2019 CED-IADR SUMMER SCHOOL

"Methods in Dental and Orofacial Tissues Research" JULY 1-5, 2019 | Zagreb, Croatia

List of procedures students will personaly perform

Module 1

Students will get to know two different cell populations (oral lamina propria stem cells and pluripotent fibroblasts/mesenchimal mucosal cells) and compare with neural stem cells. They will change cell growth medium, perform enzymatic dissociation of cells, resuspending and seeding cells in Evos live cell imaging system.

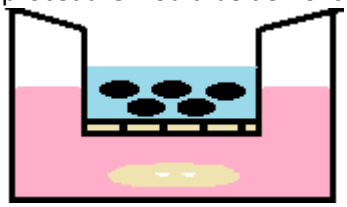
Module 2

In the first practical, participants will have a hands-on experience of isolating cells for single-cell RNA sequencing, involving murine dental pulp dissections, enzymatic dissociation, removal of dead cells from the single-cell suspension using magnetic-activated cell sorting, cell counting using hemocytometer and calculating cell viability.
In the second practical, participants will perform basic analysis and exploration of a publicly-available scRNA-seq dataset using web-based bioinformatic tools.

Module 3 (Detailed plan for the practical about growth factors)

Part 1: Cell migration towards differently pre-treated dentin disks

Each student will get 24-well plate with Transwell inserts. The day prior the experiment, cells would be seeded on the top of the insert and differently treated dentin disks would be placed in the well under the insert (Fig. 1). This procedure would be demonstrated and explained to the students due to lack of time and necessary 24h of incubation.



The students will remove the medium from the inserts and wells, wash the inserts with PBS, fix cells (4% formaldehyde) and permeabilize them with methanol, stain the cells using Crystal violet and remove the cells from the upper part of the inserts using cotton swabs. After all, students will observe migrated cells on the bottom of the inserts, take pictures and count them using program ImageJ on their computers.

Figure 1. Transwell insert with cells on the top of the membrane

Part 2: Cell attachment

Students will disinfect dentin disks with NaOCl and treat them with different conditioning solution. After washing with PBS, the disks are ready for the next step.
Meanwhile, the students have to prepare cells. They will get T75 flask with the cells and the medium. They have to remove the medium, wash the cells with PBS to get rid of all the medium, detach the cells using Trypsin, add the new medium with serum to neutralize Trypsin, centrifuge the cells and resuspend the pallet. They have to count the cell density and calculate how much medium to add. This cell suspension is going to be used for cell seeding.
Students will apply the same amount of cell suspension on the top of the prepared dentin disks and leave them in the incubator for 4h. After incubation, the disks will be washed, cells would be detached and counted. The numbers represent the cells that managed to attach to the dentin surface during incubation time and results between different groups would be compared and discussed.

Module 4

Students will perform: tooth extraction (mouse molars); preparation of periodontal ligament (PDL) cells digestion for cell culture, preparation of cells for Flow cytometry, and labelling using antibodies, preparing sections for imaging (section attachment), and fluorescent imaging.