SMALL INTESTINAL SUBMUCOSA FOR NUCLEUS PULPOSUS AUGMENTATION: A FEASIBILITY STUDY IN RABBITS

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Introduction
Discogenic back pain is associated with alterations of the disc and abnormal turnover of the disc extracellular matrix. Surgical treatment to relieve pain includes lumbar discectomy and spinal fusion. One alternative therapeutic approach aiming to retard the degeneration process is to transplant a matrix-rich tissue into the disc. Porcine small intestine submucosa (SIS), a biodegradable extracellular matrix scaffold, has shown promise in promoting tissue regeneration. We hypothesize that this acellular scaffold, which contains entrapped growth factors, may stimulate disc cells to synthesize extracellular matrix, thereby arresting the degeneration, or even promoting the regeneration, of the disc. The purpose of this study was to determine if porcine SIS is a potential bioactive scaffold for rescuing degenerative disc cells and to evaluate the efficacy of applying SIS as a disc replacement.

Methods
Porcine small intestine submuoscosa was prepared by DePuy AcroMed (Raynham, MA). SIS membranes (~50mg, 200 µm thick) were implanted in 25 New Zealand rabbits (4kgs). Lumbar intervertebral discs were exposed. Nucleotomy was performed in 2 non-consecutive levels by aspiration of the nucleus through a syringe. SIS membrane was implanted in one disc whereas the other level was left empty. Animals were monitored for any sign of abnormal behavior for up to two months before sacrifice. Lateral plain radiographs of the lumbar spine were obtained to measure disc height. MRI (1.5T spectrometer) for T2-weighted images before sacrifice was used to identify inflammation and other tissue abnormalities. After sacrifice, MRI imaging was performed on spine specimens using a 4.7T for measuring the disc water content on T2-weighted images. Discs were harvested and fixed in 10% formalin before paraffin embedding and H&E and toluidine blue staining were performed on sections. Total RNA was isolated from rabbit discs and the PCR was carried out with the following primers: collagen I and II, aggrecan, decorin, metalloproteinases MMP9 and 13, Fas. Rabbit GAPDH was used as the housekeeping gene. PCR resulting products were analyzed by agarose gel electrophoresis then imaged with a Versadoc Imaging System (Biorad). The gene expression level for all different primers was normalized to the GAPDH expression in each disc. Statistical analysis was performed with a Student’s t test to compare with gene expression levels in normal discs and p<0.05 was considered significant.

Results
The acellular scaffold used in this study consisted of a single-layered, thin extracellular matrix membrane that was successfully implanted in rabbit disc after nucleotomy. Animals showed no signs of pain or restrained mobility after surgery and were sacrificed after 2 months. Both operated levels were identified on X-Ray images by the absence of a nucleus. Disc height of nucleotomy sites was significantly reduced in comparison with controls (0.51±0.021 vs 0.89±0.030 mm, respectively). A similar reduction of disc height to 0.47±0.018 mm was observed in the SIS-implanted site. However, 7 out of 14 rabbits that were implanted with SIS presented a lower disc reduction as compared to animals with nucleotomy alone. No local inflammatory response was observed on MRI. High resolution MRI revealed a low water content for both operated levels as compared to the control level. However, SIS implanted levels presented a higher water content than the nucleotomy levels, even though the difference was not significant. Careful examination of H&E stained sections revealed the formation of fibrocartilagenous tissue. We couldn't evidence any remaining SIS material in the implanted levels. Gene expression profile was examined for both operated and control levels. A total number of 18 discs were analyzed by RT-PCR conducted at the two month time point. Each group (normal, nucleotomy and SIS) consisted of 3 to 4 discs. Although the amount of extracted RNA in the nucleotomy groups was significantly lower than in the other groups, it was sufficient enough to perform the analysis without pooling the disc samples. The gene expression level for all different primers was then normalized to the GAPDH expression in each disc and is represented in Fig.1. Compared to normal discs, all operated levels presented an up-regulated expression of collagen types I and II and metalloproteinases MMP9 and 13, even though only the SIS implanted discs demonstrated a significant difference for collagen type I, type II, and MMP 13. The discs that underwent nucleotomy showed a significantly lower level of expression of both aggrecan and decorin as compared to normal discs (~37% and 27% of normal level of expression, respectively), whereas expression levels of aggrecan, decorin in SIS implanted discs were similar to those of a normal disc.

Discussion
We have previously demonstrated that SIS can support in vitro the proliferation of human degenerative disc cells for up to three months, with the attachment and migration of the disc cells into the scaffold. This bioactive scaffold may stimulate the in situ disc cells to proliferate and synthesize a new extracellular matrix, thereby forestalling the depletion of proteoglycans and arresting the degeneration, or even promoting the regeneration, of the disc. As a first step towards disc regeneration using a biologically active scaffold, we investigated the fate of SIS implanted into a rabbit degenerative disc. SIS group was slightly better than the nucleotomy group in the maintaining the disc height as evidenced by X-ray, as well as increasing the water content as evidenced by MRI imaging. The biodegradability of SIS was confirmed as well as the formation of fibrocartilagenous tissue observed in some discs. The SIS implanted discs also showed increased MMP9 and MMP13 production. While these enzymes are commonly associated with matrix degradation and disc degeneration, they may also be upregulated for matrix remodeling. Moreover, expression levels of aggrecan, decorin in SIS implanted discs were restored to those of a normal disc. The results confirm the SIS biological activity in restoring the initial gene expression profile of normal disc cells and thus in enhancing the synthesis of a new matrix. In this study, we have shown the feasibility of implanting SIS into the disc space in rabbits and confirmed the acute biocompatibility of this scaffold. SIS is a promising bioactive material that could potentially serve as a temporary scaffold for intervertebral disc regeneration.

References

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