

# ADVANCED FUNCTIONAL MATERIALS

## Supporting Information

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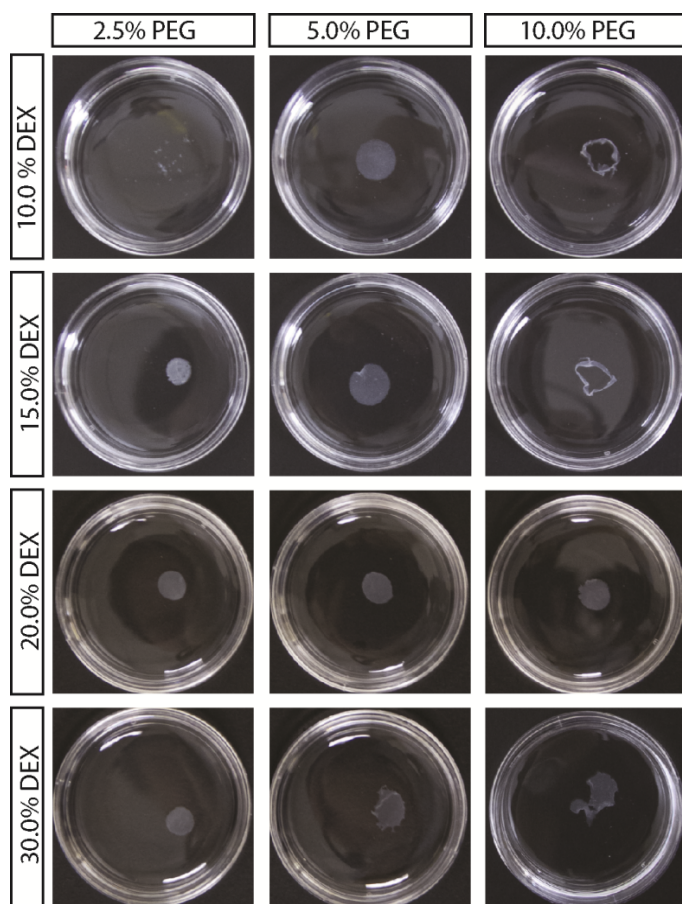
### Rapid Self-Assembly of Macroscale Tissue Constructs at Biphasic Aqueous Interfaces

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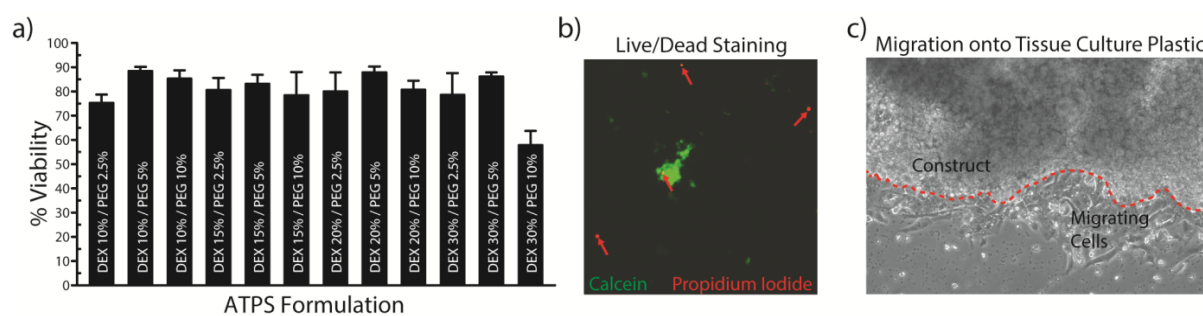
## Supporting Information

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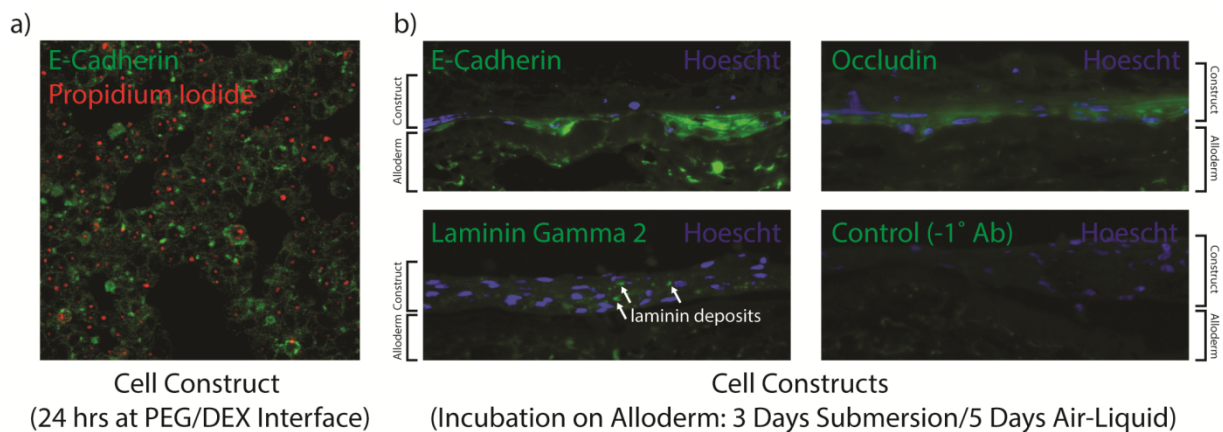
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**Figure S1.** Optimization of ATPS polymer concentrations. If the concentrations of PEG and DEX (as % wt.) are too high or too low, non-optimal interfacial forces can result in constructs with irregular morphologies. Constructs are shown in 35 mm dishes.



**Figure S2.** Viability of cell constructs formed at the ATPS interface. a) The percentages of viable cells from the constructs were measured by live/dead staining for calcein (green) and propidium iodide (PI, red), using phase contrast images for reference. Images were quantified by a blinded examiner. The percentages of live cells were calculated according to  $\% \text{Viability} = 100 \times (\text{Total Cells} - \text{PI}^+ \text{ Cells}) / \text{Total Cells}$ . Bars represent standard error of the mean. b) A representative image of calcein/PI-stained cells from a MCF10A construct dissociated using trypsin and gentle pipette tip trituration. The dissociation protocol may have contributed to a small reduction in overall cell viability. PI-positive cells are indicated by red arrows. c) Cell constructs readily attached to tissue culture plastic. Viable cells were observed to migrate outwards from the construct edges after 1-2 days.



**Figure S3.** Formation of cell-cell junctions after construct formation. a) Confocal fluorescence imaging (top-view optical sections) of MCF10 cell constructs immunolabeled for E-cadherin (green) revealed the formation of adherens junctions between cells after 24 hours of incubation at the PEG/DEX interface. PI (red) was used as a nuclear counterstain post-fixation. b) Primary human keratinocyte constructs integrated with Alloderm (3 days submersion culture followed by 5 days at an air-liquid interface) expressed E-cadherin, along with occludin (tight junctions) and laminin gamma 2 (basement membrane), as observed by immunofluorescence staining (side view epifluorescence images of tissue sections). The following antibodies were used: mouse anti-CDH1/E-cadherin (Sigma), rabbit anti-occludin (Sigma), rabbit anti-laminin gamma 2 (Thermo-Fisher), Alexa-488 goat anti-mouse (Life Technologies), Alexa-488 goat anti-rabbit (Life Technologies) and Alexa-594 goat anti-rabbit (Life Technologies). Samples incubated with only the secondary antibody (-1° Ab) were used as controls.