BIOE 476: Tissue Engineering, Fall 2018

In-class exercise, October 2, 2018

1. Embryonic stem (ES) cells require specific culture conditions in order to support cell survival, promote proliferation, and maintain pluripotency *in vitro*. You are developing a synthetic 3D platform for ES cell culture based on PEG-diacrylate hydrogels, which provide you with the flexibility of incorporating acrylate-conjugated proteins or acrylate-conjugated peptides.

   You perform controlled experiments testing a panel of acrylate-conjugated factors with both human and mouse ES cells and make an interesting finding. Human ES cells perform best (better survival, elevated markers of pluripotency) when acrylate-conjugated E-cadherin is incorporated into the hydrogel and all other factors exhibit a minimal effect. In contrast, mouse ES cells exhibit a small benefit from E-cadherin, but perform best when acrylate-conjugated RGD peptide is incorporated into the hydrogel.

(a) What does this data suggest regarding the relative dependence of human ES cells on cell-cell versus cell-extracellular matrix interactions?

Human ES: Cell-cell > Cell-ECM

E-cadherin is a cell-cell adhesion molecule

What about for mouse ES cells?

Mouse ES: Cell-ECM > Cell-cell

RGD is a peptide which is part of the ECM molecule fibronectin and is involved in cell adhesion to extracellular matrix.

(b) You plan to transduce both human and mouse ES cells with viral constructs that will downregulate the expression of cell surface receptors for E-cadherin and RGD. This will demonstrate specificity and provide insights into the mechanism.

What surface receptors should you target (i.e. what are the common surface receptors for E-cadherin and RGD)?

E-cadherin: Receptor is E-cadherin (homotypic adhesion)

RGD: Receptors are integrins (several integrins involved, alpha5beta1 integrin is one example)

Question 1 continued

(c) What are TWO other ways that you could block E-cadherin-mediated interactions?

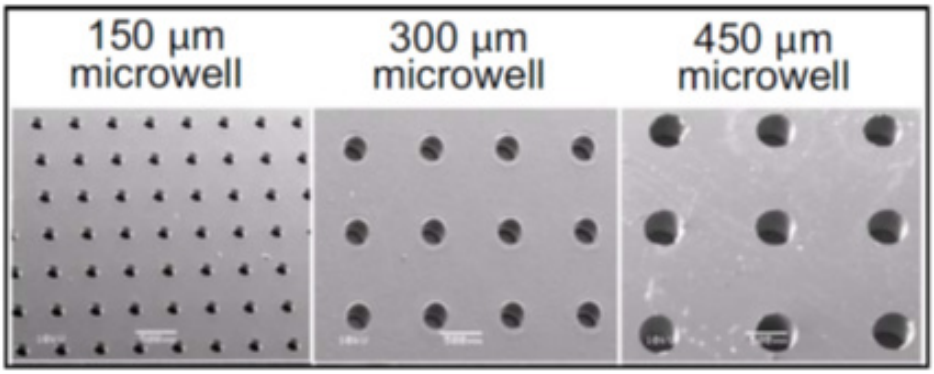
(1) Add recombinant E-cadherin in solution
(2) Add blocking antibody to E-cadherin
(3) Add calcium chelator (e.g. EDTA) which would remove calcium from E-cadherin. Cadherin-cadherin adhesion is calcium dependent.

(d) Despite the addition of E-cadherin or RGD, proliferation in the 3D PEG hydrogels is limited due to the minimal degradation. You run across a paper in which the authors identify unique matrix metalloproteinase (MMP) enzyme expression profiles exhibited by human and mouse ES cells.

How could you use this information to tailor the degradation of your PEG hydrogels for human or mouse ES cells?

Introduce sequences that are cleaved by MMP enzymes into the PEG hydrogel backbone. This would make the hydrogel degradable by cell/tissue MMP enzymes. Based on your knowledge of the MMP enzymes expressed by human and mouse ES cells, you could select specific MMP-cleavable sequences that would be selectively cleaved by human or mouse cell enzymes.

2. In a 2009 paper from the Khademhosseini lab, the authors engineer aggregates of ES cells (embryoid bodies) of different sizes and study the effects of size on differentiation. They start by fabricating microwells of different diameters in a scaffold material, as shown below. Embryonic stems cells are poured into the wells and coalesce to form embryoid bodies.

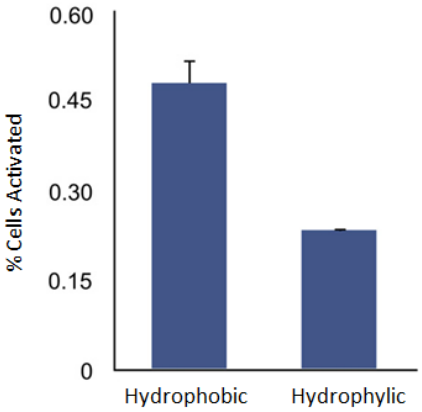


In order for proper formation of embryoid bodies, the ES cells should NOT stick to the scaffold. Which of the following materials are most appropriate? (Circle the TWO best answers).

A. Collagen
B. Fibrin
C. Alginate
D. PLGA
E. PEG

Answer: C and E

3. Two different materials, one which is hydrophobic and one which is more hydrophilic, are directly compared in an in vitro cell activation assay (below). The % of cells activated, based on a measurement of cytokine secretion, is quantified in both conditions.



Due to the distinct protein-surface interaction properties exhibited by hydrophobic and hydrophilic materials, what is one possible explanation for this observed difference in cell activation?

Hydrophobic materials would adsorb more proteins compared to hydrophilic materials. A possible explanation is that these adsorbed proteins lead to enhanced cell adhesion or activation directly.

4. Mixed cellular aggregates of mammary luminal epithelial cells (LEP, white) and myoepithelial cells (MEP, black) rapidly sort into the configuration shown to the right, with LEP cells sorting to the center and MEP cells on the outside.

(a) What does this configuration indicate about the relative magnitudes of the homotypic work of adhesion between cells of the same type, $W_{LEP\text{-}LEP}$ and $W_{MEP\text{-}MEP}$ (i.e. is $W_{LEP\text{-}LEP}$ greater or less than $W_{MEP\text{-}MEP}$?)

$W_{LEP\text{-}LEP} > W_{MEP\text{-}MEP}$

Cells with relatively greater homotypic work of adhesion will sort to the center and will be covered by cells with relatively lower work of adhesion. This behavior is similar to two immiscible liquids, in which the liquid with lower surface tension will be spread over the other liquid.

(b) Since the work of adhesion corresponds to the surface tension, how could you experimentally test the relationship in part a?

Form separate aggregates of LEPs and MEPs. Then use a parallel plate tensiometer, which compresses the aggregates with a defined force, to measure the surface tension of these separate aggregates. Based on the configuration above, you would expect that LEP aggregates would have an increased surface tension compared to MEP aggregates.