

## BIOE 476: Tissue Engineering, Fall 2018

### Homework 4- Due Tuesday, October 30<sup>th</sup> (in class)

- Read the following article: Yang et al. Mechanical memory and dosing influence stem cell fate, *Nat Materials* (2014), and answer the following questions (a-g). For some questions, you may need to look into referenced papers or outside material for additional information.  
<http://www.nature.com/nmat/journal/vaop/ncurrent/full/nmat3889.html#figures>

- a) (3 points) Briefly describe what the data in Figure 1D demonstrate.

(1.5 points) Figure 1D shows the quantification of the % of cells with nuclear localization of YAP and RUNX2 following culture on a stiff TCPS surface for a varied number of days.

(1.5 points) As the number of days on TCPS is increased, the amount of nuclear YAP and RUNX2 increased. This is indicative of increased differentiation towards the osteogenic lineage. Hence, the amount of mechanical dose (days on stiff substrate) affects the amount of osteogenic differentiation.

- b) (3 points) Why was it important for the authors to examine the nuclear localization of YAP (compared to overall expression), and how does the image data in Figure 1E support the need to examine this?

(1 point) Activated YAP translocates to the nucleus, thus the amount in the nucleus is indicative of the degree of activation.

(2 points) The data in Figure 1E illustrates the localization of YAP following 1 day or 7 day culture on TCPS. The 7 day condition shows an increased amount of YAP in the nucleus, indicating more YAP activation. However, the total amount of YAP is not significantly changed. Therefore, if only total amount was measured the effect resulting from TCPS culture time would have been missed.

- c) (3 points) Briefly describe what happens to the phototunable PEG substrates upon exposure to UV light (i.e. what changes take place to the chemistry and physical properties of the gel)?

(1.5 points) Upon exposure to UV light, the PEG chains, which contain a photocleavable domain (PEGdiPDA), are cleaved and leads to a reduction in the crosslinking of the hydrogel network.

(1.5 points) The reduction in the crosslinking following UV exposure causes a decrease in the stiffness (i.e. Young's modulus) of the hydrogel.

- d) (3 points) What is a major benefit of the phototunable PEG system (Figure 3) compared to the experiments using pre-treatment and transfer from TCPS (Figure 1 and Figure 4)?

(3 points) The phototunable system allows one to alter the mechanical environment of the cells without trypsinizing and replating the cells onto another substrate.

- e) (4 points) Based on the data in Figure 3, what is the main difference between mechanical dosing of hMSC on stiff hydrogels for 1 day (DSt1) versus mechanical dosing of hMSC on stiff hydrogels for 10 days (DSt10)?

(2 points) Mechanical dosing for 1 day (DSt1) leads to an increase in YAP and RUNX2 activation compared to soft hydrogels. This change is reversible- activation of YAP and RUNX2 decreases to the level of soft gels if the cells are moved to soft gels after 1 day.

(2 points) Mechanical dosing for 10 days (DSt10) leads to an irreversible increase in YAP and RUNX2 activation. Activation levels of YAP and RUNX2 remain at levels comparable to stiff substrates even after subsequent culture for 1-10 days on soft gels.

- f) (4 points) In Figure 4, what effect does an INCREASED mechanical dosing (culture time on TCPS) have on the ability of hMSCs to differentiate towards osteogenic and adipogenic lineages?

(2 points) Increased mechanical dosing leads to INCREASED osteogenic differentiation.

(2 points) Increased mechanical dosing leads to DECREASED adipogenic differentiation.

- g) (3 points) The authors do not explicitly discuss any role of cell shape in the differentiation of hMSCs in their system. Is it possible that it could play a role, why or why not?

(1 point) Yes, cell shape could play a role.

(2 points) Although they did not examine it explicitly, the stiffness of substrates can lead to changes in cell spreading/cell shape, which can contribute to the effects on cell differentiation. Images of cell morphology (Figure 2, Figure 4) do show some differences in cell shape on the various substrates with the stiffer substrates appearing to support increased cell area (increased spreading).