

## **Link-N Peptide: A Stepping Stone towards Biological Repair of Intervertebral Disc Degeneration**

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**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. 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 the 16 amino acid sequence from the N-terminus 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 suggested to play a role in matrix homeostasis by promoting new matrix synthesis. We evaluated its regenerative potential in intact human intervertebral discs.

**Materials and Methods:** Lumbar IVDs were obtained through organ donations via Transplant Quebec. Discs from 7 individuals, 5 discs per spine, were harvested within 6 hours after death. Cells were isolated from nucleus pulposus (NP) and inner annulus fibrosus (iAF) regions of the discs. Single cells were beaded in 1.2% alginate and cultured in DMEM containing 1g/L glucose and 10% FBS. Alginate beads were exposed to 10-10000ng/ml Link-N peptide for 48 hours. Intact 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. Link-N was conjugated with 5-TAMRA dye then injected into the center of the disc. The distribution of Link-N in the medium and within the disc was studied to determine whether Link-N is retained in the disc. Discs from adjacent levels were matched for the degree of degeneration and were injected in their NP region with 50 $\mu$ Ci  $^{35}$ SO<sub>4</sub> along with 0.1mg or 1mg of Link-N in 100 $\mu$ l of medium per disc and harvested after 48 hours. Sustained effect of Link-N was evaluated by injecting the disc with Link-N and injecting  $^{35}$ SO<sub>4</sub> one week later. Proteoglycan synthesis was evaluated by measuring  $^{35}$ SO<sub>4</sub> incorporation.

**Results:** When human lumbar disc cells from NP and iAF regions beaded in alginate were exposed to Link-N peptide for 48 hours, proteoglycan synthesis was observed to increase in a dose dependent manner with the maximal response at 1000ng/ml Link-N. Fluorescently labeled Link-N peptide was injected into the discs to determine if Link-N is retained in the discs matrix or freely diffuses throughout the tissue and equilibrates with surrounding medium, . Samples were taken continually from the surrounding medium and from the disc tissue at the termination of the experiment. Fluorescent-Link- N was detectable in the medium at 24 hours and reached equilibrium after 48 hours. The fluorescent peptide was found to in the NP and NP/iAF junction but not in the remaining AF. Thus loss of Link-N appears to occur by diffusion through the endplates. Cell viability was maintained in the NP, at > 96%, after injection of 1 mg of Link-N/disc, Discs injected with Link-N showed increased proteoglycan synthesis in the NP and iAF compared to adjacent level control discs matched for grade of degeneration. To evaluate the duration of the effect, discs were injected with  $^{35}$ SO<sub>4</sub> one week after the injection of Link-N. Proteoglycan synthesis remained elevated in Link-N injected discs compared to adjacent level

control discs suggesting a sustained effect.

**Conclusion:** Link-N peptide has previously been shown to promote matrix protein synthesis by bovine disc cells in monolayer and pellet cultures. In this work we show that Link-N can promote proteoglycan synthesis not only in human disc cells cultured in 3D constructs, but also in intact adult human discs where the cells in their native environment. Recently, an increase in disc height measured by 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 intervention for early stage disc degeneration. Link-N has a significant cost advantage over growth factors, such as BMP7, TGF $\beta$  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. Therefore, Link-N peptide injection could be both an effective and cost-efficient therapy for retarding the ongoing degenerative process in early stage disc disease and help relieve back pain.

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