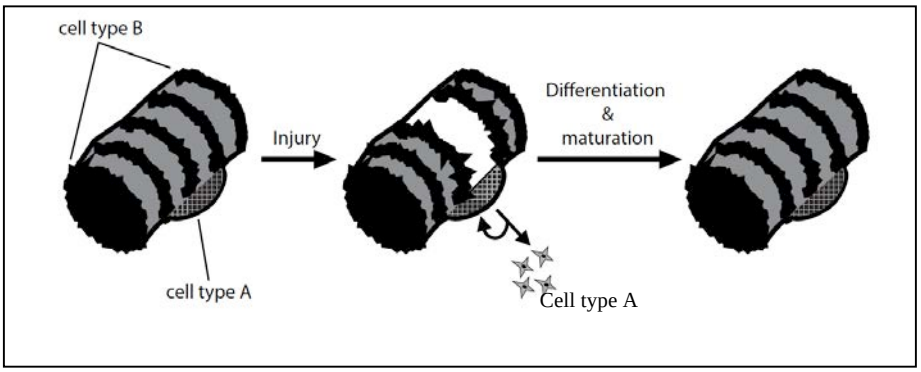
The key: 7.013 Recitation 16 – Spring 2018

Skeletal muscle is a dynamic tissue that is capable of mounting an orchestrated regenerative response to physiological stimuli (extensive exercise) or severe injury as is shown below with Type A being the stem cells.



a) Why is it disadvantageous for the stem cells to divide uncontrollably?

If these cells were always dividing, they may acquire harmful mutations, which would be propagated to all the cells that will arise from these cell type A in this lineage.

b) Cell type A expresses CD56 (a cell surface protein) and Pax7 protein (a transcription factor). You want to purify these stem cells and use them for muscle regeneration. Which protein (***CD56*** or *Pax7*) would you use as a marker to purify the live stem cells from a mixed cell population? **Explain** why you circled this protein.

You would use cell surface protein CD56 since you want to get live cells with which you can work. You can add a fluorescence conjugated CD56 antibody, which will bind only toCD56 on the surface of the SC and NOT any other cells in the population. These antibody labeled cells can be separated from the remaining cell population through Fluorescence activated cell sorter (FACS)

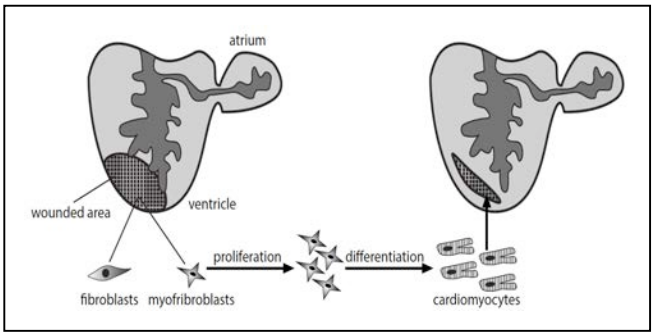
c) Recently, scientists inserted the extracellular matrix (with cells removed removed) from pig muscle into damaged human muscles to attempt regeneration. **Explain** why it is important to remove all cells attached to pig ECM prior to inserting it in humans.

Since the pig's cells are foreign/ non-self cells (xenografts), they if not removed will be attacked by host immune system resulting in life threatening graft rejection.

d) You decide to clone animals by somatic cell nuclear transfer (SCNT) by transferring the nucleus from an **adult muscle cell** into an enucleated egg. In a separate experiment, you first treat the muscle cell genome with 5-aza-cytosine (5-azaC) a nucleotide that prevents DNA methylation. You insert the 5-azaC treated nucleus into an enucleated egg. You observe an increase the efficiency of cloning. How can you **explain** this?

The 5-azaC treatment de-methylates the DNA thus bringing the methylation pattern of the cell closer to the embryonic state.

e) The following schematic shows zebrafish heart regeneration as described below.



- *After injury, expression and secretion of TGFβ ligand begins in the wounded area.*
- *Two cell types (fibroblasts and myofibroblasts) appear in the wounded area.*
- *TGFβ ligand binds to and activates TGFβ cell surface receptors, which activate SMAD3, a transcription factor.*
- *Active SMAD3 promotes proliferation of myofibroblasts and their differentiation into new cardiomyocytes.*

iv. Do any cells described in the schematic above show stem cell properties? **Explain** your answer.
Myofibroblasts, since they self renew and also differentiate into cardiomyocytes,

v. Where would you expect to see the localization of TGFβ receptor (*choose from* ***cardiomyocytes, ECM, myofibroblasts, blastema***)?

vi. Where would you expect to see the blastema? *In the wounded region or at the site of injury*

vii. Constitutive expression of active SMAD3 in a TGFβ receptor null mutant results in cardiomyocyte regeneration after heart injury. **Explain** this result.
In the absence of TGFβ receptor, active SMAD substitutes for the normal signaling sequence that leads to its activation and promotes myofibroblast proliferation and differentiation.

7.013 Introductory Biology

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