Fabrication Of Canine-Sized Alginate/Collagen Tissue-Engineered Intervertebral Discs

Jorge A. Mojica-Santiago¹, Peter Grunert, MD², Roger Hartl, MD², Lawrence J. Bonassar, PhD¹,²

¹Department of Biomedical Engineering, Cornell University, Ithaca, NY, USA, ²Neurological Surgery, Weill Cornell Medical College, New York, NY, USA, ³Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca, NY, USA.

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Introduction: Whole tissue-engineered intervertebral disc (TE-IVD) scaffolds have been produced in the last decade as a biological alternative to treat degenerative disc disease (DDD). Investigators have proposed diverse approaches to construct composite scaffolds with distinct nucleus pulposus (NP) and annulus fibrosus (AF) regions designed for total disc replacement [1-6]. Efforts led to in vivo studies in athymic mice and rats that have yielded promising results, in terms of tissue integration, biochemical composition, and mechanical function [5,7]. However, small animal models limit the composite TE-IVDs to a different biomechanical environment from what occurs in the human spine and have a considerably different anatomy. To date, a larger animal model with comparable loading patterns and anatomical features remain to be studied. Small breeds of dogs develop spontaneous cervical disc degeneration and have been used as animal models for biomechanical studies of disc diseases and surgical procedures [8-10]. The current work seeks to utilize our previously established methods [2] to scale up the manufacture of composite alginate and collagen TE-IVDs for a canine cervical spine model and characterize the mechanical and morphological properties of canine-sized TE-IVDs.

Methods: Direct measurements of the C2/C3 and C3/C4 IVDs were obtained from three-year old beagles and the dorsoventral, latero-lateral, and disc height dimensions were used to create customized models of molds with NP-sized cavities in Solid Works. The CAD mold models were fabricated in UV-cured acrylic plastic using 3D printing technology. Three groups of TE-IVDs were produced by varying the size of the disc and the number of AF layers (Fig.1B) [2]. NP and AF cells were isolated from the lumbar spine of skeletally mature Finn/Dorset cross male sheep and cultured for 3 weeks. NP cells were encapsulated in 3% alginate and injected into the molds to produce NP scaffolds, while AF cells were seeded in 2mg/mL solution of collagen type I gel to create the AF layers. Two groups consisted of double-layered discs - mold cavity dimensions were 1:1 and 3:4 of the CAD model - and a group of single layered discs. The composite discs were cultured for 2-4 weeks and disc size measurements were taken twice a week using ImageJ to measure the annular contraction and the NP/AF ratio. Samples were tested under unconfined axial compression using multi-step stress relaxation methods, and the instantaneous and equilibrium moduli were obtained to assess their viscoelastic response [11]. Statistical significance in all results was determined at a 95% level of confidence with a p-value lower than 0.05, using a single factor analysis of variance followed by a post-hoc Scheffé Test.

Results: The single-layered discs had significantly greater ratios of NP:AF (0.6±0.1 %) than both double-layered groups by the end of the culture period (Fig.1C). A contraction ranging from 13.8 to 17.9% of the original size was observed in both single-layered and double-layered TE-IVDs by the first week (Fig.2A-B). With a mean equilibrium modulus of 1.4±0.6 kPa, the large double-layered discs resulted significantly greater than the 0.3±0.2 kPa of the single-layered group, but there were no significant differences between the mean equilibrium modulus of small double-layered discs (0.7±0.6 kPa) and the other groups (Fig.3A). Within the double-layered groups, the large discs presented a significantly greater instantaneous modulus (2.1±0.9 kPa) than their 25% smaller counterparts, which yielded mean modulus values of 1.0±0.7 kPa (Fig.3B). The mean elastic modulus of the single layered discs (0.4±0.1 kPa) resulted significantly different to the large multilamellar disc only.

Discussion: The present studies showed that canine-sized composite TE-IVDs can be successfully produced by the same methods used for our in vivo rat TE-IVDs [7]. The implants were based of direct measurements from beagle IVDs and their size and shape were comparable to their native counterparts (Fig.1A-B). Composite discs with double layers showed higher mechanical properties than those with a single layer, but they also exhibit lower NP:AF ratios than their unilayered counterparts. The addition of collagen-based AF layers coupled with a decrease of the NP:AF ratio accounts for the production of stiffer implants, and thus provided valuable insights with respect to how differences in fabrication parameters have an effect in the compressive properties of the tissue-engineered constructs. Fundamentally, the low relative stiffness of alginate-based NP can reduce the effect of the overall composite stiffness as its relative volume increases. Therefore, in the design and scale up of alginate/collagen composite TE-IVDs, the morphological and composition characteristics allow modifying the construct properties to a desired conditions.

Significance: This work demonstrates the use of established fabrication methods of composite alginate/collagen TE-IVDs, which have proven successful in rat in vivo models, as a scalable process to construct canine-sized cervical TE-IVDs. Such a large animal model with comparable morphology and biomechanical environment to human spine is required to translate this technology to clinical use as an alternative to total disc replacement.
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Figure 1

A. Native canine IVD

B. Tissue-engineered IVD
C. NP/AF Ratio at week 4

Figure 2

A. Multilamellar IVDs Contraction

B. Unilamellar IVDs Contraction

Figure 3
A. Equilibrium Modulus from Stress Relaxation

B. Instantaneous Modulus from Peak Stress

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