Thermally Triggered Hydrogel Induces Nucleus Pulposus Differentiation of Mesenchymal Stem Cells and Restores Mechanical Function Following Injection into Bovine Intervertebral Disc.

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Disclosures: N/A

ABSTRACT INTRODUCTION: We have previously reported the development of a synthetic Laponite® crosslinked pNIPAM-co-DMAc (NPgel) hydrogel delivery system which has the potential to deliver mesenchymal stem cells (MSCs), using small bore needles (26G) which decrease the chance of inducing damage to the annulus fibrosus[1]. We have shown *in vitro* that hMSCs incorporated into NPgel and cultured in 5% O_2, induces differentiation into nucleus pulposus (NP)-like cells without the use of additional growth factors [1]. The clinical success of this hydrogel is dependent on the capacity to restore mechanical function to the IVD, the ability to integrate with surrounding tissue to prevent extrusion as well as support the viability and differentiation of incorporated MSCs into the correct NP cell phenotype following injection into NP tissue. In the present study we investigated several IVD repair strategies following injection into bovine NP tissue explants, including: MSCs alone, acellular NPgel or hMSCs incorporated within NPgel. This study tested the hypothesis that the delivery of hMSCs within the NPgel would aid scaffold integration and promote differentiation of MSCs towards the correct NP cell phenotype within native NP tissue. In addition the ability of NPgel to restore mechanical function following injection into digested bovine IVDs was investigated. Together these data ascertain the capacity of this hydrogel to be used both as a cell delivery vehicle and provide mechanical support in treatment of IVD degeneration.

METHODS: Bovine NP tissue explants[2] were injected with media (control), hMSCs alone, acellular NPgel or hMSC incorporated within NPgel and maintained at 5% O_2 for up to 6wks. Injected MSCs were stained with a green fluorescent CFSE stain for identification following injection. Cell viability was assessed by Caspase 3 immunohistochemistry (IHC). Histological assessment of the matrix was performed using Alcian Blue for proteoglycans and Masson Trichrome for collagen, alongside IHC for aggrecan, chondroitin sulphate and collagen type II to assess phenotypic characteristics of both native NP cells and injected MSCs. The hydration degree of NP tissue explants and mechanical characterisation (via dynamic mechanical analysis (DMA), was performed following 6 weeks in culture. In addition NPgel was injected into collagenase digested bovine caudal discs and mechanical analysis performed to assess the injectability of the biomaterial and to ascertain the capacity of the NPgel to restore mechanical function of the degenerate IVD. All data (performed in at least triplicate) was found to be non-parametric and hence statistical comparisons were performed by Kruskal-Wallis with a pairwise comparisons (Conover-Inman) post hoc test performed ($p \leq 0.05$).

RESULTS SECTION: CFSE positive MSCs were identified in all NP tissue explants where MSCs had been injected either alone or incorporated within the NPgel, throughout the 6 week culture duration. Cell viability was maintained in all explants throughout the 6 week culture duration, with no significant difference in the number of Caspase 3 immunopositive cells observed between the different experimental groups at any given time point. Where hMSCs were delivered via NPgel, the hydrogel integrated with native NP tissue and cells were shown to be producing NP matrix components: aggrecan; collagen type II and chondroitin sulphate (Fig 1). No significant difference in the elastic modulus, loss modulus or tan delta was observed between NPgel injected NP tissue and media injected controls following 6 weeks in culture. The hydration degree of NP tissue explants injected with acellular NPgel or hMSC incorporated within NP gel was significantly higher than NP tissue explants injected with media or MSCs alone following 6 weeks in culture. Finally hydrogels injected into collagenase digested bovine discs, demonstrated rapid solidification, filled micro and macro fissures, were maintained within the disc during loading and significantly restored the young's modulus back to levels of non-degenerate bovine IVDs (Fig 2).

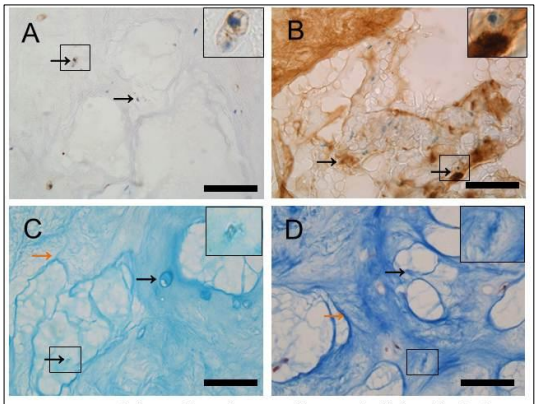

Fig 1: Representative image of hMSC incorporated into NPgel and injected into bovine NP tissue explants following 6 weeks in culture. (A) IHC for aggrecan, (B) IHC for collagen type II, (C) Alcian blue staining and (D) Masson Trichrome staining. Black arrows indicate positively stained cells and orange arrow indicate hydrogel/NP tissue interface. Enlarged cell images shown inset to aid visualisation. *Scale bar 100μm.*

DISCUSSION: Here, we have demonstrated that hMSC incorporated within NPgel and injected into NP explants, integrate with native NP tissue and promote differentiation towards the NP phenotype. Moreover we have demonstrated that the NPgel can be delivered by minimally invasive injection, maintained within the IVD during mechanical loading and restore the mechanical function of degenerate discs; thus could be used both as a cell delivery scaffold whilst providing mechanical support as a treatment strategy for IVD degeneration. The use of a combined cellular and mechanical repair approach, through the use of the NPgel developed here, is particularly promising since it is hypothesised that the mechanical support of the NPgel, to restore disc height, would provide immediate symptomatic pain relief, whilst the delivery of MSCs targets the underlying pathogenesis of IVD degeneration and would provide a long term, gradual regeneration of an extracellular matrix which biologically functions akin to native NP tissue as a treatment strategy for LBP.

SIGNIFICANCE: The NPgel developed here has the potential to deliver regenerative cells for repair of the IVD and simultaneously restore mechanical function in degenerate discs for the treatment and pain relief of chronic low back pain.

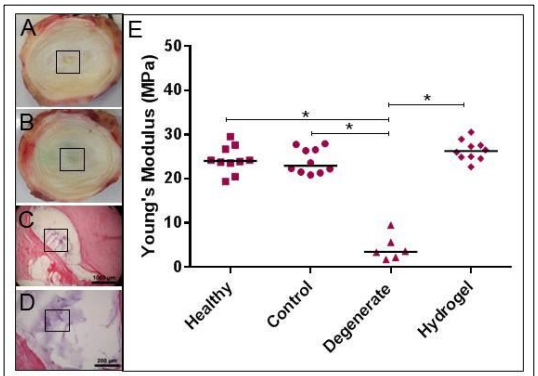

Fig 2: Macroscopic images of bovine IVDs following: (A) collagenase digestion (degenerate), (B) NPgel injected (with green dye for visualisation), (C) Microscopic section stained with H&E and (D) magnified microscopic section stained with H&E. *Scale bar 200μm and 1000μm.* (E) Young's modulus mechanical analysis of healthy, control (needle punctured), degenerate (collagenase digested) and NPgel injected bovine IVD.

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ACKNOWLEDGEMENTS: Biomolecular Sciences Research Centre, Materials and Engineering Research Centre, Sheffield Hallam University and School of Engineering, Manchester Metropoilitan University for funding.