Tutorial 3: Regeneration in humans?

This tutorial is going to involve watching a video and answer questions related to the video. You will stop and start the video at various points in order to address the questions.

Tell students the model that will be followed for each:

You will watch a clip from the video – as you are watching think about:

What is the question?
What is the testable hypothesis?
What was the experiment?
What were the results?
What was the conclusion?

After the video clip, students will go into their groups to discuss the answers to these questions – 5 minutes each time.

To take up the answers, you can ask students to volunteer answers from any group. Try to make sure that groups are participating equally. For example, if one group has not answered recently, simply say, we haven't heard fom the group at the back recently, can you offer an answer?

(If you prefer, you could rotate around the groups in a pattern to obtain the answers.)

Students will also be encouraged to discuss the results and to think about the following questions:

What questions stem from these results? What hypotheses could you test?
What experiments would you do next?
I have provided questions for each part that can help to facilitate the discussion. Again, you can take these answers from the whole class.

TAs: Please watch the video and look at the questions and answers that I have given to you. You may wish to watch the entire video including questions from the audience as a preparation for your tutorial as you may receive similar questions from your students.

Also, practice finding the correct video segments that I have indicated so that you can present the tutorial smoothly.

Part A: The ability to repair/regenerate muscle as we age.
(4:21 – 24:20 – begin by clicking number 4 on the right panel.)

General question:
What is the relationship between aging and stem cells – do stem cells track aging or cause aging?

For Part A, there are three questions that follow from one another. I think that it is probably easier to ask the questions after each section of the video – I have indicated the time frames for each set of experiments.

Total time? 20' video, 3 X 5' break-out into groups, 3 X <10' for taking up the answers and follow-up questions - perhaps about 1 hour)

After each part/question, ask the students:
What is the question? Or questions?
What is the testable hypothesis?
What was the experiment?
What were the results?
What was the conclusion?

1) **Segment 1 (4:21 to 7:03 is introduction - click 4 on the right panel to go to the beginning of this section - and then this is followed by the first question which falls between 7:03 to 16:10 or click 6 on the right panel to go to the beginning of this section. I would just play the introduction and the first section together and then go to the discussion below.)**

Ask the students:
What is the question?
What is the testable hypothesis?
What was the experiment?
What were the results?
What was the conclusion?

Answers for TAs:

Question: Do decreasing numbers of stem cells contribute to aging?

Testable hypothesis: There are fewer stem cells/satellite cells in old versus young muscle.
Experiment: Identify and count the stem cells.
Note: "identify" is an important part of the experiment. Questions to ask: How did the researchers identify the cells? *(Histology – cell morphology; stem cells are the small bright pink cells in the first images, compared to the larger, pale muscle cells. In the second set of images, the satellite cells are fluorescently-labelled.)* How did they show that these were functional stem cells? *(Removed satellite cells and placed them in culture.)*

Result: Yes, there are fewer satellite cells.. Are you convinced by the results?

Conclusion: Satellite cell/stem cell number decreases with age.

Note: this is a correlation – what does it tell us about the requirement for stem cells in repair? (*Actually, it does not tell us anything about a causative relationship*.) What experiment could you perform to demonstrate a causative relationship between stem cell number and the ability to regenerate? *(Remove stem cells. In fact, irradiation of salamanders to kill neoblasts (stem cells required for limb regeneration) prevents regeneration.)*

2) **Segment 2 (11:10 to 16:10 or click 10 on the right panel to go to the beginning of this section)**

Ask the students:

What is the question?
What is the testable hypothesis?
What was the experiment?
What were the results?
What was the conclusion?

Answers for TAs:

Question: Does a change in the capacity of stem cells contribute to aging?

Testable hypothesis: There is change in the signalling molecules required for stem cell function.
Experiment: Assay for the signalling molecule, Delta
Note: Delta is the signalling molecule produced by the satellite cell – how was its presence assayed? (*It is not actually stated, but it may have been using an antibody that recognizes the protein or tagging the protein with Green fluorescent protein (GFP).)*

Questions to ask: What is meant by capacity? You may want to agree upon a definition of capacity. (*e.g. The ability of the cells to induce the mechanism of regeneration.*) What are Notch and Delta? (*Signalling molecules; Notch is a transcription factor that activates the gene expression required for the regenerative process*.) Why were Notch and Delta signalling proteins chosen? (*Three reasons were given in the lecture: i) required for muscle development in the embryo, ii) regulates satellite cell proliferation and differentiation, iii) decreases with age. An incorrect answer would be, because these decrease in older cells – this is in fact the findings of the experiment, not a reason for choosing the molecules.*)

Result: There is a reduced expression of Delta in older tissues.

Conclusion: Delta expression is reduced in older tissues.

As pointed out in the video, this is a correlation – decreased Delta protein is seen in older tissues. How did the researcher address the issue of causation? (*Need an intervention – perturb the system and observe the effect.*)

So, ask the students, what is the next testable hypothesis?
Hypothesis A: Inhibiting the signalling pathway (inhibiting Notch) inhibits repair.
Experiment A: Inhibit Notch protein and observe tissue repair.
Hypothesis B:Activating the signalling pathway (activating Notch) activates repair.
Experiment B: Activate Notch protein and observe tissue repair.

Note: tissue damage is visible in these histological sections – the black cells indicate dead or dying cells. (A) Lower left - old tissues, control; and Top middle – young tissues, Notch inhibited show many dead cells. (B) Bottom right – old tissues, Notch activated; and Top Left – young tissues, control show repair. What is the purpose of the control (C*omparison – so that you can observe what repair or lack of repair looks like in the absence of the intervention and compare it to the results of the intervention/experiment.*)

Conclusion: The Delta-Notch signalling pathway is required for regeneration of the muscle cells.

3) **Segment 3 (16:10 to 23:07 or click 14 on the right panel to go to the beginning of this section)**

Ask the students:

What is the question?
What is the testable hypothesis?
What was the experiment?
What were the results?
What was the conclusion?

Answers for TAs:

Question: Does an altered stem cell environment contribute to aging?

Testable hypothesis: Changing the satellite cell environment can change the regenerative response.
Or, two hypotheses: A) A young animal can reprogram old tissue to respond to injury.
B) An old animal reduces the ability for young tissue to respond to injury.
Experiment A: Provide young satellite cells with an old environment
Experiment B: Provide old satellite cells with a young environment.

Questions to ask: How was this done? (*Connecting circulatory systems of two animals.*)
Why were old with old and young with young animals connected? (*Controls. You may ask students to elaborate – what is the effect in the normal scenarios, old in old, young in young*.) Why not just look at a young animal and an old animal that has not be connected to another animal? (*Control for the effect of the surgery.*)

Results A: The young animal can regenerate just as well when it is connected to another young animal or to an old animal (i.e. exposed to the older environment).

Results B: The old animal regains the ability to regenerate when it is connected to a young animal (i.e. exposed to the younger environment), but not when connected to the old animal.

Conclusion: Something in the young animal environment (circulating in the blood) affects regeneration.

Ask the students, what were the follow-up questions and hypotheses?
How do we know that this is an effect of the environment and not simply the supply of more stem cells?
Hypothesis A: The young animal can activate the old stem cells through the Notch/Delta pathway.
Hypothesis B: The young animal provides young stem cells to the old animal

Experiment A: Cells in culture are exposed to cell-free serum. (Same pairings and controls as earlier.)
Result A: The frequency of old satellite cells expressing Notch is higher when the cells are exposed to young serum compared to old serum.
Conclusion A: The environment can activate the Notch/Delta pathway.

Hypothesis B: Young stem cells are being transferred from young to old in the first experiment.
Experiment B: Label all of the cells in the young animal with green fluorescent protein (GFP).
Result B: There are no green (young) cells found in the older animal with which it is connected.
Conclusion: The old animals muscle cells are regenerating in response to young factors.

Overall Summary: (23:07 or click 19 on the right panel to go to the beginning of this section)

It is noteworthy that in each case, the researcher uses the term "contributes to aging". Why?

(Answer: M*any factors contribute to the process – not just one answer to the question.*)

Part B. Heart regeneration – can mammals regenerate their heart in the same way that we see in the zebrafish? (30:38 to 55:36 or click 24 on the right panel to go to the beginning of this section)

Just play the 25 minutes of video and ask the students to address the same questions on their own at home.

Or, depending upon time, you can just play part of this video, the 17 minutes from 30:38 (click on 24 to start) to 47:24 (when the second question is addressed – can we stimulate the heart to make new cells).

What is the question?
What is the testable hypothesis?
What was the experiment?
What were the results? What were the controls?
What was the conclusion?

This will then leave sufficient time to (1) complete a short quiz (2) sign up in pairs for the oral presentation project.

Part A:	20' total for video (broken into segments) 3 X 5' break-out into groups to discuss questions 3 X <10' for taking up the answers and follow-up questions **(1 hour?)**
Part B	25' to play this part of the video straight through. They can address the same questions that they did in tutorial for Part A. Answers will be posted on A2L. **(25 minutes or 17 minutes)**
Sign-up	The video on heart regeneration is a good lead-in to their presentation topics: how stem cells might be used in the treatment of a human disease – 12 topics will be provided to choose from. They will have these posted this week so that they can plan a first and second choice before arriving to tutorial. **(10 minutes)**
Quiz	A short quiz that will simply ask them to outline the hypothesis, test, results and conclusions for one of the experiments – <u>they can have their books open</u>, but they will have had to pay attention and understood the concepts in tutorial to get this right. **(15 minutes)**