**Intervertebral Disc Repair By A Combination Of Genipin-enhanced Fibrin Hydrogel And Growth Factor-enriched Silk-fleece**

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**ABSTRACT INTRODUCTION:** It is well accepted that two incidents are causing discogenic back pain: trauma or disc degeneration. In the cases of disc material protrusion of the inner annulus fibrosus (AF) and/or injuries of the outer AF, we aimed to repair the intervertebral disc (IVD) from an “inside-out” approach. Therefore, we propose using hydrogel in combination with a genetically modified but GMP-compliant silk. The silk’s fibrinogen contains the human growth and differentiation factor 6 (GDF6), directly produced by the baculovirus transduced Bombyx mori larva in culture. GDF6 was shown to drive stem cells towards an IVD-like phenotype. Within this study, we investigated the feasibility of a genipin cross-linked fibrin hydrogel as a filling material for the IVD as well as a glue for the silk membrane-fleece using an ex vivo organ culture approach. Additionally, different physiological loading regimes were applied to investigate the IVDs cellular response in situ. Furthermore, cytotoxicity and proliferation potential of human mesenchymal stem cells (MSC) within the silk material was assessed.

**METHODS:** Bovine IVDs of 10-14 month old animals were harvested under aseptic conditions. After inducing an IVD injury by a circular 2 mm biopsy punch, the cavity was filled with an FDA-approved human based fibrin hydrogel (Baxter Tisseel) enhanced with 4.2 mg/ml of a cross-linker; genipin (Wako Chemicals GmbH). The defect was closed with a GMP-compliant silk membrane-fleece composite (Spinteec Engineering GmbH) that was placed on the hydrogel. Subsequently, the IVDs were subjected to in vitro organ culture for 14 days using three different and independent loading regimes: 1) complex loading of 0.2MPa compression and 0 ± 2° torsion at 0.2Hz for 8h/day, 2) static diurnal loading of 0.2MPa and 3) no loading (free swelling control). For complex loading a custom built two-degree of freedom bioreactor was used. At the end of culture, the discs were harvested and controlled for seal failure, disc height, metabolic activity (alamar blue), cell death by necrosis (LDH assay) and apoptosis (Caspase 3/7), DNA, GAG and collagen content under static load hydrogel.

**RESULTS SECTION:** Macroscopic inspection revealed that the silk seal was not displaced throughout the culture period. Further, cellular metabolic activity (data not shown), DNA and GAG content and disc height of the repaired discs did not differ significantly from the injured IVDs. Except for a higher DNA content under static loading for the repaired discs compared to the injured IVDs (p-value ≤ 0.004, Fig. 1). Examination of the histological sections indicated that the injury created a cavity in the injured discs. Whereas in the repaired discs the induced injury was closed and the cavity was filled with tissue (Fig. 2). In vivo experiments on the cellular level attributed a good cell compatibility within the silk and GDF6 silk. Also proliferation, DNA and GAG content did not reveal significant differences among the different silks (data not shown). qPCR of MSC revealed a trend towards a higher ACAN to COL2 ratio. This indicated the differentiation of the MSC towards a nucleus pulposus phenotype (Fig. 3).

**DISCUSSION:** Strikingly, the discs responded to the injury on the opposite sides equally, suggesting exchange of cytokines either through the disc or the culture media. The in vivo silk experiments attribute the silk a bio-compatibility. Further, GDF6 silk thrives MSC towards a NP-like phenotype. The silk and the hydrogel offer a promising approach to repair and regenerate the IVD after nucleotomy upon disc herniation.

**SIGNIFICANCE:** Exploring the possibilities of combining natural biomaterials with growth factors might lead towards new treatment approaches in the field of IVD regeneration. Which is of importance due to the lack of satisfying options and a high incidence rate.


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**Figure 1:** Bovine IVD ex vivo organ culture under three different loading regimes: A DNA content and B GAG content compared on the injured and intact sides of the discs. The results are presented as means ± standard error of the mean (SEM) for N = 5 bovine IVDs. p-values: *<0.0038 and **<0.0005

**Figure 2:** Histological sections of injured (top, transversal cuts) and repaired (bottom, sagittal cuts) discs. In the injured disc a cavity is created in contrast to repaired discs where the injury site is closed. Scale bar 1 cm

**Figure 3:** ACAN vs COL2 ratio of MSC seeded on control silk (cSilk), silk with growth factors (GDF6-Silk and TGF3-Silk) or in the presence of exogenous GDF6. The higher ratio of MSC on GDF6 silk indicated a trend of differentiation towards an NP-like phenotype (N = 5 donors).