

## TOWARDS BIOENGINEERING A SCAFFOLD-FREE NUCLEUS PULPOSUS

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**INTRODUCTION:** Intervertebral disc (IVD) degeneration is a common problem and the treatment options for persistent back pain are limited. Recent studies have shown that reinsertion of nucleus pulposus can delay disc degeneration<sup>1</sup>. Our goal is to bioengineer an IVD *in vitro* that can be used to repair diseased discs. In this study we investigated whether it was possible to generate a nucleus pulposus-bone substitute construct *in vitro*.

**METHODS:** To generate the bone substitute material, porous cylindrical substrates (4mm diameter, 4mm height) were formed by sintering calcium polyphosphate powder (CPP)<sup>2</sup>. Nucleus pulposus (NP) was dissected from bovine lumbar or caudal spines or sheep lumbar spines. For selected experiments, articular cartilage was harvested from bovine metacarpal-phalangeal joints. The cells were harvested by sequential enzymatic digestion and placed on the upper surface of the CPP substrate<sup>(2)</sup>. To generate multi-tissue constructs, chondrocytes were placed on the top surface of the CPP and allowed to form cartilage *in vitro* for 2 weeks and then NP cells were placed on the top surface of the cartilage layer. These were grown in culture for up to 8 weeks. The constructs were evaluated histologically. For biochemical quantification the tissues were papain digested (40 µg/ml) for 48 hr at 65°C. The digest was assayed for DNA content using the Hoechst 33258 dye binding assay and fluorometry, glycosaminoglycan content using the dimethylmethylene blue dye binding assay and spectrophotometry, and collagen content using the chloramine-T/Ehrlich's reagent dye binding assay and spectrophotometry. Interfacial shear properties of the *in vitro*-formed tissues to CPP were assessed using a specially designed shearing jig held in an Instron universal testing machine. The data was analyzed using an unpaired t-test and significance assigned at  $p < 0.05$ .

**RESULTS:** Histological evaluation of the bovine caudal cells placed on CPP showed that a continuous layer of tissue formed on the

substrate surface by 2 weeks and attained a thickness of about 2mm by 6 weeks. The matrix contained sulfated proteoglycans and similar to the *in vivo* tissue, scattered individual cells had greater staining intensity suggestive of localized enhanced pericellular proteoglycan accumulation. Notochordal cells were present in the tissue formed by the bovine caudal cells. Scanning electron microscopy of the NP-cartilage-CPP (triphasic) constructs demonstrated that at 24 hours after the NP cells were placed on the cartilage layer the cells maintained their rounded morphology, similar to NP cells placed directly on CPP. At 8 weeks of culture histological examination of the triphasic constructs by light microscopy showed that a continuous layer of NP tissue had formed and was fused to the underlying cartilage tissue, which itself was integrated with the porous CPP. The incorporation of a cartilage layer stabilized the construct by improving tissue attachment to the CPP, as demonstrated by increased peak load and increased energy required for failure during shear loading.

**DISCUSSION & CONCLUSIONS:** *In vivo* the intervertebral disc is exposed to a range of mechanical stresses due to torsion, flexion, and extension of the spine. Since these forces are absorbed by all the tissues of the IVD and as they are all involved in degeneration, an implant should attempt to reconstruct the normal structural anatomy of the IVD including nucleus pulposus and cartilage endplate to ensure proper function. This study demonstrates that it is possible to generate a multi-component construct while maintaining the integrity of the different tissues.

**REFERENCES:** <sup>1</sup> T. Nomura et al (2001) *Clin Orthop* **389**:94-101. <sup>2</sup> C. Seguin et al (2004) *Spine* **29**:1299-1306

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