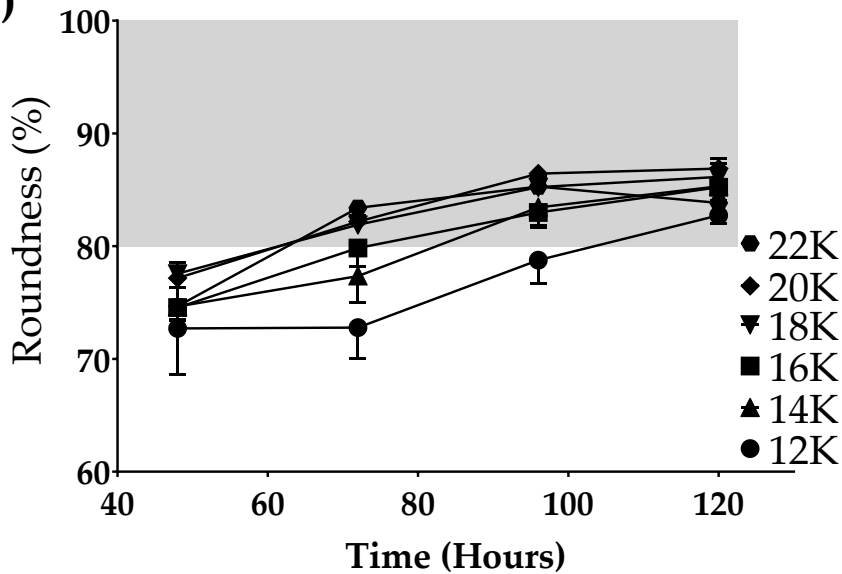
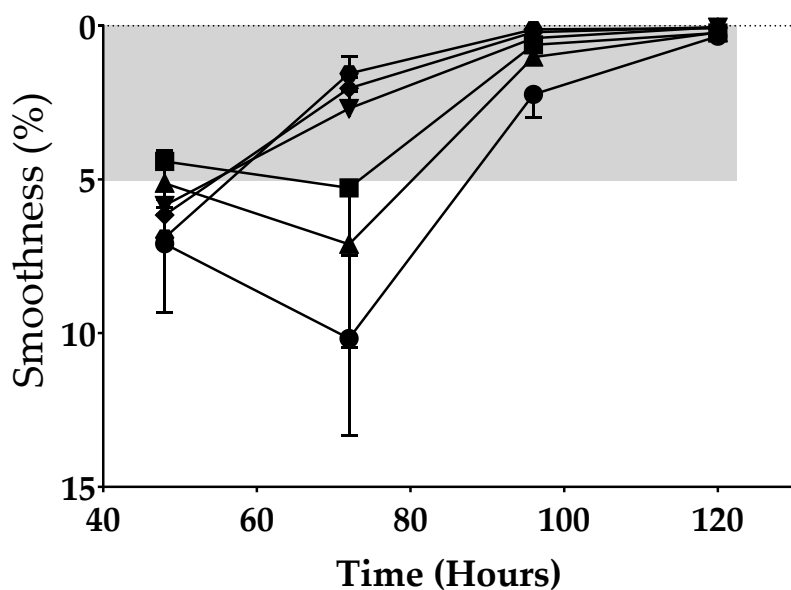
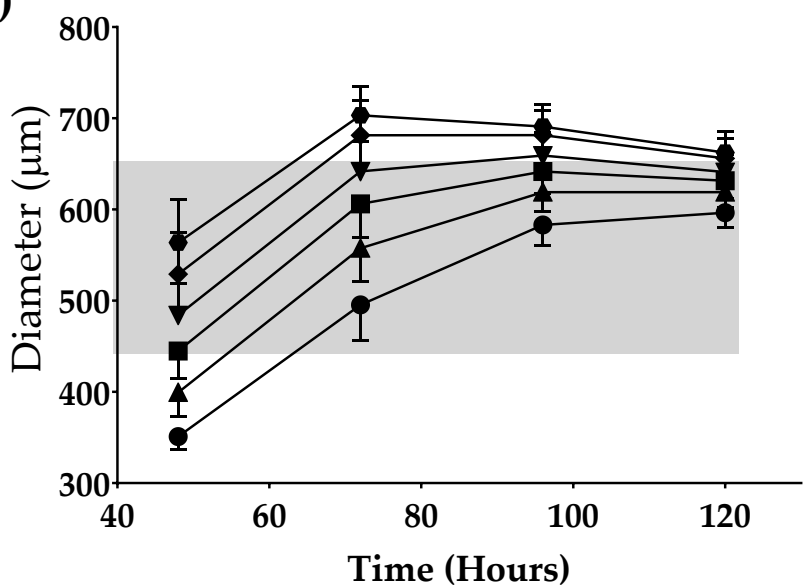


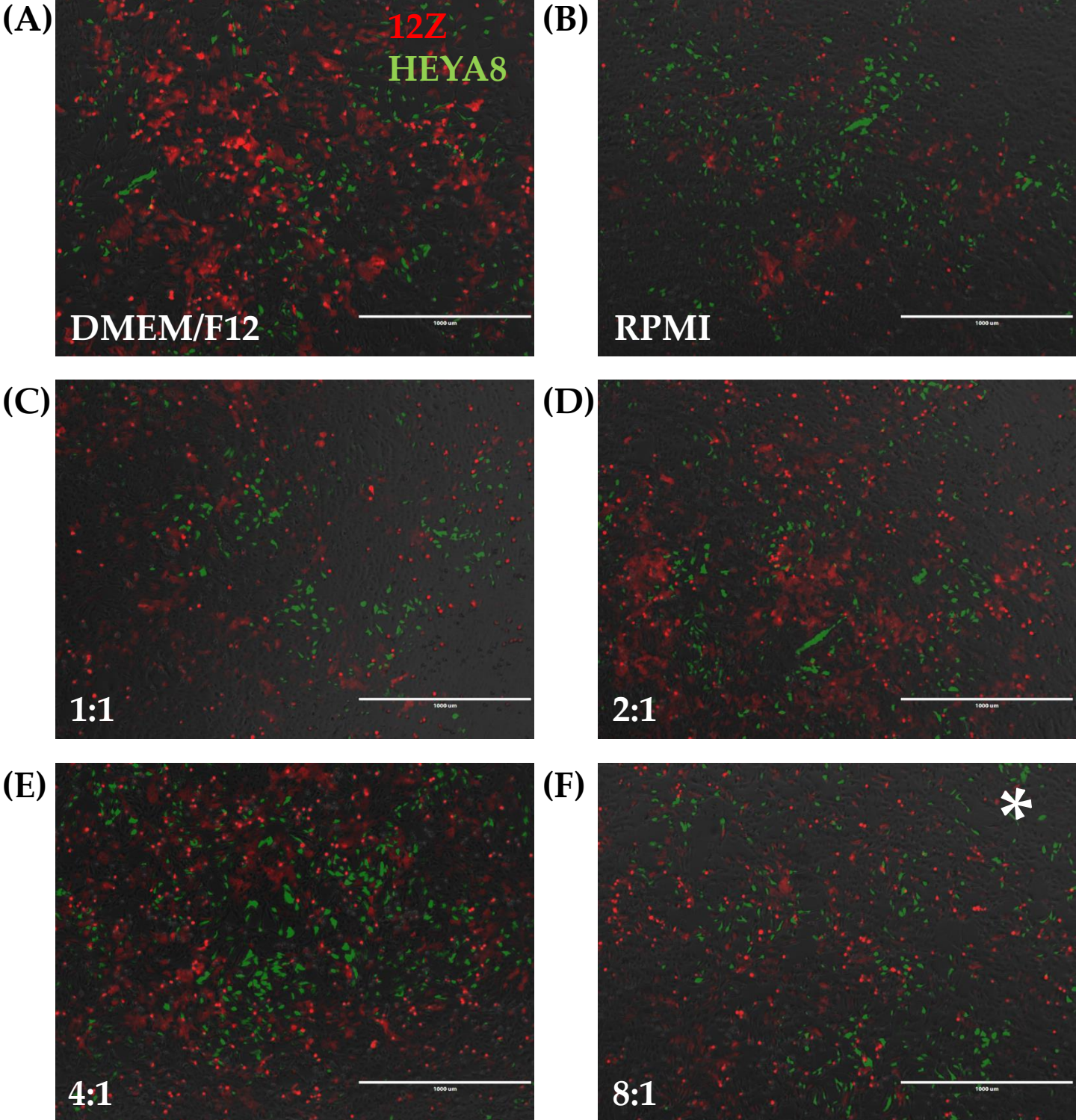
Supplemental Figure S1. 12Z cell spheroid size was sensitive to changes in serum. A) At 72 hours, 12Z spheroids showed no differences in roundness or smoothness. However, 12Z spheroids were significantly larger when grown in serum from Atlanta Biologicals than Corning. **B)** At 72 hours, spheroids have no statistically significant differences in roundness, smoothness, or diameter as passage number increases. Early passage was < +5, middle = +5-+15, and late < +15. N = 3. **, P < 0.01.

(A)

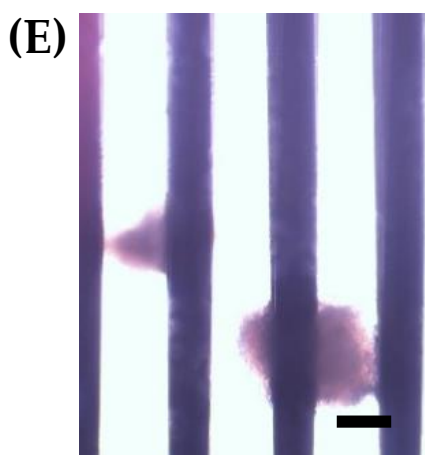
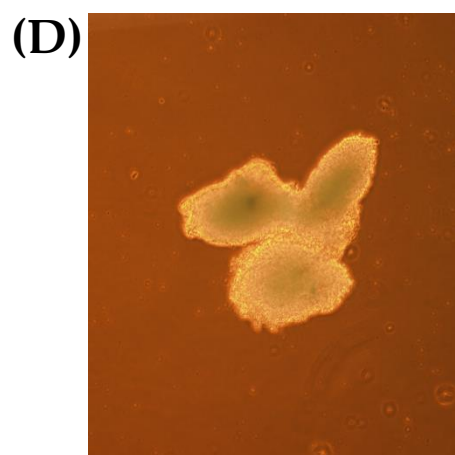
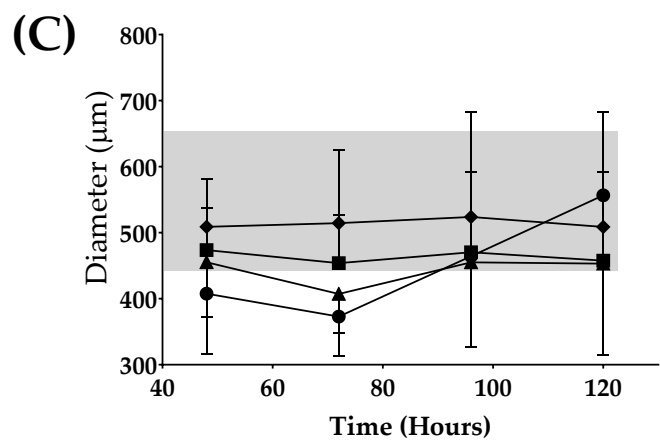
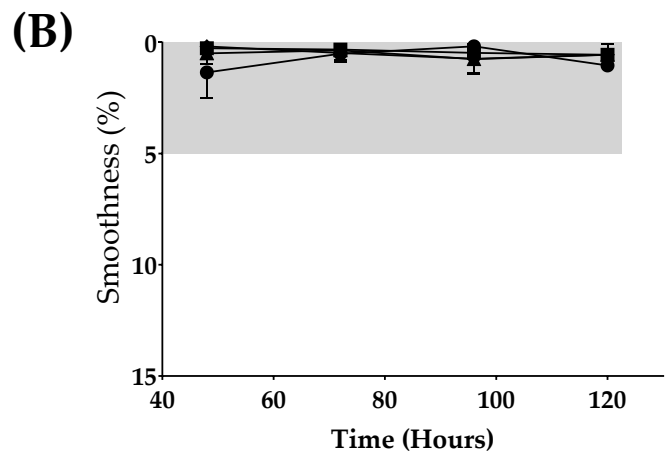
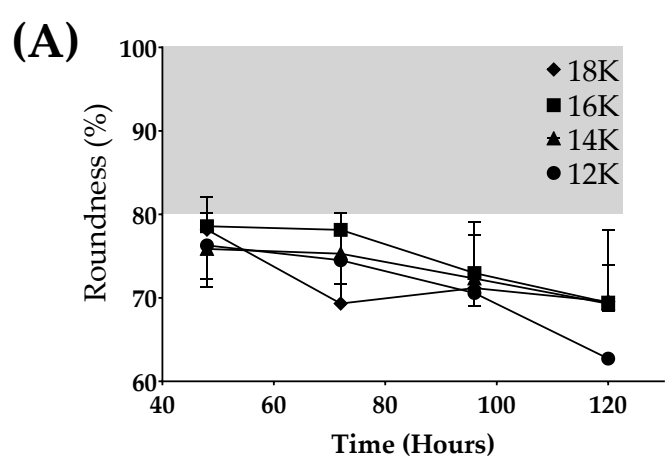
Supplemental Figure S2. HEYA8 spheroid characteristics.

Optimization for time post-seeding and number of cells seeded for A) roundness, B) smoothness, and C) diameter. Grey shading indicates tolerability range of Regenova technology. N = 3.

(B)**(C)**



Supplemental Figure S3. Co-culturing cells in monolayer to determine optimal media. Cells were cultured for 96 hours in a 6-well plate with a total of 100,000 cells at a 7-12Z:1-HEYA8 ratio. Media used was **A)** DMEM/F12, **B)** RPMI, **C)** 1-DMEM/F12:1-RPMI, **D)** 2-DMEM/F12:1-RPMI, **E)** 4-DMEM/F12:1-RPMI, or **F)** 8-DMEM/F12:1-RPMI. Scale = 1000 μm . * Indicates media that appropriately supports growth of both cell lines in co-culture.



Supplemental Figure S4. T-HESC cells formed spheroids but failed to meet all of the Regenova Bio 3D Printer criteria for biofabrication. Optimization for time post-seeding and number of cells seeded for **A)** roundness, **B)** smoothness, and **C)** diameter. Grey shading indicates tolerability range of Regenova technology. N= 3. **D)** Spheroids made from T-HESC cells alone become misshapen from the suction or **E)** shear on contact with the Kenzan. Scale = 200 μm .