

# Flexible Support Materials Maintain Disc Height and Support the Formation of Hydrated Tissue Engineered Intervertebral Discs in Vivo

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**Introduction:** An enduring challenge in implementing a tissue-engineered replacement for end-stage intervertebral disc (IVD) degeneration is mechanically stabilizing the construct at early timepoints following implantation<sup>1</sup>. Previously, polylactic acid (PLA) cages were used to provide mechanical support for tissue engineered intervertebral disc (TE-IVDs) in a pilot minipig study<sup>2</sup>. While PLA possesses mechanical properties that should far exceed those needed to support loads in the spine, stress concentrations arising from bony protrusions at the posterior side of adjacent vertebrae led to failure of these implants in vivo. As such, identifying materials with sufficient flexibility is key to the successful implementation of a TE-IVD support cage. FPLA (FormFutura), a flexible thermoplastic co-polyester, is a 3D printer filament that is amenable to the fabrication of TE-IVD support structures<sup>3</sup>. We have previously shown that cages comprised of FPLA withstood nearly twice the deformation of PLA without any detrimental changes in mechanical performance and minimal damage<sup>4</sup>. However, no present study has evaluated FPLA's ability to maintain disc height or tissue hydration in vivo, both of which are necessary for the long-term stabilization and integration of TE-IVDs. In this study, we evaluated the long-term success of TE-IVDs cultured in FPLA in a clinically relevant large animal model, hypothesizing that FPLA implants would maintain disc height and tissue hydration in the minipig spine.

**Methods:** *TE-IVD fabrication:* Nucleus pulposus (NP) cells were encapsulated in 3% (wt./vol.) alginate at  $10 \times 10^6$  cells/mL, and NP plugs were placed in the center of FPLA cages. Annulus fibrosus (AF) cells were encapsulated in 10 mg/mL type I collagen at  $10 \times 10^6$  cells/mL and pipetted around NP plugs. Resulting implants were cultured within printed cages for 18 days. *Implantation:* Following IACUC approval, empty FPLA cages (n=4), and TE-IVDs cultured in FPLA (n=4) were implanted at C3-4 or C5-6 following complete discectomy (DX) in skeletally mature Göttingen minipigs (n=4) with one level at C6-7 left as a DX control (**Fig. 1**). *Imaging and quantification:* X-rays were taken each week for up to 6 weeks or until cage fracture. Terminal disc height indices (DHI) were calculated using a previously described method and results were compared to DHIs calculated from the PLA pilot study (n=6 animals, n=4 implants, n=3 DX) (**Fig. 1**)<sup>5</sup>. DHIs for both cage materials were normalized to adjacent native discs. Terminal T2 MRI scans were taken of pigs that received TE-IVDs to quantify disc hydration. TE-IVDs were segmented from surrounding tissues by applying a Gaussian mixture model to first-echo T2 images and T2 maps as previously described (**Fig. 3**)<sup>6</sup>. The mean relaxation time of discectomy or implant treated segments were normalized to adjacent healthy discs. *Statistics:* Analysis of cage DHIs and TE-IVD hydration were performed using a one-way ANOVA. DHIs of stabilized and displaced TE-IVDs were analyzed using a one-way nested ANOVA. All statistical analysis confirmed by Tukey's HSD ( $\alpha=0.05$ ).

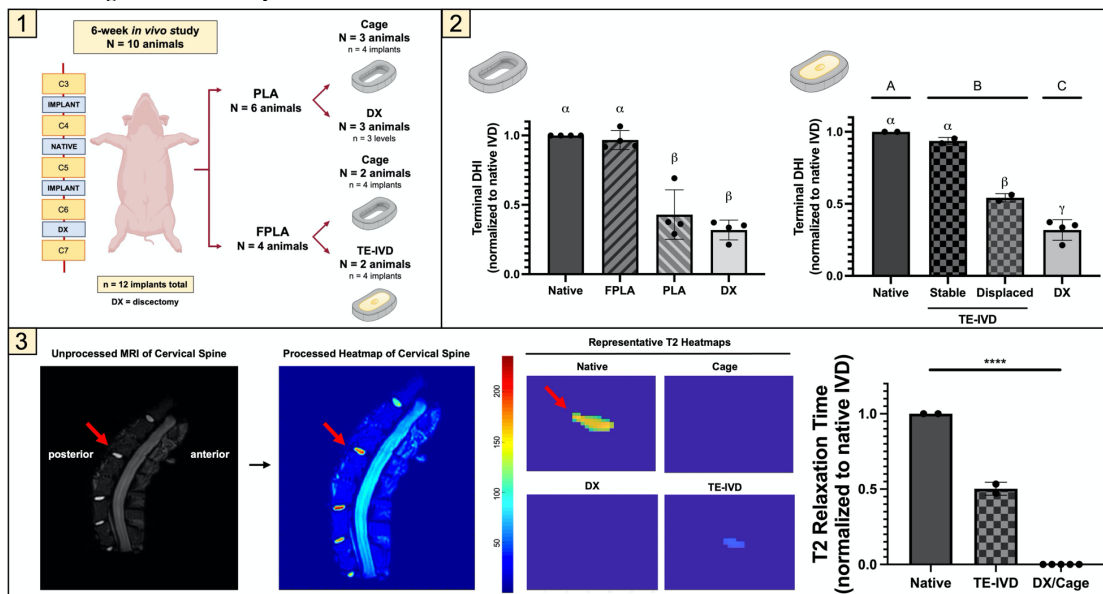
**Results:** FPLA cages restored DHIs to native levels until study endpoint as confirmed by x-ray imaging. Moreover, FPLA cages maintained DHI significantly better than DX levels ( $P<0.0001$ ). In contrast, PLA cages fractured before reaching study endpoint, and terminal DHIs were statistically similar to DX levels (**Fig. 2**). Of the four levels treated with TE-IVDs, 2 levels remained stable and 2 were displaced from the disc space. Levels which remained stabilized yielded DHIs which were statistically similar to native IVD and significantly greater than both displaced and DX levels ( $P<0.05$ ). Displaced levels yielded DHIs which were significantly lower than native and stabilized levels, but which were significantly greater than DX levels ( $P<0.05$ ) (**Fig. 2**). T2 MRIs of stabilized TE-IVDs revealed that levels treated with a construct maintained tissue hydration which was significantly greater than levels treated with an empty cage or DX levels ( $P<0.0001$ ), but which was about half the hydration of native disc tissue (**Fig. 3**).

**Discussion:** In this study, we found that FPLA support cages maintained disc height and supported the formation of hydrated TE-IVD constructs for up to 6 weeks. FPLA cages maintained disc height at native levels throughout the study, suggesting that flexible materials are superior for preserving disc height without failure. This resistance to failure is consistent with FPLA's ability to conform to surface features of adjacent vertebrae. Levels treated with TE-IVDs remained stabilized in the disc space or were displaced before study endpoint. Although displacement led to a decrease in DHI, displaced levels had a greater disc height when compared to DX levels, implicating the therapeutic benefit of implanting a TE-IVD support structure. Stabilized TE-IVDs maintained tissue hydration up to 6 weeks at half the T2 relaxation time of native disc. This level of T2 relaxation time is consistent with previous findings in other large animal models<sup>7</sup>. Our work confirms that FPLA is a suitable material for fabricating TE-IVD support structures by maintaining disc height and supporting the formation of hydrated TE-IVDs for 6 weeks in the minipig spine.

**Significance:** Implanting TE-IVDs with FPLA support cages leads to disc height maintenance and the stabilization of hydrated tissues in the spine, enhancing the long term success of TE-IVD implants and providing a basis for clinical translation.

**References:** 1. Mojica-Santiago+ 2018, 2. Lintz thesis 2021, 3. Marshall+ 2021, 4. Fidai+ 2022, 5. Jarman+ 2015, 6. Hussain+ 2019, 7. Gullbrand+ 2018

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**Figure 1:** In vivo study design and treatment schematic.

**Figure 2:** Left) Terminal DHIs of levels treated with empty support cages normalized to native disc. Right) Terminal DHIs of levels treated with TE-IVDs cultured in FPLA normalized to native disc. Shared letters or symbols denote no statistical difference.

**Figure 3:** Left) Effect of MATLAB algorithm on unprocessed MRI. Mid) Representative T2 heatmaps of native, DX, and treated levels. Right) T2 relaxation times of native, DX, and treated levels normalized to native disc.