Statement of Purpose: The field of tissue engineering has made great strides in the last three decades, especially in demonstrating the ability to create geometrically complex tissues with multiple cells and some physiologic functionality. However, achieving satisfactory cell alignment in complex tissues remains a challenge. Current techniques allow for unidirectional alignment, but complex structures like the heart or the gastroesophageal junction are composed of multiple wrapping alignments of muscle cells which are critical to their function (1-4). In this work, we demonstrate that previous work in unidirectional cell alignment can be extended to these complex problems by engineering microtissues composed of cells that are pre-aligned. These microtissues can then be assembled into larger structures by 3D bioprinting (Fig. 1a). This method promises to allow direct printing of tissues with either simple or complex alignments.

Methods: Human primary esophageal smooth muscle cells (SMCs) were seeded into collagen or collagen/fibrinogen pre-gels then cast into geometrically restrictive molds. Molds included posts or other retention features which the tissues could suspend between as the cells remodeled the hydrogel. Alternatively, cells suspended in pre-gels were encapsulated in long fibers by coaxial wet spinning with sodium alginate, resulting in a shell of alginate restricting an inner core of gel and cells. After the cells proliferated and contracted their hydrogel to form aligned microtissues, the tissues were cut to remove them from their molds. They were then suspended in a variety of viscous bioinks, including gelatin methacrylate (GelMA). These bioinks were extruded using Cellink Inkredible and BioX 3D bioprinters.

Results: Both mold casting and wet spinning (Fig. 1b) resulted in microtissues with aligned smooth muscle cells. We found that the molding methods resulted in unstable tissues which were prone to failure prior to full cellularization of the gel. Growth in the 3D aligned environment resulted in better expression of alpha smooth muscle actin and collagen 1A1 compared to unaligned gels and 2D controls (Fig 1c). The wet spinning process was considerably easier to control and produce large amounts of microtissues once the cell fiber was cut into small pieces. 3D bioprinting of microtissues suspended in GelMA or Pluronic F127 demonstrated the feasibility of aligning the microtissues by flow through a restrictive nozzle (Fig 1d) and revealed possible failure modes, including “hairpinning” and “looping” of the tissues as they were extruded.

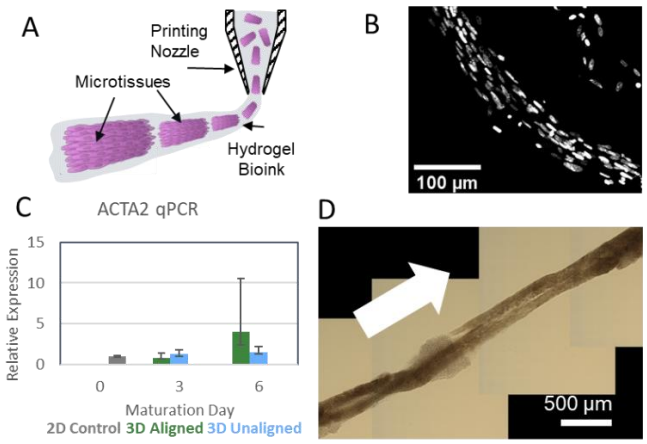

Figure 1. (A) Bioprinting approach (B) DAPI image of aligned SMC nuclei in a coaxial wet spun fiber (C) enhanced smooth muscle actin expression (D) Multiple microtissues printed in alignment with each other (white arrow shows movement direction of printing nozzle).