Potential of Link-N Peptide for Biological Repair of the Human Intervertebral Disc

Introduction
Back pain is a fairly common problem which affects a large portion of the population across all ages and has an impact on quality of life. It has a major financial impact on the health system and industry due to loss of productive labor hours. Intervertebral disc degeneration is the single most common implicated cause of back pain. Presently there is no medical treatment or therapeutic agent to address this problem and surgery is the only offered option. Link-N peptide represents a 16 amino acid sequence from the N-terminal of the Link protein that stabilizes the proteoglycan aggregates present in cartilage and disc. Link-N peptide is released from the Link protein as a result of proteolysis, and has been speculated to play a role in matrix homeostasis by promoting new matrix synthesis. We evaluated its regenerative potential in intervertebral discs by means of a whole organ culture model for intervertebral disc developed by us.

Methods
Coccygeal discs (Co 2-4) from healthy 22-24 months old steers were obtained from a local abattoir. Cellular studies were performed on cells isolated from nucleus pulposus (NP) and annulus fibrosus (AF) regions of the bovine coccygeal discs. Cells were isolated by sequential digestion with Pronase followed by Collagenase I digestion for NP and Collagenase II digestion for AF, respectively. Cells were beaded in 1.2% alginate and cultured in DMEM containing 1g/L glucose and 10% FBS. Alginate beads were stabilized for 5 days and cell viability estimated by Live/Dead assay (Live/Dead®, Invitrogen). The beads were then exposed to Link-N peptide (CanPeptide, Montreal) for 48 hours. Link-N peptide was commercially prepared with 99% purity. Link-N concentrations used for alginate cultures were from (10-10000) ng/ml for dose response studies. Response was evaluated by monitoring 35S incorporation.

Lumbar IVDs, from 4 individuals, 5 discs per spine, were obtained through organ donations via Transplant Quebec within 6 hours after death. The spines were assessed by X-ray to exclude specimens where the majority of the discs were associated with loss of disc height or vertebral osteophyte formation. The discs were prepared for organ culture by parallel cuts through the adjacent vertebral bodies close to the end plates, and the remaining bone and the calcified part of the cartilage endplates were removed using a high-speed bone burr. Discs were maintained and cultured with no external load applied in DMEM containing 1g/L glucose and supplemented with 1% FBS. Discs from adjacent levels were matched for the degree of degeneration and were injected in their NP region with 50µCi of 35S along with 1mg of Link-N and harvested after 48 hours. Live/Dead assay was performed on 4mm punches taken from the NP to verify that the selected dose of Link-N is not lethal to cells. Response to Link-N was evaluated by monitoring 35S incorporation. Human tissue used in the study was obtained after consent and the procedure was approved by local Research Ethics Board.

Results
When bovine caudal disc cells from NP and AF regions beaded in alginate were exposed to Link-N peptide for 48 hours, proteoglycan synthesis was observed to increase with 10mg/ml and 100ng/ml and to achieve a maximal response at 1000ng/ml of Link-N (Fig.1). Proteoglycan synthesis decreased after removal of Link-N peptide stimulation and the controls and experimental beads were comparable within 48 hours of withdrawal of stimulation.

Discussion
Link-N peptide has previously been shown to stimulate matrix synthesis in articular cartilage, and to promote matrix protein synthesis by bovine disc cells in monolayer and pellet cultures. In this work we also show that Link-N can promote proteoglycan synthesis not only by the bovine disc cells in alginate but also by adult human disc cells in their native environment. Recently an increase in disc height on MRI was shown in an in vivo rabbit model, where degenerate discs were injected with Link-N. If a similar restoration of disc function could be achieved in the human, then Link-N could be a promising candidate for biologically induced disc repair, and could provide an alternative to surgical interventions for early stage disc degeneration. In principle many growth factors also have the potential of being able to stimulate disc repair. However, Link-N has a significant cost advantage over bioactive proteins such as BMP7, TGFβ1 and GDF5 previously tested in other systems. Based on prior in vivo studies in the rabbit, Link-N is over 100 times less expensive than recombinant growth factors that have a similar repair response.

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When intact human discs were injected with 1 mg of Link-N/disc, cell viability was maintained at > 96%. Discs injected with Link-N showed 30-50% more proteoglycan synthesis compared to adjacent level controls discs matched for grade of degeneration. (Fig.2). Analysis of AF at a distance of 1 cm from the site of injection demonstrated increased 35S incorporation at these sites too, thus indicating that Link-N diffuses within disc.

![Fig.1 Dose response by bovine NP cells in alginate to 48 hour Link-N stimulation.](image1)

![Fig.2 Proteoglycan synthesis by human discs injected with Link-N.](image2)