

Engineering of Three-Dimensional Microenvironments to Promote Contractile Behavior in Primary Intestinal Organoids

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Supplemental Figures

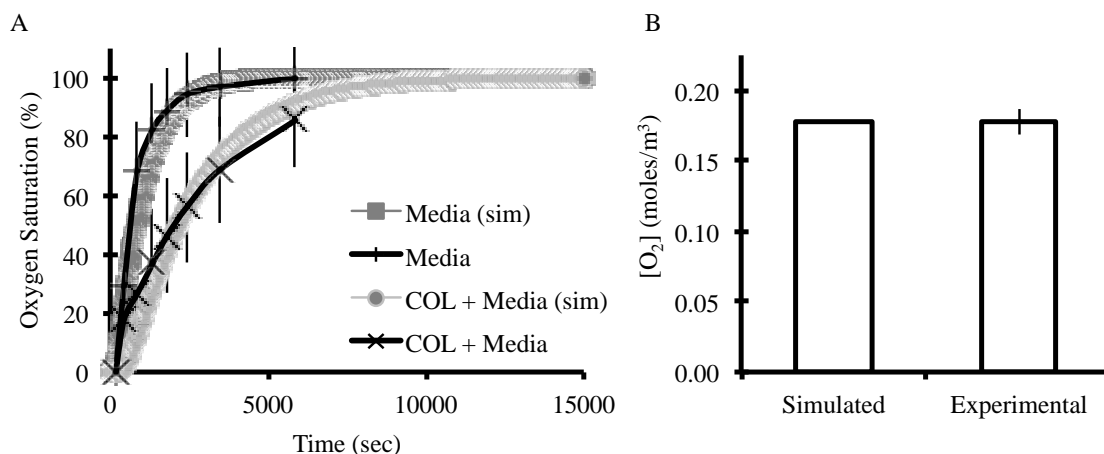


Figure S1. A) Overlay of simulated and experimentally measured oxygen saturation of cell media and collagen matrices of varying density, hydrated with cell culture media. B) Comparison between simulated and experimentally measured equilibrium oxygen concentration for organoid cultures, to experimentally determine the maximum oxygen consumption rate of 0.0006 moles/m³.

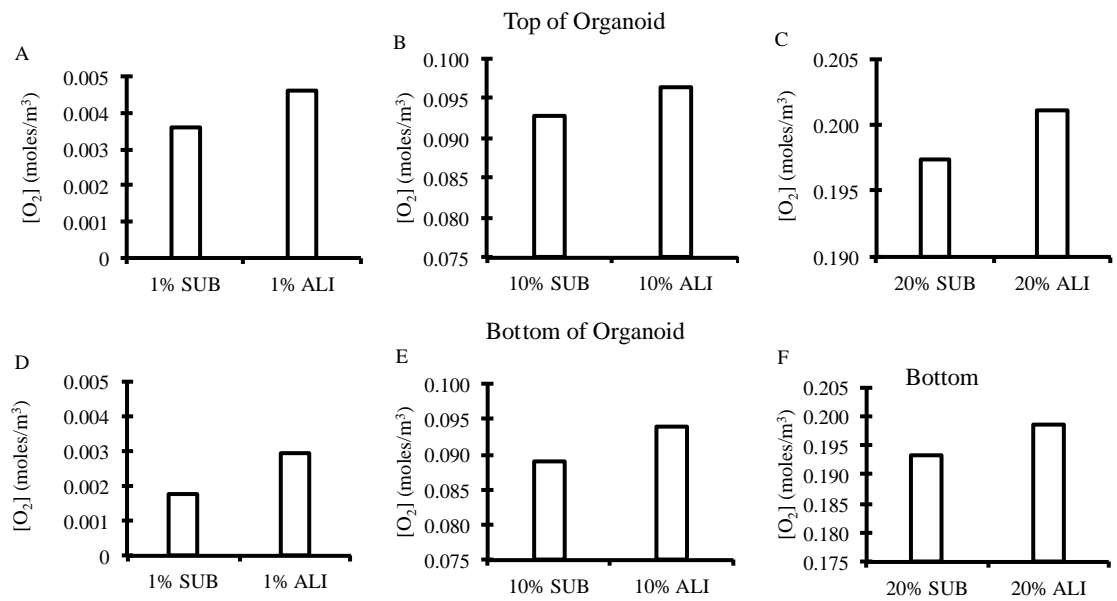


Figure S2. Finite element model predicted equilibrium oxygen concentrations at the A-C) top and D-F) bottom of the central organoid body within submerged and air-liquid interface culture configurations equilibrated to 1%, 10%, and 20% ambient oxygen concentrations.

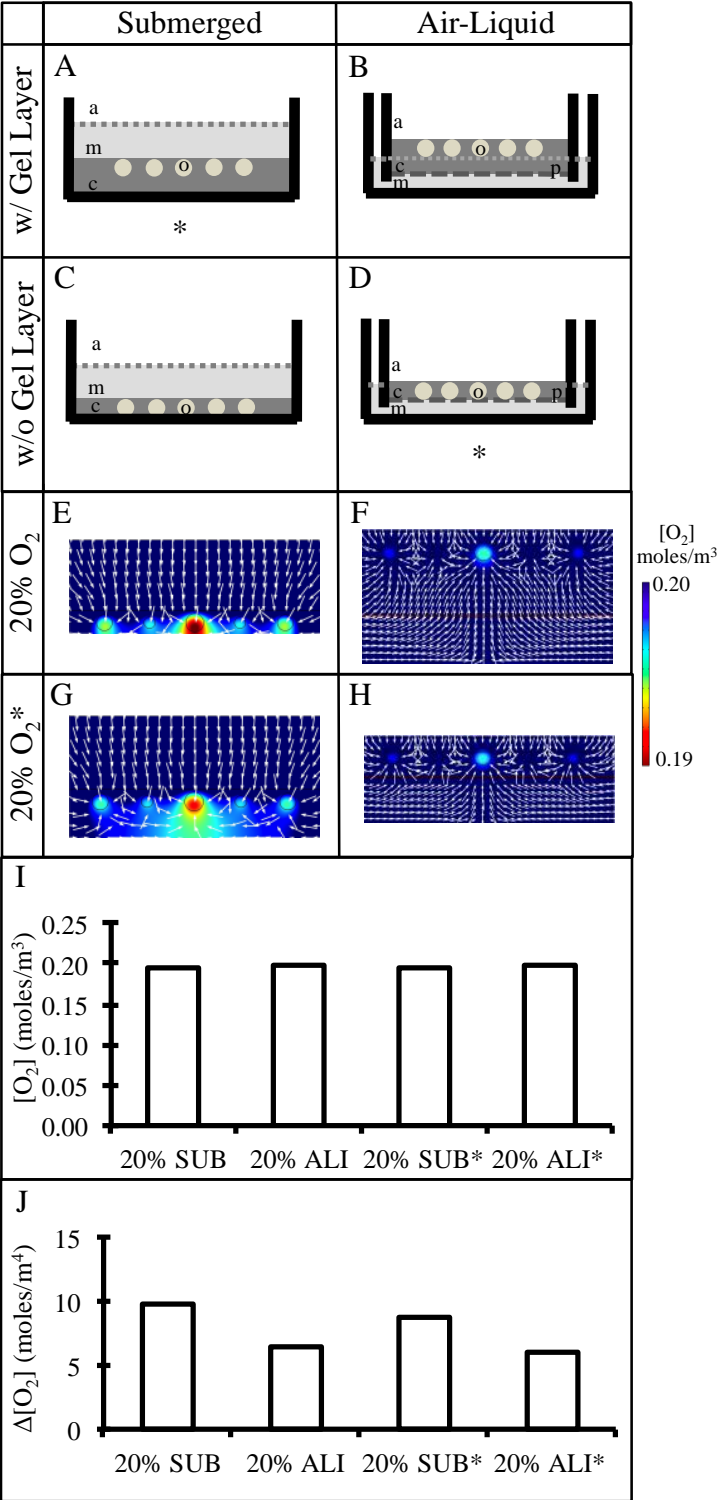


Figure S3. A-D) Schematic of submerged and air-liquid interface cultures with and without an underlying acellular layer of collagen gel, and E-H) corresponding simulated equilibrium oxygen concentration when exposed to normoxic ambient conditions. The I) average equilibrium oxygen concentration and J) oxygen gradient experienced across the central organoid body are also shown for these culture conditions.

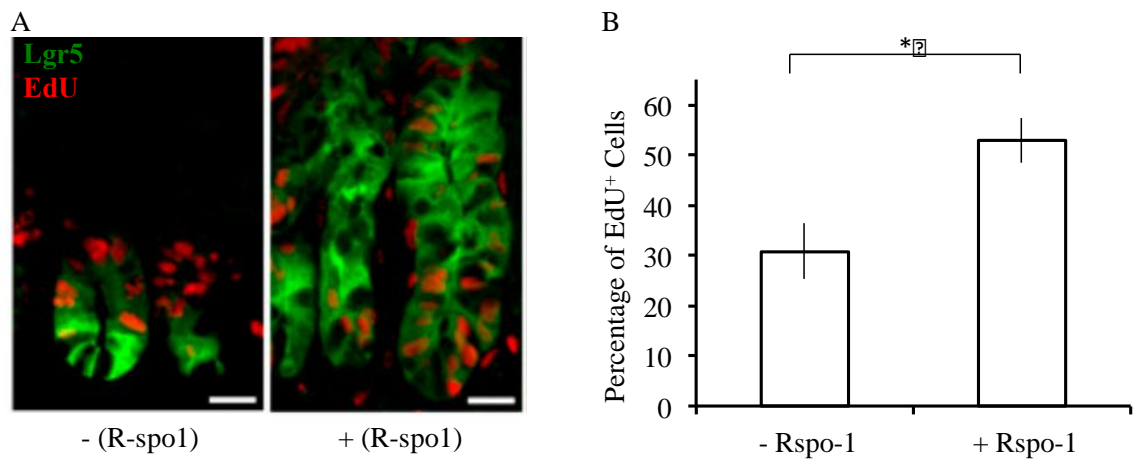


Fig. S4. Recombinant R-spondin1 (R-spo1) elicited expansion of Lgr5-eGFP⁺ intestinal stem cells as evidenced by A) fluorescence microscopy and B) EdU incorporation by Lgr5-eGFP⁺ cells. * denotes statistical significance ($p<0.05$).³⁸

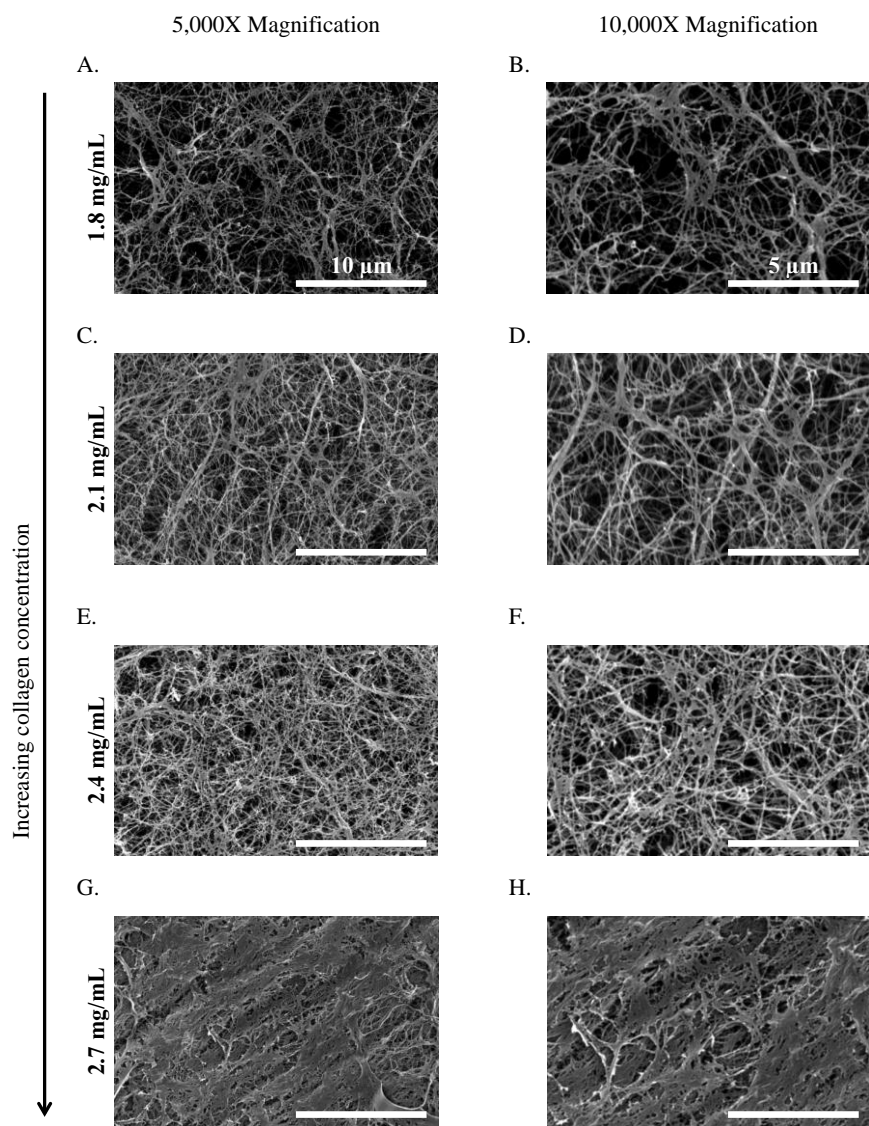


Figure S5. Characteristic scanning electron micrographs of collagen matrices at concentrations of A,B) 1.8, C,D) 2.1, E,F) 2.4, and G,H) 2.7 mg/mL at 5,000X and 10,000X magnification, respectively.

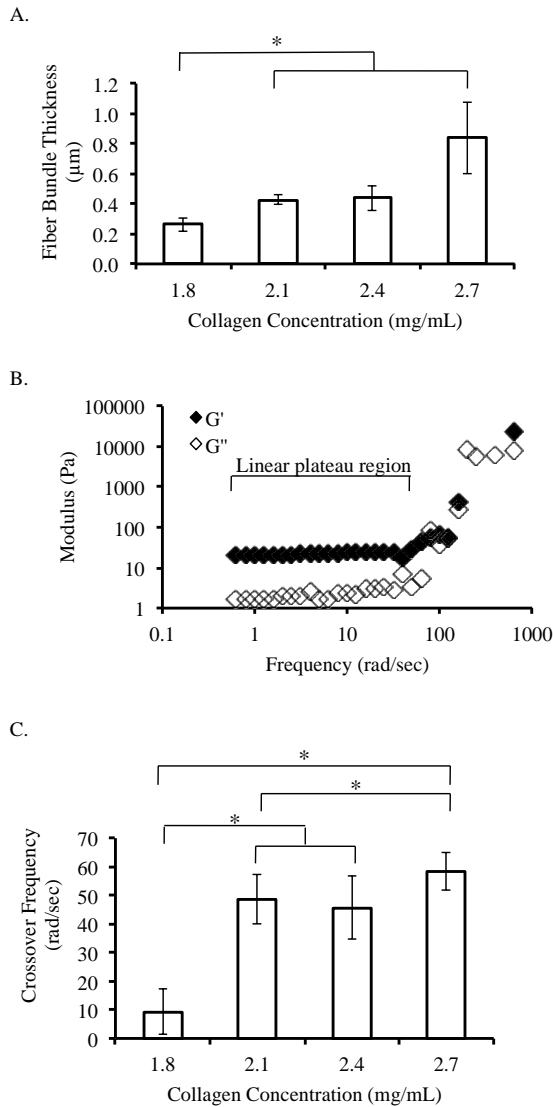


Figure S6. A) Matrix fiber bundle thickness was found to increase at higher collagen densities. B) A representative frequency sweep (collagen concentration of 2.4 mg/mL) demonstrating how the storage modulus, G' , and crossover frequency of collagen matrices were assessed through identification of the plateau region using oscillatory rheology. C) Crossover frequency was found to increase with collagen density. * denotes statistical significance, $p < 0.01$.

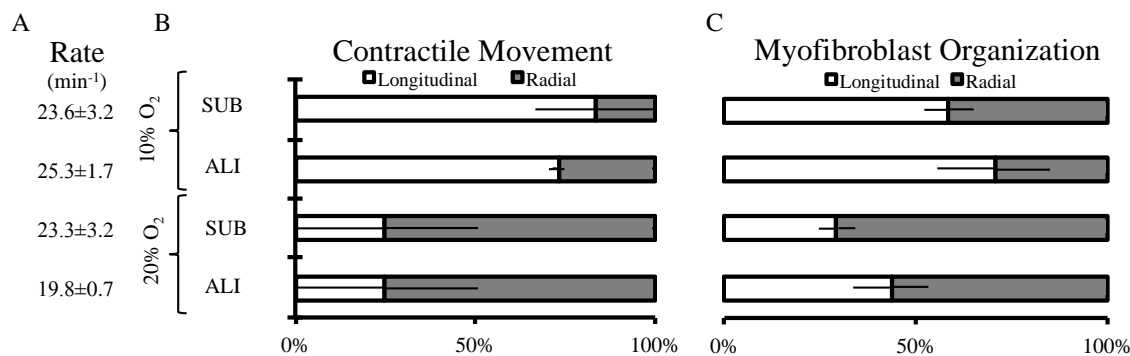
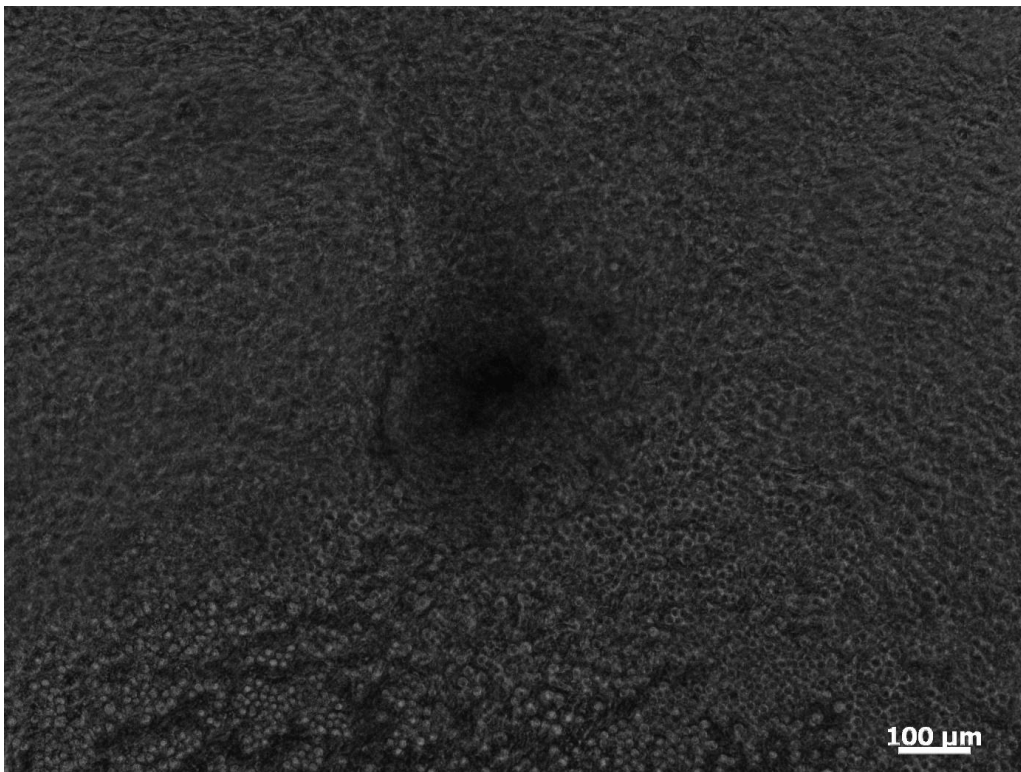


Figure S7. A) Rate of contraction, B) direction of contractile movement, and C) direction of myofibroblast organization for submerged and air-liquid interface cultures under 10% and 20% ambient oxygen concentrations. No statistically significant differences were found in the rate or direction of contractile movement or in the direction of myofibroblast organization surrounding the primary organoids between air-liquid and submerged culture configurations (student's T test, $p < 0.05$).

Supplemental Videos

Supplemental Video 1:



Title: *In vitro* contraction of primary neonatal murine intestinal organoid

Keywords: *in vitro* contraction, intestinal, organoid, primary cell culture, murine organoid, neonatal organoid

Video S1. Contraction of neonatal primary murine intestinal organoid *in vitro*. Video is played at twice the recorded speed.

Supplemental Video 2:

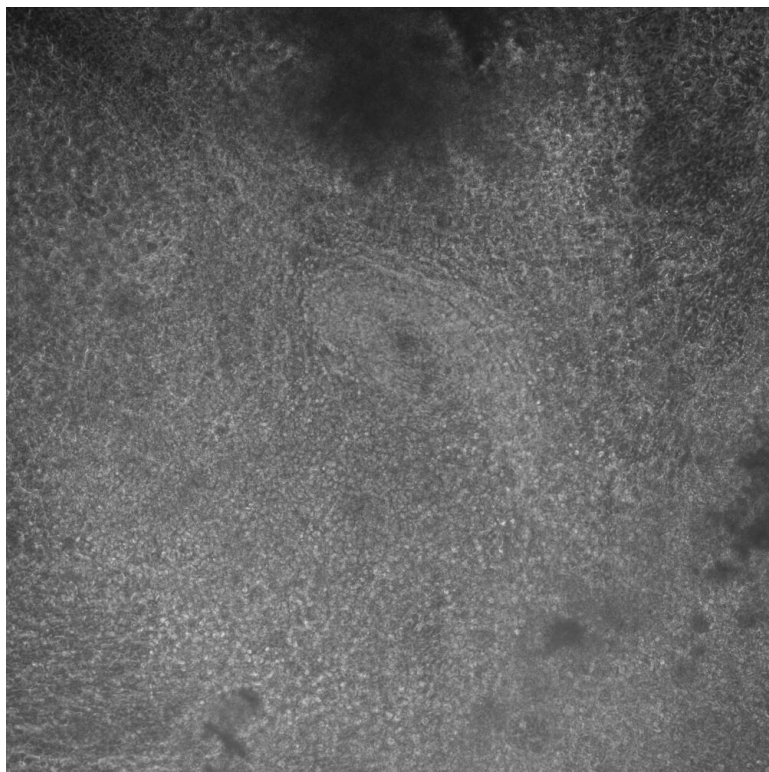


Title: Longitudinal *in vitro* contraction of primary neonatal murine intestinal organoid

Keywords: *in vitro* contraction, intestinal, organoid, primary cell culture, murine organoid, neonatal organoid, longitudinal contraction

Video S2. Longitudinal contractile movement of neonatal primary murine intestinal organoid *in vitro*. Video is played at twice the recorded speed.

Supplemental Video 3:



Title: Radial *in vitro* contraction of primary neonatal murine intestinal organoid

Keywords: *in vitro* contraction, intestinal, organoid, primary cell culture, murine organoid, neonatal organoid, radial contraction

Video S3. Radial contractile movement of neonatal primary murine intestinal organoid *in vitro*. Video is played at twice the recorded speed.