBG : HA ratios (2, 4) and combined with thrombin for gelation; constructs were cultured for 14 days. Constructs of pure FBG and non-conjugated FBG-HA mixtures served as controls. The FBG and FBG-HA mixtures degraded faster than the conjugate constructs, and glycosaminoglycan (GAG) accumulation was higher in the conjugate constructs. B2 conjugates showed the least degradation and retained the highest GAG by day 14. There was a trend for highest gene expression of collagen II and transcription factor Sox9 in 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. To conclude, HA-FBG conjugate provides the cells with a 3D environment of HA as a natural NP matrix component and FBG, which facilitates gelation and provides cell adhesion and stability. This study indicates that HA-FBG conjugate may be a suitable injectable hydrogel for biological NP regeneration.

05.P08
In vitro and in vivo biological performance of modified gellan gum-based hydrogels for nucleus pulposus tissue engineering

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Ionic- (iGG-MA) and photo-crosslinked (phGG-MA) methacrylated gellan gum hydrogels have been proposed as biomaterials for supporting nucleus pulposus (NP) regeneration and/or repair. In this study, the mechanical stability and biocompatibility of these hydrogels have been evaluated in vitro. Human intervertebral disc cells obtained from herniated patients were cultured within both hydrogels, for 1–21 days. Dynamic mechanical analysis and biological characterization (Live/Dead assay, ATP and DNA quantification, PCR and immunocytochemistry) were performed after specific times of culturing. The in vitro study showed that both cell loading and culturing time do not affect the mechanical properties of hydrogels. In addition, the iGG-MA and phGG-MA hydrogels showed to be effective on supporting cells encapsulation and viability up to 21 days of culturing. In vivo biocompatibility screening was also performed, by subcutaneous implantation of both hydrogels in Lewis rats for the period of 10 and 18 days. Haematoxylin & eosin staining revealed that the hydrogels do not elicit necrosis, calcification or acute inflammatory reaction. The present study demonstrates that the iGG-MA and phGG-MA hydrogels support cells encapsulation and viability, and are well-tolerated, stable and non-cytotoxic in vitro and in vivo, thus possessing promising features for finding application as viable NP substitutes.

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05.P09
The effect of different defects and hydrogels for nucleus replacements on the biomechanical response of the intervertebral disc

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Hydrogels offer unique opportunities for regenerative strategies of the intervertebral disc. A nucleotomy necessary for the implantation of hydrogels, however, disrupts the annulus integrity and destroys natural interfaces in the disc. To clarify whether hydrogels can restore the mechanical competence of the disc an experimental test was used. Intradiscal pressure(IDP) in an ovine disc was measured in vivo for 24 h and adapted to an axial compressive test consisting of three cycles 15-min diurnal and 30-min night load. To study the fluid mechanics, 30 motion segments in different defect conditions were used: (i) INTACT; (ii) DEF-ANN: isolated annulus defect; (iii) DEF-NUC: re-implanted nucleus; (iv) DDAHA and (v) iGG-MA: two hydrogels. DEF-ANN showed no significant difference in disc height loss or IDP compared to INTACT, while DEF-NUC reduced the IDP by ~30%($p = 0.03$) and tended to increase the height loss($p = 0.2$). Both DDAHA and iGG-MA better reflected the height loss of INTACT, but caused an even stronger loss in IDP than DEF-NUC(~34%). Neither the hydrogels nor the re-implanted nucleus, assumed to be the ideal implant, could restore the mechanical functionality of the disc. Hydrogels designed to mimic the mechanical behavior of the native nucleus may fail in restoring IDP due to the destruction of natural interfaces and an inappropriate annulus closure. To regain a biomechanical equivalent of the natural nucleus, more attention needs to be paid to the anchoring of the substitute inside the disc.

05.P10
Cell-loaded gellan gum-based hydrogels for nucleus pulposus regeneration

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Limitations of current treatments for intervertebral disc (IVD) degeneration encourage the development of tissue engineering approaches. Injectable hydrogels loaded with cells can be used as substitute material for the inner part of the IVD, the nucleus pulposus (NP), and provide an opportunity for minimally invasive treatment of IVD degeneration. The NP is populated by chondrocyte-like cells, therefore, chondrocytes or mesenchymal stem cells (MSC), stimulated to differen-