Intervertebral Disc Regeneration using a Biomimetic Injectable Hydrogel

*Sidong Yang[1,2,3], Sergio Garcia Joya[4], Madeleine Rumble[4], Ruby Cooper-White[4], Felicity Han[5], Samuel Rudd[4], Nyoman Kurniawan[6], Farhad Soheilmoghaddam[4], Stephanie Belen Michelena Tupiza[4], Peter Pivonka[7], Joan Li[8], Justin Cooper-White[1,4]**

[1] Australian Institute for Bioengineering and Nanotechnology (AIBN), University of Queensland, St Lucia, 4072, Qld, Australia.
[2] Department of Spine Surgery, The Third Hospital of Hebei Medical University, Shijiazhuang, 050051, China.
[3] Hebei International Joint Research Centre for Spine Diseases, Shijiazhuang, 050051, China.
[4] School of Chemical Engineering, University of Queensland, St Lucia, 4072, Qld, Australia.
[5] Australian Institute for Bioengineering and Nanotechnology (AIBN), University of Queensland, St Lucia, 4072, Qld, Australia.
[6] Centre for Advanced Imaging, University of Queensland, St Lucia, 4072, Qld, Australia.
[7] School of Mechanical Medical & Process Engineering, Queensland University of Technology, Brisbane City, 4000, Qld, Australia.
[8] Faculty of Medicine, University of Queensland, St Lucia, 4072, Qld, Australia.
** j.cooperwhite@uq.edu.au*

Intervertebral disc (IVD) degeneration is an age-related problem triggering chronic spinal issues, such as low back pain and IVD herniation, resulting in compression of the spinal cord and/or nerve roots. Standard surgical treatment is a discectomy to decompress the nerve roots, with or without fusion surgery stabilising the spine structure. However, accelerated degeneration of adjacent segments is an all-too-common outcome, driven by the changes in the biomechanical properties of the discovertebral segment, a result of the inability of surgical intervention to restore the native IVD structure and functionally replace biomechanical properties. Various scaffolds encapsulating mesenchymal stem cells (MSCs), without or with cytokines, are seen as promising approaches for engineering IVD regeneration and improving surgical outcomes. Of all growth factors used in IVD tissue engineering research to date, transforming growth factor-β1 (TGF-β1) and growth differentiation factor 6 (GDF6) have seen the most attention with a range of primary and stem cell types. We have also shown that a semi-synthetic glucosaminoglycan-like molecule, pentosan polysulfate (PPS) can drive mesenchymal progenitor cells (MPCs) to differentiate towards chondrogenic phenotype or even nucleus pulposus cells (NPCs). The jury however remains out as to which cytokine/bioactive – cell – scaffold combination is the best in terms of potential clinical intervention, repairing both nucleus pulposus (NP) and annulus fibrosus (AF) tissues. In this work, we first performed combinatorial assessments of TGF-β1, GDF6 and PPS and three human cell sources (pericytes, MSCs and NPCs) as microaggregate cultures to determine their capabilities in facilitating nucleopulpogenic outcomes, in both maintenance and chondrogenic mediums. PPS was observed to be the best candidate of all bioactives, when combined with all cell types, even in maintenance medium. We next fabricated a visible light cross linkable hyaluronic acid/polyethylene (HA/PEG)-based hydrogel to encapsulate NPCs and display the PPS (on the HA backbone, HA-PPS). We discovered that a.) HA-PPS is more effective than PPS alone when using NPCs; b.) our HA/PEG/HA-PPS hydrogel enhanced matrix production and maintained phenotype of NPCs; and single NPCs secreted more ECM components (aggrecan, collagen 2) than pre-conditioned NPC aggregates when cultured in the hydrogel. Lastly, *in-vivo* validation of the combination of NPCs with HA/PEG/HA-PPS injected into a degenerated rat tail IVD model was performed, showing regeneration of the NP tissue and even recovery of the AF region of the IVD to levels equivalent to uninjured IVD.