2.785J/3.97J/20.411J/HST.523J

CELL-MATRIX MECHANICS

Homework #6 Answers

1.

a) What could explain retraction of the ends of the ruptured tendon?

The contraction of α-smooth muscle actin-expressing tendon cells in the ruptured tissue results in "retraction" of the fragments, and widening of the gap.

b) What connection might there be between the acute inflammation induced by the injury that resulted in the rupture of the tendon, and the retraction of the ends?

TGF-β1, which is elevated in the acute inflammatory response induced by the injury, stimulates the expression of α-SMA.

c) What explanation could you offer for the fact that the retraction of the ends occurs for a certain period (*e.g.*, 6 weeks) and then there is no additional retraction of the ends?

Just as in the closure of dermal wounds, the contraction of the α-SMA-expression cells may be temporal, reaching a peak after a few to several weeks post-injury, and then decreasing. α-SMA-expressing cells may die or migrate from the reparative tissue, and the replacement tendons cells do not express α-SMA (because there is no longer elevated TGF-β1). Alternatively, the α-SMA in the cells is broken down to be replaced by β- and γ-cytoplasmic actin.

d) Assuming that an appropriate animal model exists for this condition what method would you use to directly test your answers?

The method for demonstrating the presence of α-SMA-containing cells would be immunohistochemistry.

2. The researcher has conducted experiments to study the contractile behavior of cells in this reparative tissue with healing time. She has removed pieces of the reparative tissue from an experimental animal model after 2, 8, and 26 weeks for laboratory testing of the stress-strain behavior of the extracellular matrix (Fig. 2) and the contractile behavior. For the latter, she pulls the tissue to increasing lengths and excites the cells to contract using a chemical regulator, and measures the force (Fig. 3). She interprets the findings as showing that the contractile behavior of the cells decreases through the 26-week period of the experiment. Do you agree? Explain. Show on Fig. 3 how you arrived at your answer, and hand in Fig. 3 with your answer book.

The "total" contraction curves are shown in Fig. 3. In order to obtain the "active" contraction is it necessary to subtract the "passive" curves in Fig. 2 from the curves in Fig. 3 (see attached Fig. A). The conclusion could be drawn that the contraction remains about the same for the 26-week period (depending on how you subtract the curves).

3. What is the explanation at the molecular level for the contractile behavior of the reparative tissue in Fig. 3?

The molecular level explanation is based on the overlap of the myosin and actin filaments.

4. In order to investigate the contractile behavior of the reparative tissue cells, the researcher has isolated the cells for growth in culture.

a) Describe one *in vitro* assay that she could use to investigate the contractile behavior of the cells, other than the cell force monitor.

Answers could be cells in a gel or sponge-like scaffold or wrinkling of a flexible membrane (*viz*., silicone).

b) Note the principal limitation of the assay that you have proposed to use in (a).

For the gel or sponge-like scaffold, one limitation would be determining the number of cells actually contracting. For the cells on the flexible membrane it is the calculation of the force for the wrinkling.

c) In order to obtain enough cells to use in the *in vitro* assay in (a) she needs to allow time for the cells to grow in culture and then she needs to subculture them into additional culture dishes. How might this process of increasing the number of cells affect her contractile results?

It has been shown that the longer many types of cells are in culture (and sub-cultured) the higher their contents of α-SMA.

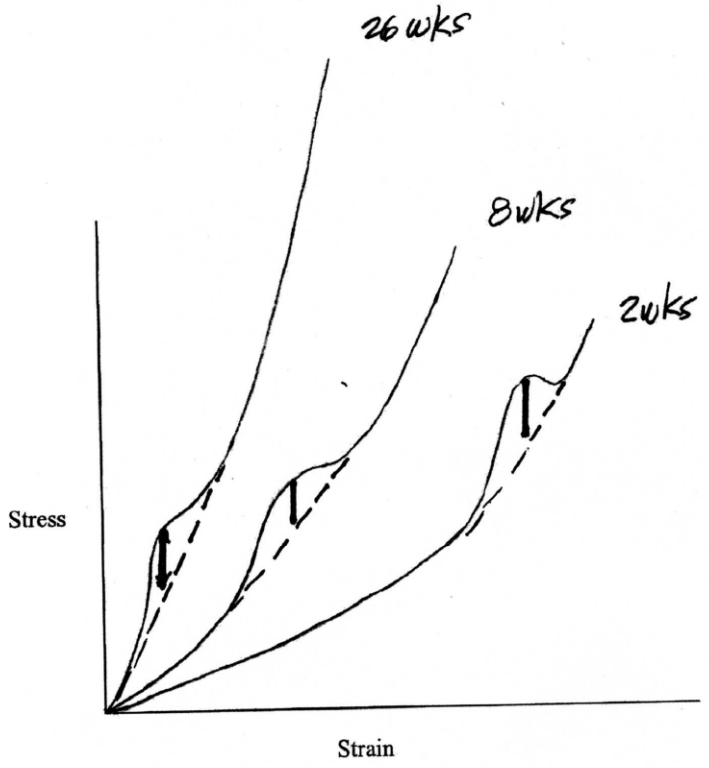

Fig. 3

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Fall 2014