

# Programmed Bending of a 3D Bioprinted Heart Tube Inspired by Morphogenesis

Jacqueline Bliley<sup>1</sup>, Joshua Tashman<sup>1</sup>, Maria Stang<sup>2</sup>, Brian Coffin<sup>2</sup>, Andrew Lee<sup>1</sup>, Annie Behre<sup>1</sup>, Andrew Hudson<sup>1</sup>, Dan Shiowski<sup>1</sup>, T.J. Hinton and Adam W. Feinberg<sup>1,2</sup>

Regenerative Biomaterials and Therapeutics Group

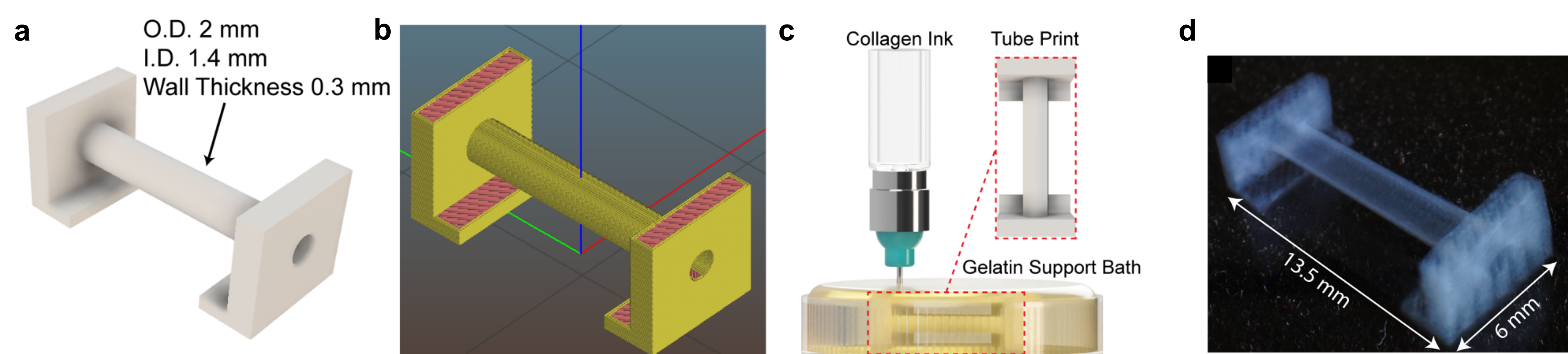
<sup>1</sup>Dept. of Biomedical Engineering, <sup>2</sup>Dept. of Materials Science and Engineering, Carnegie Mellon University

## OVERVIEW

- A long-term goal of cardiac tissue engineering is to engineer whole heart organs to replace the damaged heart.
- Cardiac bioprinting has emerged as a potential tool to generate whole heart macroscopic structures, but current bioprinted hearts display reduced function compared to the adult *in vivo* heart.
- During embryonic heart development, the heart undergoes morphogenesis starting as a linear tube, which then bends, loops, and septates to form its mature three-dimensional structure. This morphogenesis process is thought to impart specific mechanical stresses and strains, which are critical to later heart organ structure and function.
- Here, we sought to use embryonic heart morphogenesis as a guiding principle with the ultimate goal of building heart-like organs with enhanced contractile function.

## APPROACH

### (1) 3D Printing of Collagen Tube



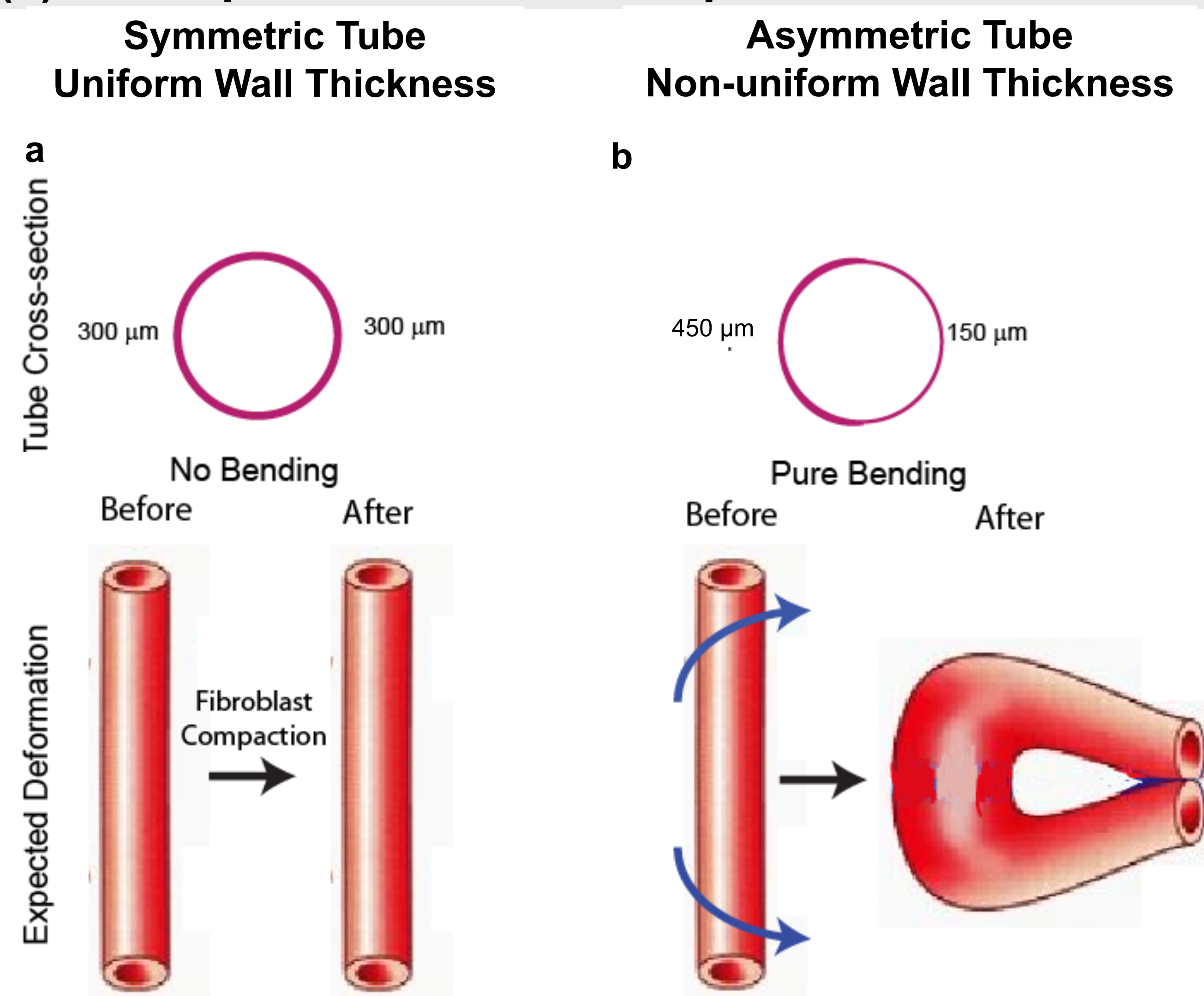
- Computer aided design (CAD) model of tube.
- CAD model converted to sliced model using Slic3r to enable 3D printing.
- The tube was printed out of type I collagen using Freeform Reversible Embedding of Suspended Hydrogels (FRESH).
- Image of 3D printed collagen tube.

### (2) Generation of Heart Tube



- 3D printed collagen tube in polydimethylsiloxane (PDMS) well to enable heart muscle tissue casting around the tube. SB= 2 mm.
- Heart muscle tissues consisting of 90% embryonic stem cell derived cardiomyocytes and 10% cardiac fibroblasts within a collagen/Matrigel mixture were cast around 3D printed collagen tubes. SB= 2 mm.
- Fibroblasts within the matrix compact the matrix around the tube to form an engineered heart tube. SB= 2 mm.

### (3) Concept of Fibroblast Compaction Force Driven Heart Tube Bending

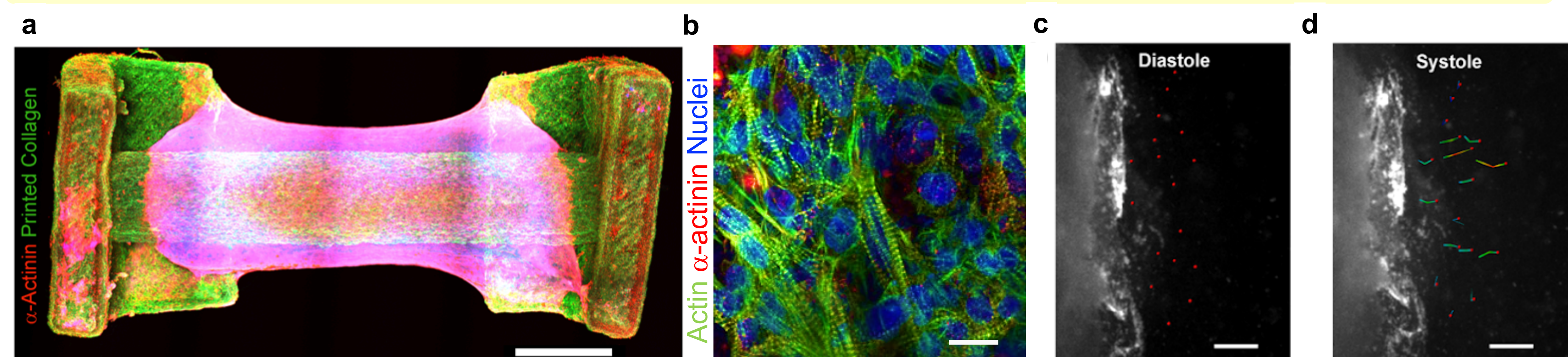


We hypothesized that we could alter the dimensions of the tube and use the force applied during fibroblast compaction to drive heart tube bending similar to early heart morphogenesis.

- Symmetric tubes have a uniform wall thickness. Following fibroblast compaction, we expect no significant tube bending or deformation.
- Asymmetric tubes have a thinner wall on one side (150  $\mu$ m) and a thicker wall on the other side (450  $\mu$ m). We hypothesize that following the applied fibroblast compaction force asymmetric tubes will bend similar to early heart morphogenesis.

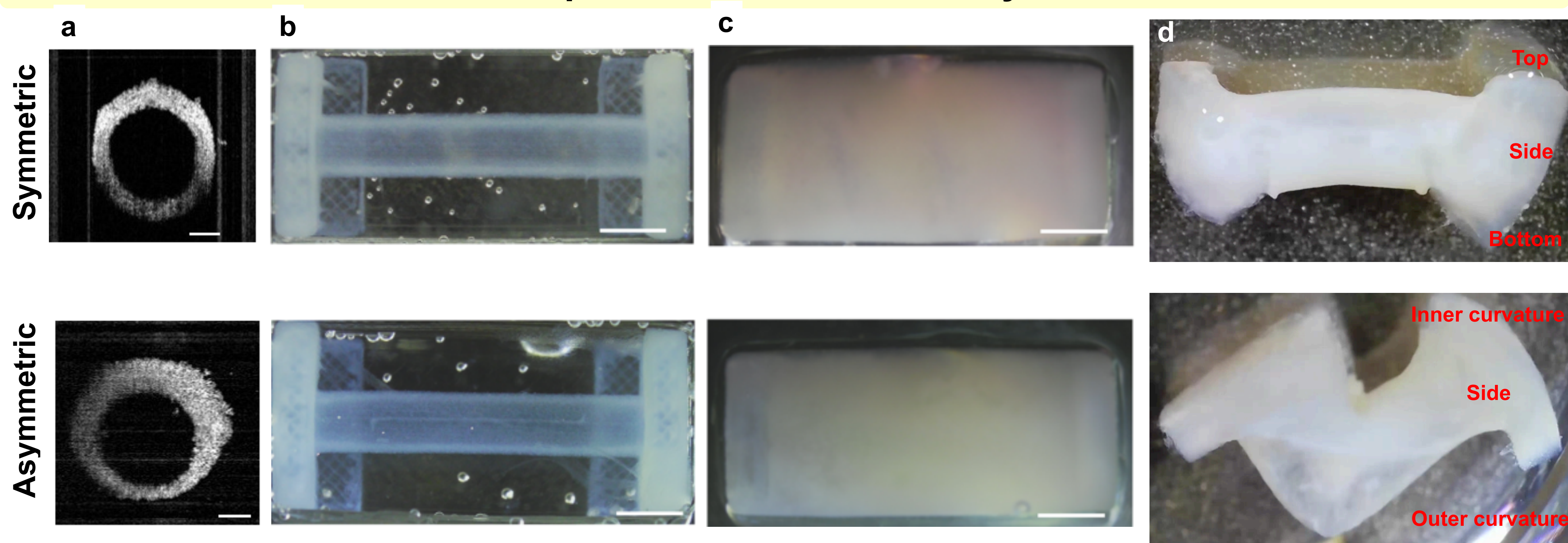
## RESULTS

### Linear Heart Tubes Can Be Generated From 3D printed Symmetric Collagen Tubes



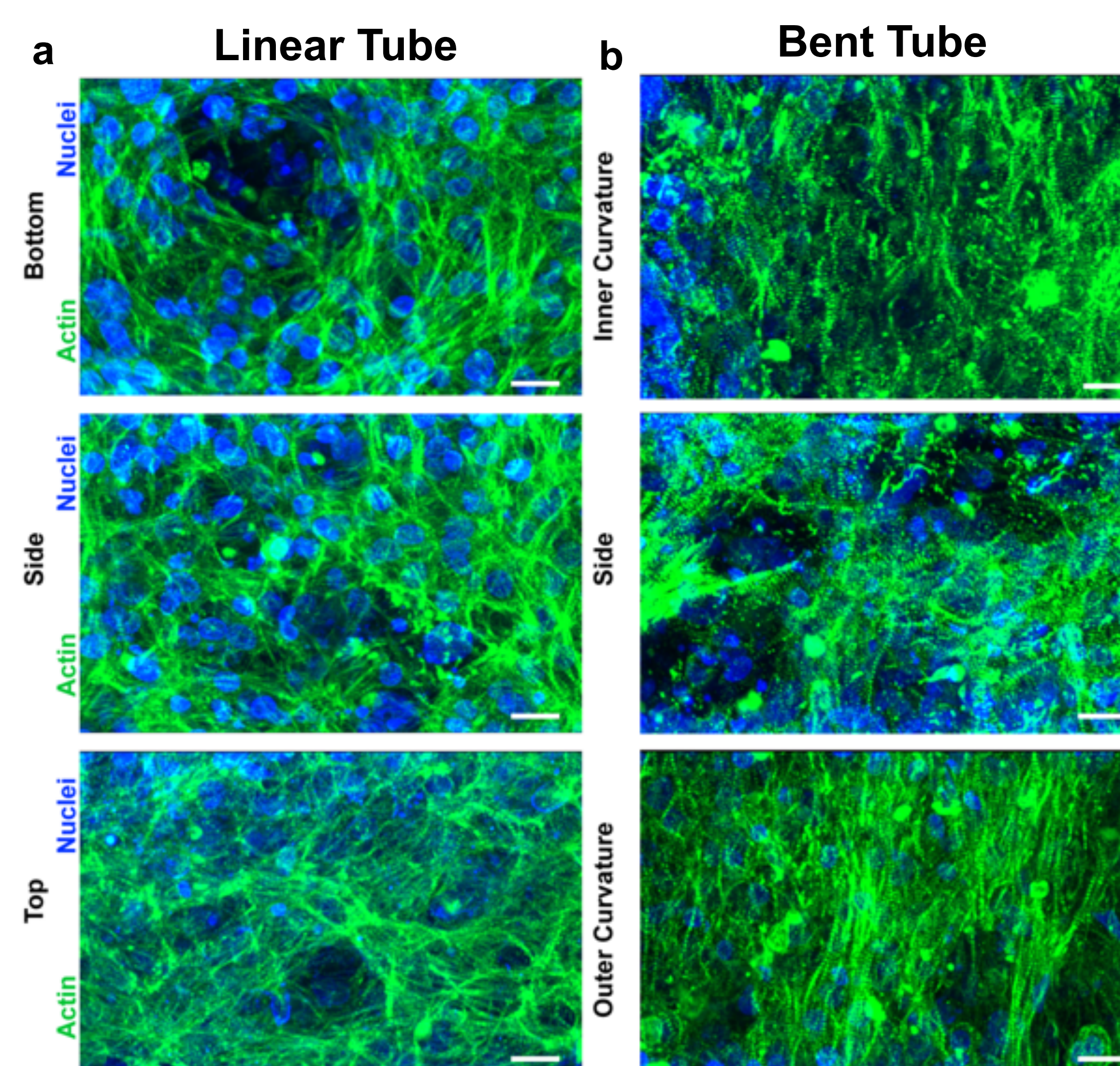
- Confocal image of heart tube showing heart muscle tissue (red) wrapped around 3D printed collagen tube (green). SB= 2 mm.
- Confocal image of tube surface showing dense layers of interconnected cardiomyocytes on tube surface. SB= 20  $\mu$ m.
- Engineered heart tubes can contract and generate pressure within their lumens to displace fluorescent beads. Panel shows initial location of beads (red) during heart muscle relaxation (diastole). SB=300  $\mu$ m.
- Displacement of beads during active engineered heart tube contraction (systole). SB=300  $\mu$ m.

### Fibroblast Compaction Force Bends Asymmetric Tubes



- Cross-sections of symmetric and asymmetric tubes. SB= 300  $\mu$ m.
- Brightfield image of tubes in well prior to casting. SB= 2 mm.
- Heart muscle tissues cast around tubes. SB= 2 mm.
- Heart tubes in culture. Asymmetric tubes bend significantly following fibroblast compaction. SB= 2 mm.

### Heart Tube Bending Creates Regional Differences in Cardiomyocyte Alignment



Previous research has suggested that heart tube bending morphogenesis drives increased cardiomyocyte alignment on the outer curvature of the bent heart tube (Aumon et al. PLoS Biology. 2007, Chi et al. PLoS Biology. 2008) and we were interested in investigating this in our simulated morphogenesis model.

- Symmetric (linear) tubes displayed no significant difference in cardiomyocyte alignment in different regions of the heart tube. SB= 20  $\mu$ m.
- Asymmetric (bent) tubes in general displayed a higher degree of cardiomyocyte alignment. The outer curvature of the bent heart tube demonstrated a trend towards increased alignment ( $p=0.0547$ ) when compared to the top region of the symmetric tube. SB=20  $\mu$ m.

## SUMMARY

- Engineered heart tubes can be created using a combination of 3D printing and casting approaches and display dense cardiomyocytes on their surface in a largely isotropic orientation.
- Asymmetric heart tubes bend following fibroblast compaction whereas symmetric tubes stay linear.
- Heart tube bending results in regional differences in cardiomyocyte alignment with a higher degree of alignment being observed on the outer curvature of the bent heart tube.
- This work was funded by the Additional Ventures Cures Collaborative [to A.W.F], the Dowd Fellowship [to J.B], and the Carnegie Mellon Presidential Fellowship [to J.B].