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Total disc replacement using a tissue-engineered intervertebral disc in vivo: new animal model and initial results

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ABSTRACT

Study type: Basic science

Introduction: Chronic back pain due to degenerative disc disease (DDD) is among the most important medical conditions causing morbidity and significant health care costs. Surgical treatment options include disc replacement or fusion surgery, but are associated with significant short- and long-term risks [1]. Biological tissue-engineering of human intervertebral discs (IVD) could offer an important alternative [2]. Recent in vitro data from our group have shown successful engineering and growth of ovine intervertebral disc composites with circumferentially aligned collagen fibrils in the annulus fibrosus (AF) **(Figure 1)** [3].

Objective: The next step is to investigate if biological disc implants survive, integrate, and restore function to the spine in vivo. A model will be developed that allows efficient in vivo testing of tissue-engineered discs of various compositions and characteristics.

Methods: Athymic rats were anesthetized and a dorsal approach was chosen to perform a microsurgical discectomy in the rat caudal spine **(Figures 2 and 3)**. Control group I (n = 6) underwent discectomy only, Control group II (n = 6) underwent discectomy, followed by reimplantation of the autologous disc. Two treatment groups (group III, n = 6, 1 month survival; group IV, n = 6, 6 months survival) received a tissue-engineered composite disc implant. The rodents were followed clinically for signs of infection, pain level and wound healing. X-rays and magnetic resonance imaging (MRI) were assessed postoperatively and up to 6 months after surgery **(Figures 6 and 7)**.

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A 7 Tesla MRI (Bruker) was implemented for assessment of the operated level as well as the adjacent disc (hydration). T2-weighted sequences were interpreted by a semiquantitative score (0 = no signal, 1 = weak signal, 2 = strong signal and anatomical features of a normal disc). Histology was performed with staining for proteoglycans (Alcian blue) and collagen (Picrosirius red) (**Figures 4 and 5**).

Results: The model allowed reproducible and complete discectomies as well as disc implantation in the rat tail spine without any surgical or postoperative complications. Discectomy resulted in immediate collapse of the disc space. Preliminary results indicate that disc space height was maintained after disc implantation in groups II, III and IV over time. MRI revealed high resolution images of normal intervertebral discs in vivo. Eight out of twelve animals (groups III and IV) showed a positive signal in T2-weighted images after 1 month (grade 0 = 4, grade 1 = 4, grade 2 = 4). Positive staining was seen for collagen as well as proteoglycans at the site of disc implantation after 1 month in each of the six animals with engineered implants (group III). Analysis of group IV showed positive T2 signal in five out of six animals and disc-height preservation in all animals after 6 months.

Conclusions: This study demonstrates for the first time that tissue-engineered composite IVDs with circumferentially aligned collagen fibrils survive and integrate with surrounding vertebral bodies when placed in the rat spine for up to 6 months. Tissue-engineered composite IVDs restored function to the rat spine as indicated by maintenance of disc height and vertebral alignment. A significant finding was that maintenance of the composite structure in group III was observed, with increased proteoglycan staining in the nucleus pulposus region **(Figure 4d–f)**. Proteoglycan and collagen matrix as well as disc height preservation and positive T2 signals in MRI are promising parameters and indicate functionality of the implants.

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2. **Mizuno H, Roy A, Zaporojan V, et al** (2006) Biomechanical and biochemical characterization of composite tissue-engineered intervertebral discs. *Biomaterials;* 27(3):362–370.
3. **Bowles RD, Williams RM, Zipfel WR, et al** (2010) Self-Assembly of Aligned Tissue-Engineered Annulus Fibrosus and Intervertebral Disc Composite Via Collagen Gel Contraction. *Tissue Eng Part A;* 16(4):1339–1348.

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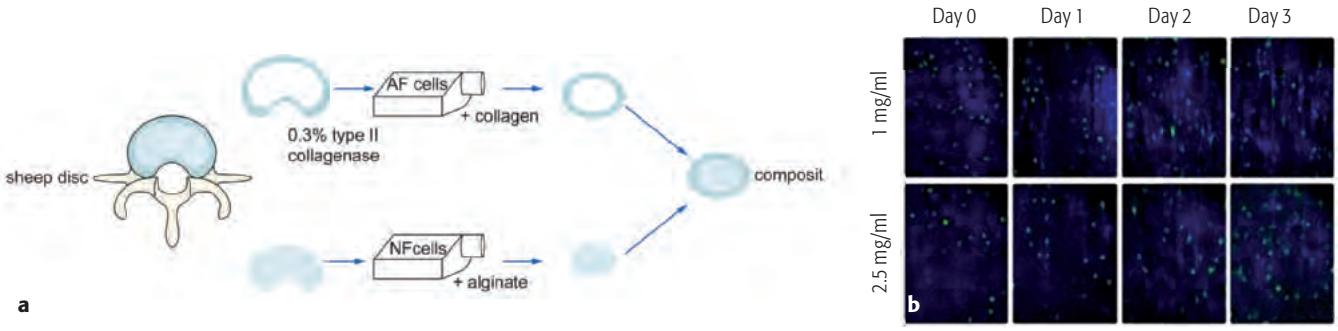
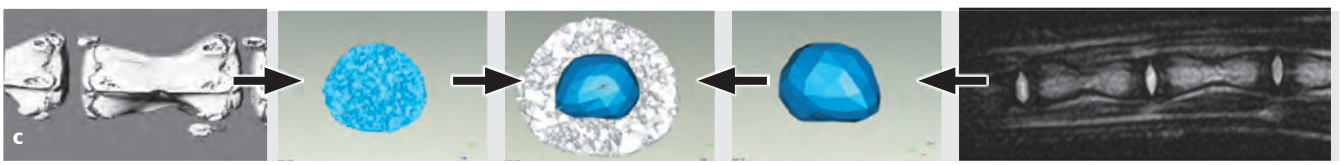
Figure 1 **Tissue-engineered composite disc**

a **Experimental steps to generate composite tissue-engineered IVDs [3]**

b **Example of different AF formulations on collagen alignment in the AF. Second harmonic generation and two-photon excited fluorescence images of seeded collagen gels (for AF) of 1 and 2.5 mg/ml over time. At seeding, cells and collagen were homogenously distributed in the gels. Over time, AF cells elongated and collagen aligned parallel to cells. Less contraction and less alignment is noted after 3 days in the 2.5 mg/mL gel.**



c **Imaging-based creation of a virtual disc model that will serve as template for the engineered disc. Total disc dimensions (AF and NP) were retrieved from micro-computer tomography (CT) (left images), and nucleus pulposus (NP) dimensions alone were retrieved from T2-weighted MRI images (right images). Merging of MRI and micro-CT models revealed a composite disc model (middle image)—Software: Microview, GE Healthcare Inc., Princeton, NJ; and slicOmatic v4.3, TomoVision, Montreal, Canada.**



d **Flow chart describing the process for generating multi-lamellar tissue engineered IVDs. IVDs are produced by allowing cell-seeded collagen layers to contract around a cell-seeded alginate core (NP) over time**

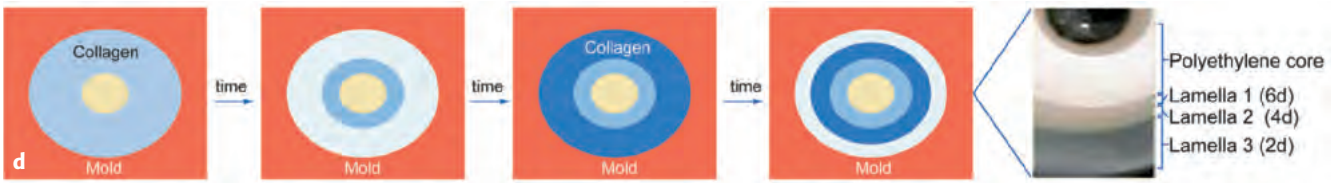


Figure 2 **Disc replacement surgery**

a **Operative situs with native disc that has been disassociated from both adjacent vertebrae**

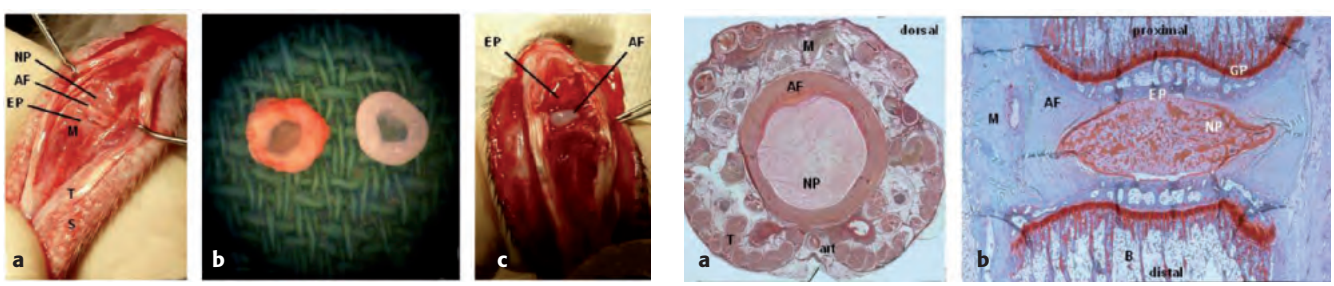
b **Native disc (left) and tissue-engineered implant (right)**

c **Implant in situ before wound closure**

Figure 3 **Disc replacement surgery. Anatomy of the rat caudal disc space**

a **Pircrosirius red stained axial cut of native disc space**

b **Saffranin-O stained sagittal cut of native disc space**



AF: Annulus fibrosus, NP: Nucleus pulposus, EP: Endplate, M: Muscle, T: Tendon, S: Skin, art: artery, GP: Growth plate, B: Bone

Figure 4 **Histologies of three separate motion segments from three different rats. Animal one = native IVD, Animal two = status after discectomy, Animal three = tissue-engineered implant (1 month)**
a–c **H&E (overall tissue staining for light micrsocopy)**
d–f **Alcian blue (proteoglycans)**
g–i **Picrosirius red (collagen I and II)**

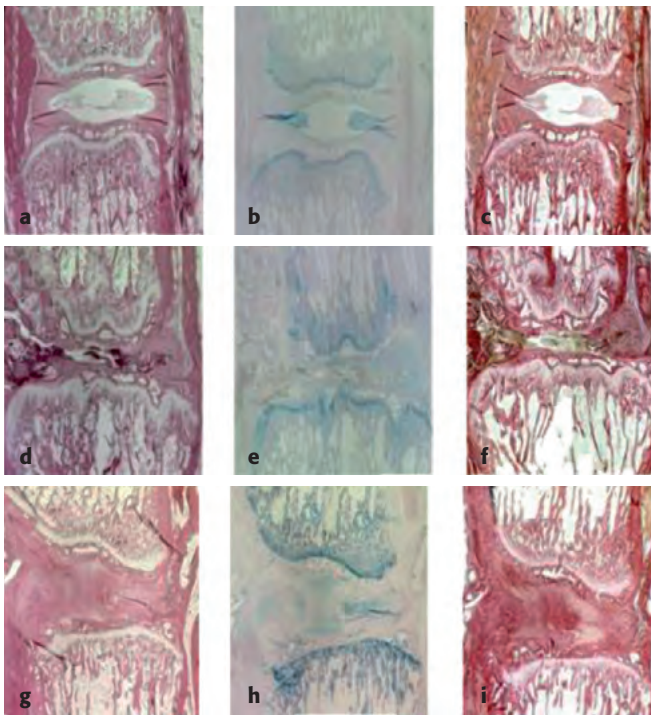


Figure 5 **Histology from one motion segment four months after implantation of a bio-engineered disc construct**
a **Picrosirius red staining (collagen)**
b **Polarized light microscopy showing collagen staining and collagen organization in AF region**
c **Increased Safranin-O staining (proteoglycans) in NP region of the disc implant**
d **Higher magnification of figure 5c: Integration between implanted tissue-engineered total disc replacement and vertebral body bone**

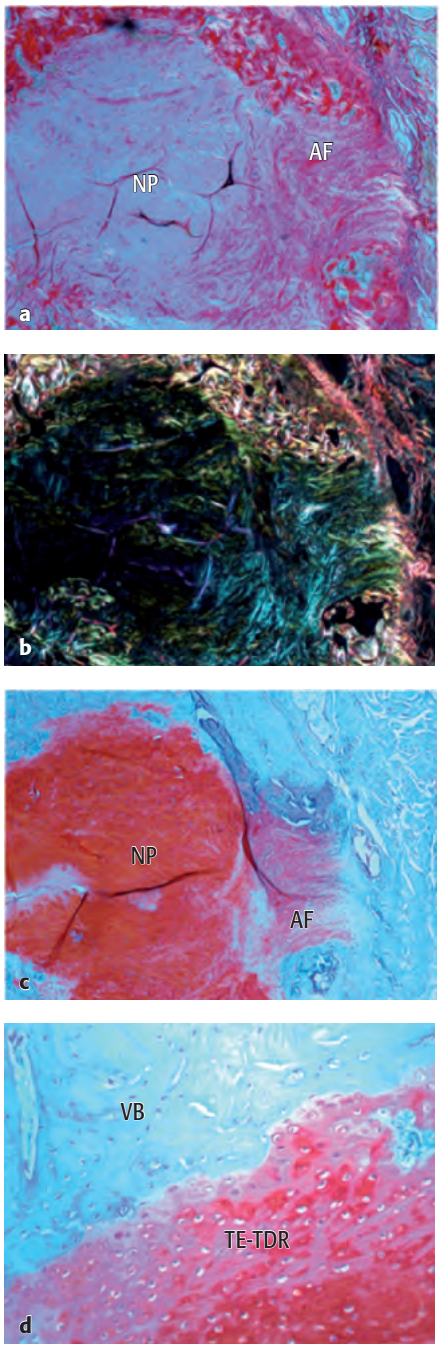


Figure 6 **MRI**
a **Disc space height measurements in flash/T1 sequence (top: implant (714.0 micrometer), bottom: native disc (823.5 micrometer)**
b **T2 sequence, red circle surrounding the implant NP**

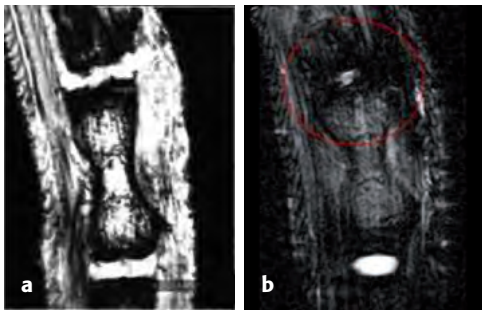


Figure 7 **7 Tesla MRI imaging of rat tail IVDs showing axial images (preliminary pilot data)**
a **Diffusion tensor imaging (DTI) on two explanted rat tail discs in Formalin**
b **Higher magnification of a, showing directional alignment of collagen fibers (red and green) when compared to the color ball on top which maps fibers' directional alignment (eg, fibers directing from left to right: red, from top to bottom: blue)**
c **Native IVD in vivo (successful imaging of top and bottom of the IVD (red)**
d **Gradient echo sequence (GE) showing differentiation between NP (light grey) and AF (dark margin)**
e **GE of reimplanted tail IVD at the explantation level**
f **T1Rho sequence demonstrating the NP (grey) within the AF (dark margin), containing the yellow marked region of interest for value acquisition (preliminary data are consistent with values reported in the literature).**
g **T2 image of native IVD in vivo for monitoring of hydration (white: NP)**