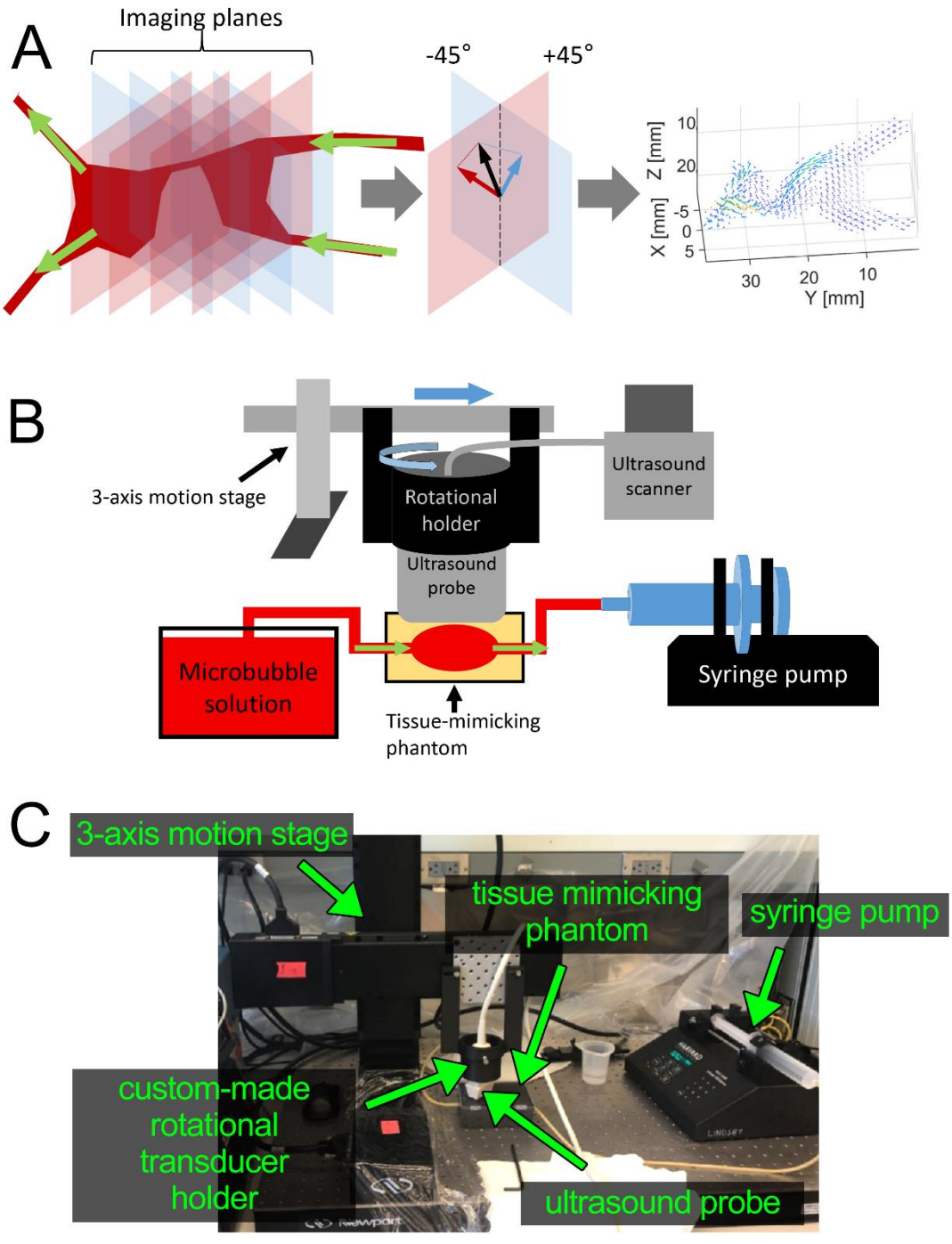
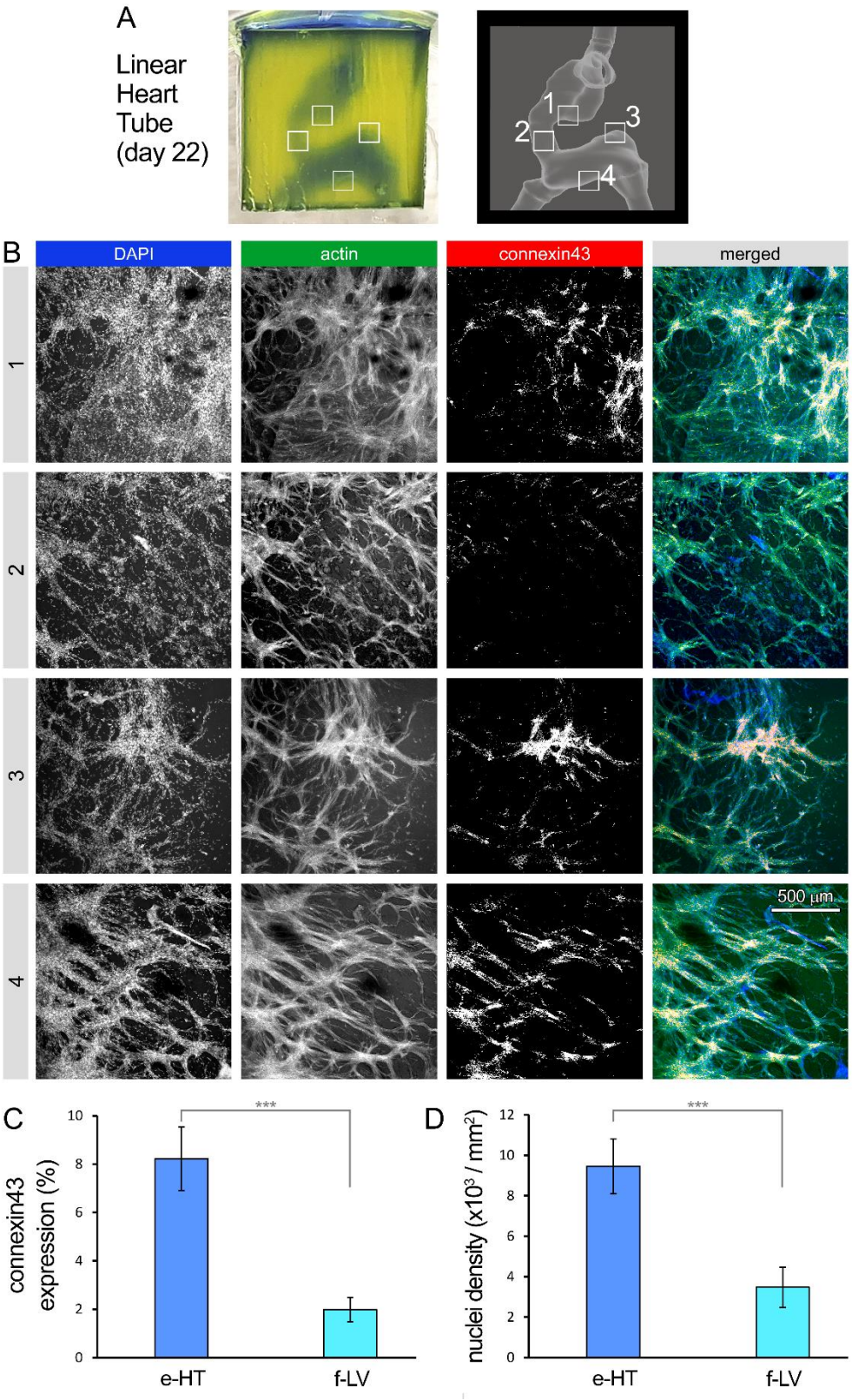
Online Supplements

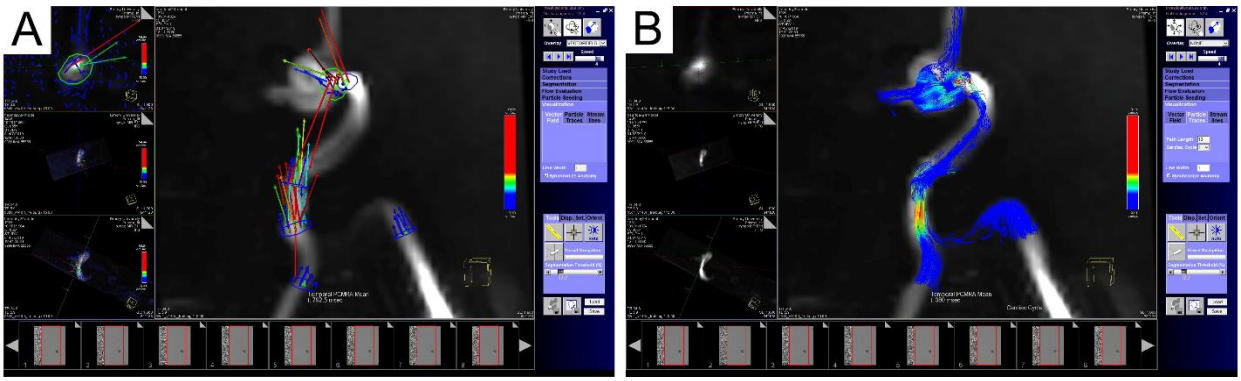


SI Figure S1: Schematic presentation of the experimental set-up for 3D ultrasound imaging of flow in bioprinted constructs. A: Illustration of methodology for the 3D ultrasound velocimetry. **B** and **C:** Illustration and photo of the 3D particle imaging velocimetry system and experiment set-up.

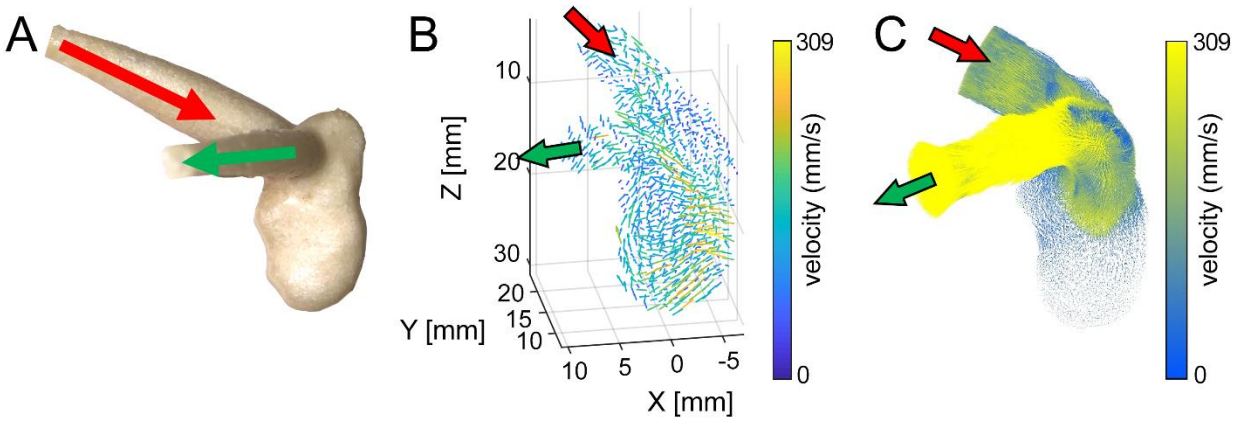


SI Figure S2: Additional immunohistochemical analyses of cellularized models of developing human heart. A-Left: A bioprinted e-HT construct, made using gelMA-

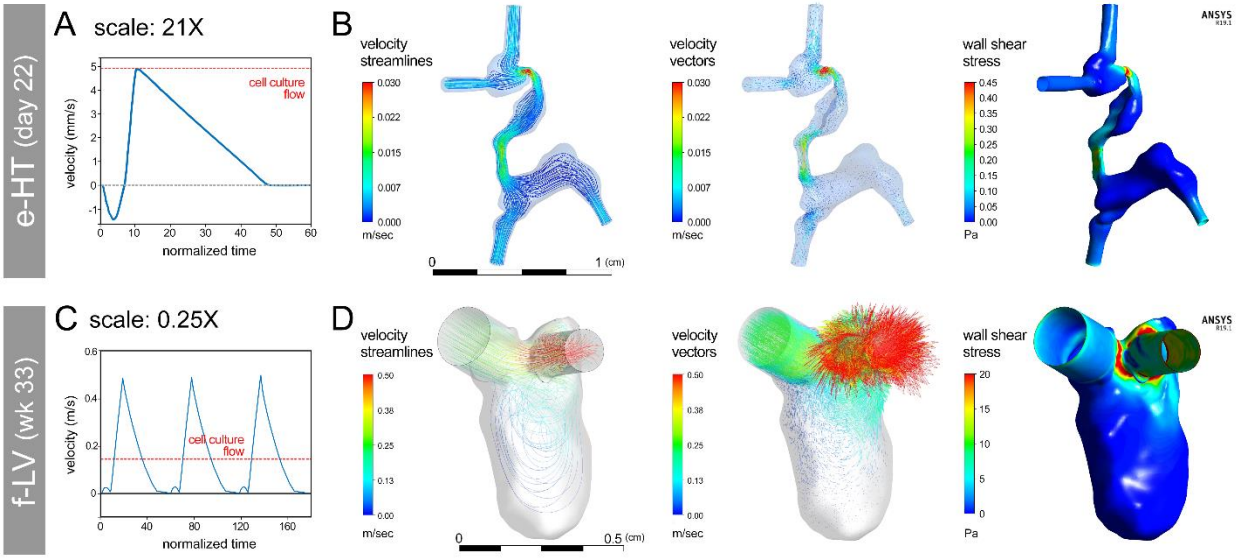
based bioinks and perfused with PBS (a blue food coloring used for visualization). **A-**Right: corresponding CAD model used to bioprint the e-HT constructs. The four insets in panel (**A**) highlight the areas in which the cellular constructs were fixed, sliced, and imaged using immunohistochemical (IHC) assay. **B:** IHC imaging of bioprinted, cellular e-HT constructs performed after 14 days of *in vitro* 3D culture. Rows 1 to 4 correspond to the windows 1-4 depicted in the panel (**A**). From left to right, columns show immunostaining results for DAPI, actin, connexin43, and merged, respectively. **C-D**: Quantification of connexin43 expression (**C**) and nuclei density (**D**) obtained using IHC images in panel **B**. Note ***: $p < 0.001$.



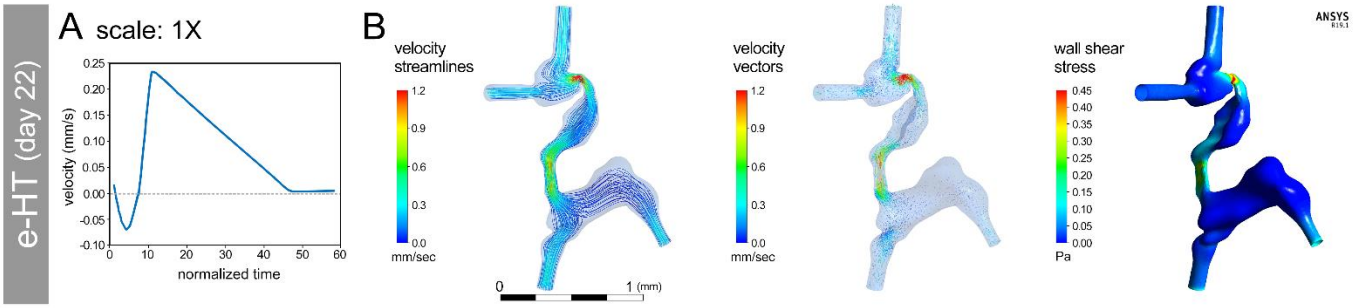
SI Figure S3: A-B: 4D flow MRI of fluid particle tracings in embryonic heart tube structure (246X scale).



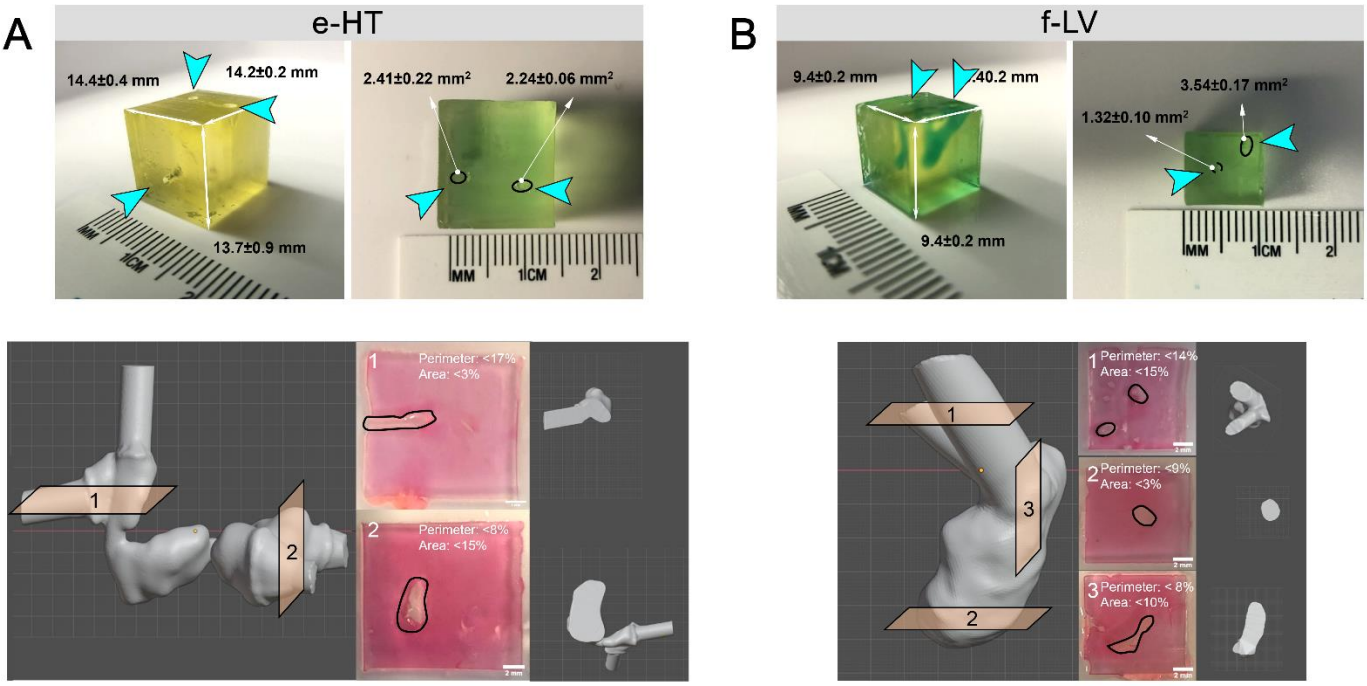
SI Figure S4: A-C: A representative comparison between ultrasound results (**B**) and CFD simulations (**C**) for the flow velocity vectors in the fetal left ventricle (f-LV, 1X scale). The CFD result is a snapshot of the turbulent flow presented in Fig. 3 (F) at the inlet velocity of ~0.22 m/sec during deceleration.



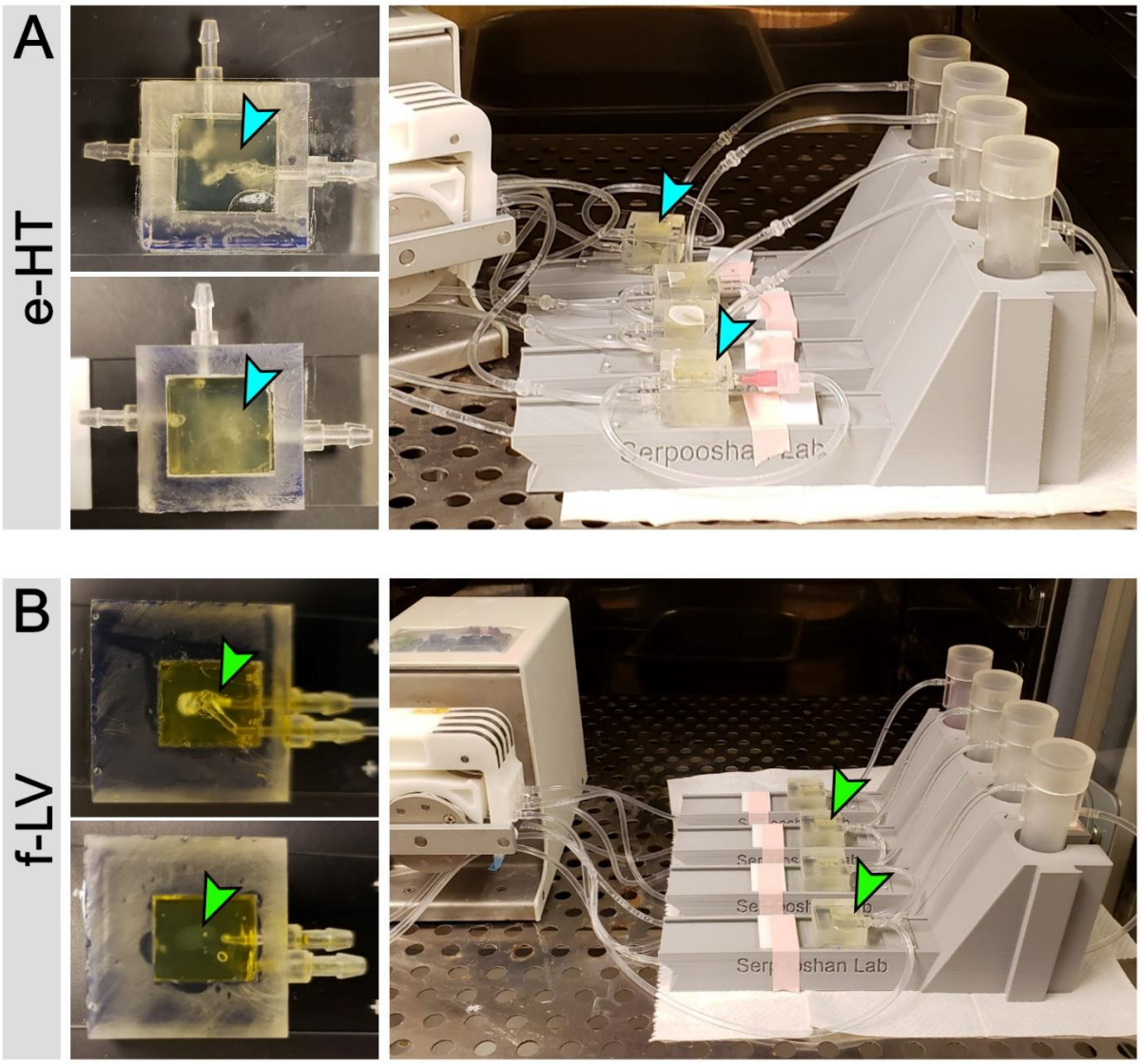
SI Figure S5: A-B: CFD results for flow in the human e-HT constructs at 21X scale. The same scale was used to bioprint e-HT constructs, using gelMA hydrogel, and conduct the cellular assays. Results demonstrated the flow velocity and wall shear stress at peak flow. **C-D**: CFD results for flow in the human f-LV constructs at 0.25X scale. The same scale was used to bioprint f-LV constructs, using gelMA hydrogel, and conduct the cellular assays. Results demonstrated the flow velocity and wall shear stress at peak flow.



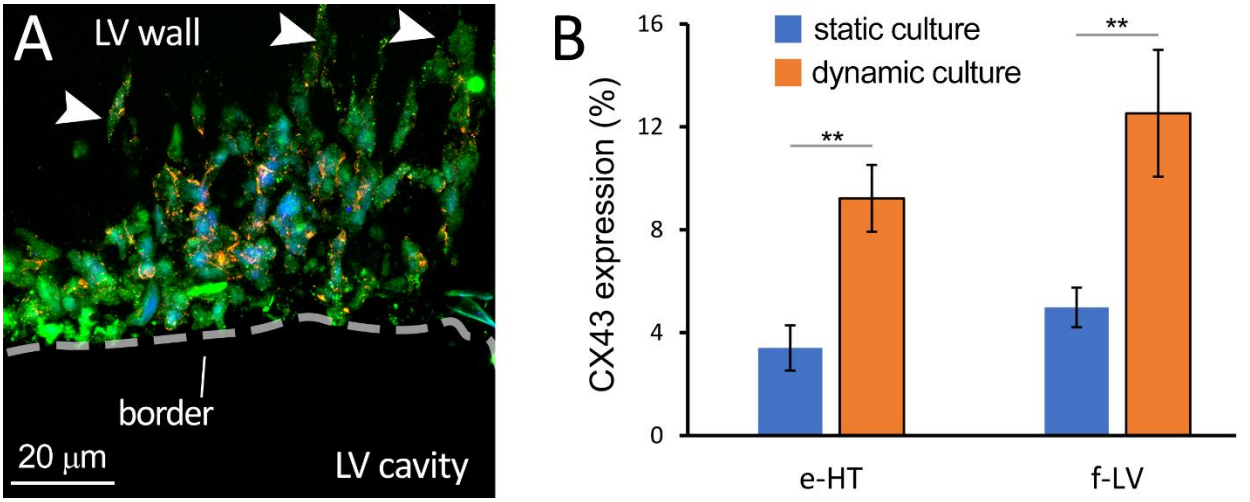
SI Figure S6: A-B: CFD results for flow in the human e-HT construct at 1X (natural) scale. This scale was used to determine the range of wall shear stress in the native tissue under physiologic conditions.



SI Figure S7: Bioprinting fidelity assessment for the e-HT (A) and f-LV (B) constructs. Bioprinted constructs underwent precise structural measurements, including the length of the entire cubic structure in X, Y, and Z directions, the diameter of inlets and outlets in each construct (arrows, top row), as well as some of the internal cavity dimensions (bottom row). Measurements were compared to the CAD design, as reference. A total number of n = 4 was used for each group.



SI Figure S8: A: Experimental setup used for the perfusion of e-HT tissues via a 4-channel bioreactor, enabling parallel flow of four constructs. **B**: Representative assembly for the f-LV constructs. Arrows point to the printed tissue within the perfusion chambers. A total number of *n* = 4 was used for the perfusion culture of cellular cardiac models for a total duration of 2 weeks.



SI Figure S9: A: Immunohistochemical image of the HUVECs cultured in an f-LV construct under dynamic flow conditions for 2 weeks. Immunostaining for DAPI (blue), CD31 (green), and connexin43 (red) at the luminal surface of the LV cavity shows significant EC growth and confluency on the surface, as well as the inward migration of cells towards the gelMA tissue (arrows). **B:** Quantification of CX43 expression in the e-HT and f-LV constructs cultured under static (blue) versus dynamic flow (orange) conditions for 2 weeks. Note **: $p < 0.01$. A total number of $n = 3$ was used per study group.

Online Video S1: CFD modeling of flow velocity streamlines through a perfused (84X) model of e-HT.

Online Video S2: CFD modeling of flow velocity vectors through a perfused (84X) model of e-HT.

Online Video S3: CFD modeling of wall shear stress through a perfused (84X) model of e-HT.

Online Video S4: 4D MR imaging of fluid particle tracings in embryonic heart tube structure (246X scale).

Online Video S5: CFD modeling of flow velocity streamlines through a perfused (1X) model of f-LV.

Online Video S6: CFD modeling of flow velocity vectors through a perfused (1X) model of f-LV.

Online Video S7: CFD modeling of wall shear stress through a perfused (1X) model of f-LV.

Online Video S8: Confocal imaging of HUVEC-seeded static e-HT construct after 14 days of *in vitro* culture. The 10X magnification video shows DAPI (blue), actin (green), and Connexin43 (red) staining throughout the 3D slice.

Online Video S9: Confocal imaging of HUVEC-seeded static e-HT construct after 14 days of *in vitro* culture. The 40X magnification video shows DAPI (blue), actin (green), and Connexin43 (red) staining throughout the 3D slice.

Online Video S10: Confocal imaging of HUVEC-seeded static f-LV construct after 14 days of *in vitro* culture. The 10X magnification video shows DAPI (blue), actin (green), and Connexin43 (red) staining throughout the 3D slice.

Online Video S11: Confocal imaging of HUVEC-seeded static f-LV construct after 14 days of *in vitro* culture. The 40X magnification video shows DAPI (blue), actin (green), and Connexin43 (red) staining throughout the 3D slice.

Online Video S12: Confocal imaging of HUVEC-seeded f-LV construct after 14 days of dynamic *in vitro* culture (using the bioreactor). The 40X magnification video shows DAPI (blue), CD31 (green), and Connexin43 (red) staining throughout the 3D slice.