7.013 Recitation 16 – Spring 2018

*(**Note:** The recitation summary should NOT be regarded as the substitute for lectures)*

Summary of Lectures 27 (4/23) and 28 (4/25)

The major idea behind tissue regeneration and repair is to inject the stem cells (SCs) of correct potency into the damaged organs so that they can proliferate and differentiate into cells that can replace the dead cells of the damaged tissue and thereby repair it. But adult SCs are rare and mostly very difficult to isolate/ purify. As an alternative therefore one can use the pluripotent embryonic stem cells (ES cells or **ESCs**) or induced pluripotent stem cells (**iPS cells**).

ESC are pluripotent stem cells that can be generated by using the cells from the inner cell mass (ICM) of an early embryo. The ICM cells when cultured with appropriate nutrients can result in the formation of ES **cell lines,** which are pluripotent. It is important to note that ESCs are made by culturing the cells from an early embryo cells, so they are NOT embryonic cells.

A cell line may be defined as a group of cells that can continuously proliferate in a culture plate to make more of themselves i.e. it is a continuously growing population of cells. Different cell lines can have different potencies.

If you establish the ES cell line you have to start from the embryo, which raises ethical concerns. Furthermore, since these cell lines are not autologous (from self) there is a chance that these can be rejected. These issues can be resolved by using the iPS cells. Here you start with an adult differentiated cell (such as cell from the patient's body having the same MHC-I to prevent the chance of immunological rejection) and introduce 3-4 genes that encode specific transcription factors (*sox2, nanog, wnt, oct4*) and then culture them in the presence of nutrients to generate pluripotentent autologous cell lines. These cell lines can be used for organ repair and study different diseases.

Stemness: for the stem cells to be produced the following are the points you need to keep in mind:

- -Asymmetric cell division, a process that can produce more stem cells (self-renewal) and its progenitors.
- -You need to reactivate the regulatory genes to get a "more embryo like" gene expression profile to make the cells pluripotent.
- -You need to regulate the epigenetic control such as DNA methylation and histone acetylation.
- -You need to have cells with young organelles such as young mitochondria since these can more likely be reprogrammed to stem cells.

Cloning and SCNT: Here the definition and the idea is similar to "cloning of a gene into a plasmid" as you observed in your recombinant DNA module. However, here it applies to the whole organism i.e. you try to make a replica of yourself with the same genetic makeup as yours. This may of significance in agriculture, replacement for lost animals and plant species and source of spare body parts.

Here the idea is that the cytoplasm of an egg has all the factors you need to make all cell types. So you take the somatic cell nucleus from a non-embryonic cell and implant it into an enucleated egg so that now the ploidy of the egg is 2n. You let it develop into an embryo and then onto the newborn. Here the cloning efficiency is very low i.e. 0.001% with adult somatic cell nucleus and 10% with ESC or iPS. Furthermore the newborn can have many physiological problems since it is very difficult to completely reprogram the genetic information.

Repair and Blastema: Our body for most part repairs itself; this gave rise to the notion of SCs. The process of regeneration is observed in organisms such as planaria (can regenerate the whole body),

hydra, starfish, zebrafish (can regenerate retina, heart and spinal cord) etc. Even humans can regenerate their liver or cut fingertip.

Decisions/ questions that the cells ask during regenerations and associated mechanisms:

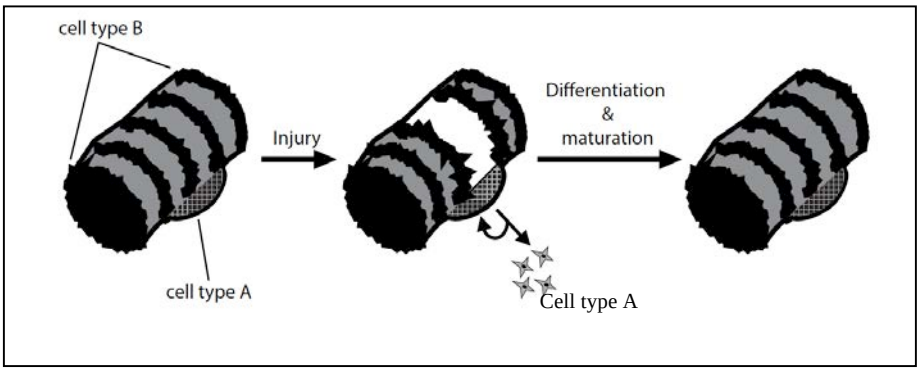
- Which tissues are missing?
- How should they position themselves?
- How much should they grow? How do they know when to stop?

In general, the remaining tissue parts have this information retained, which is used during regeneration. The regeneration involves blastema formation and signaling pathway and size control is mediated through HIPPO pathway. A blastema forms from the damaged surface. At the damaged region there is cellular mass that has undergone de-differentiation and can form the whole organ.

Tissue Engineering: This is the study of the growth of new tissues, from cells and a collagenous scaffold to produce a fully functional organ for implantation back into the donor host. This technique will allow organs to be grown from implantation (rather than transplantation) and hence free from imunological rejection. The starting point for any tissue-engineered organ is the harvesting of small amounts of tissue from the future recipient of the Tissue Engineered organ. This could be as small as a 2mm punch biopsy for some applications. Cells from the biopsy are then cultured from explants or a collagenase digestion to create a "cell bank". These cells are then further cultured on collagenous substrates, under the correct physiological conditions, to form Tissue Engineered constructs for implantation.

Questions:

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.



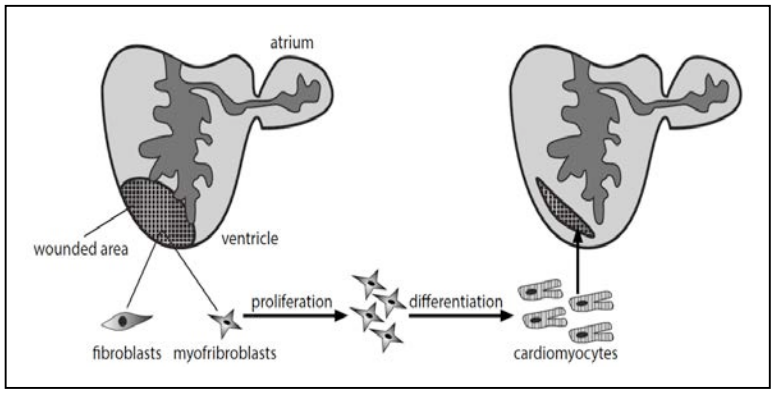
a) Why is it disadvantageous for the stem cells to divide uncontrollably?

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.

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.

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?

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.*

i. Do any cells described in the schematic above show stem cell properties? **Explain** your answer.

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

iii. Constitutive expression of active SMAD3 in a TGFβ receptor null mutant results in cardiomyocyte regeneration after heart injury. **Explain** this result.

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