Total Disc Replacement Using Tissue Engineered Intervertebral Discs In An In-vivo Beagle Model

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Introduction: Disc degeneration in the cervical spine is a prevalent clinical predicament that may require surgery. Anterior cervical decompression and fusion (ACDF) [1] is a commonly performed surgical procedure, but it poses risks for pseudoarthrosis and adjacent segment disease (ASD) [2]. Prosthetic total disc replacement (TDR) devices have been developed to maintain segmental mobility. However, it remains controversial whether the theoretical advantage of TDR truly translates into clinical or radiological superiority over ACDF [3]. Tissue-engineered intervertebral discs (TE-IVD), a form of TDR that ideally mimics the native IVD in respect to motion, stability, load bearing properties, and mechanical damping [3], are potential biological advancements for the treatment of degenerative disc disease. We have demonstrated the feasibility of creating a composite AF/NP disc-like construct with viable cells and mechanical properties analogous to the native discs in the rat tail [3]. In this study, we evaluated the viability of our TE-IVD implants in a beagle cervical model measuring radiological and histological parameters.

Methods: TE-IVD Construction: Canine-sized TE-IVD was fabricated as previously described [4]. Lumbar IVDs from skeletally mature beagles were obtained in aseptic conditions, washed in phosphate-buffered saline, and separated into AF and NP tissues by macroscopic appearance. Upon further dissection into small fragments, the tissues were digested in collagenase Type II at 37°C for 6 hours prior to being filtered and centrifuged. Cells were cultured in Ham’s F-12 media, removed from the flasks with 0.05% trypsin, and counted with a hemocytometer. Alginate (3% weight/volume) was then seeded with the cultured NP cells (2.5 × 10E6 cells/ml) and injected into a predesigned mold. Each of the molded NPs was placed in a well of 24-well culture plate as a collagen gel solution (1 mg/ml seeded with 1 × 10E6 cells/ml) brought to a pH of 7.0 was pipetted around the NP component. All components were gelled at 37°C to create the AF. Culture was kept in media for 2 weeks, facilitating collagen fibril alignment and contraction under the influence of AF cells until required diameter was attained. In-vivo surgical implantation was subsequently undertaken.

Experimental Protocol: A total of 10 skeletally mature beagles were divided into two groups: group 1 (n=4) served as discectomy control, group 2 (n=6) was implanted with TE-IVDs following discectomy. Adjacent proximal segments served as internal healthy control. For discectomy, the whole IVD was resected. All dogs were imaged post-operatively at two and four weeks.

Imaging: Postoperative imaging was performed with conventional X-rays and high-resolution 3-Tesla MRI under full anesthesia. Quantitative measurements were taken using a pre-established method
comparing the ratio of IVD height in line with the spine to that of the vertebrae proximal and distal to the IVD [5]. Additionally, all MRIs were analyzed both qualitatively and quantitatively in accordance to T2-weighted images. Utilizing a novel algorithm developed by our group, we filtered out all MRI voxels unrepresentative of NP tissue using their T2-relaxation time (T2-RT), sequestering the extent of NP hydration based on the mean T2-RT values within the NP voxels [6].

**Histological assessment:** Animals were sacrificed for histological assessment. Histological staining was obtained using Safranin-O for proteoglycans (PG).

**Results:** TE-IVDs were successfully implanted following discectomy of the cervical spine (Fig 1a, b). The dogs tolerated the procedure well and without prolonged pain. MRIs of 2-week implanted TE-IVDs showed T2 high intensity surrounded by acute inflammatory responses due to surgical invasion, which faded by 4 weeks. At 4 weeks, the TE-IVD maintained its position in the disc space with a relative increase in T2 intensity. By contrast, the discectomized segments appeared as a black disc (Fig 1c, d). These findings suggest that the implanted TE-IVDs engraft in the disc space despite significant biomechanical demands in the dog cervical environment. Histological assessment further demonstrated chondrocyte-like cell viability in the TE-IVD, abundant proteoglycan content in extracellular matrices, and robust integration into the host tissues without signs of immune reaction (Fig 2a, b). Quantitative analyses showed that by 4 weeks, the disc height indices (DHI) of TE-IVDs and discectomy dogs were 71% and 49% of healthy control discs, respectively (healthy control: 0.25±0.03; TE-IVD: 0.18±0.02; discectomy: 0.14±0.02, Fig 2c). T2 relaxation time measurements at 4 weeks further revealed that NP hydration of the implanted TE-IVDs was over 70% of that of healthy discs (healthy control: 384±49.4; TE-IVD: 271±67.7; Discectomy: 0±0, Fig 2d).

**Discussion:** Biological disc implants are exposed to a severe local milieu: mechanical loading, continual dynamic motion of the dog cervical spine, acute inflammatory response, potential immune reaction to the implant, and poor nutritional supply. Despite these conditions, our in vivo TE-IVDs in the beagle cervical spine maintained their position and remained viable at four weeks. Discs showed evidence of dynamic adaptation to the host environment, with extracellular matrix production and cell proliferation. TE-IVDs further maintained disc height as well as hydration of NP at 4 weeks with up to 70-percent viability as normal healthy discs. Experiments with long-term follow-ups are necessary to further evaluate the clinical applicability of the presented innovation.

**Significance:** The biological replacement of degenerated discs may have a significant impact on our management of spinal disease. To our knowledge, this is the first study testing tissue-engineered discs derived from allogenic NP and AF cells in a larger animal model.
Figure 2

A

B

C

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