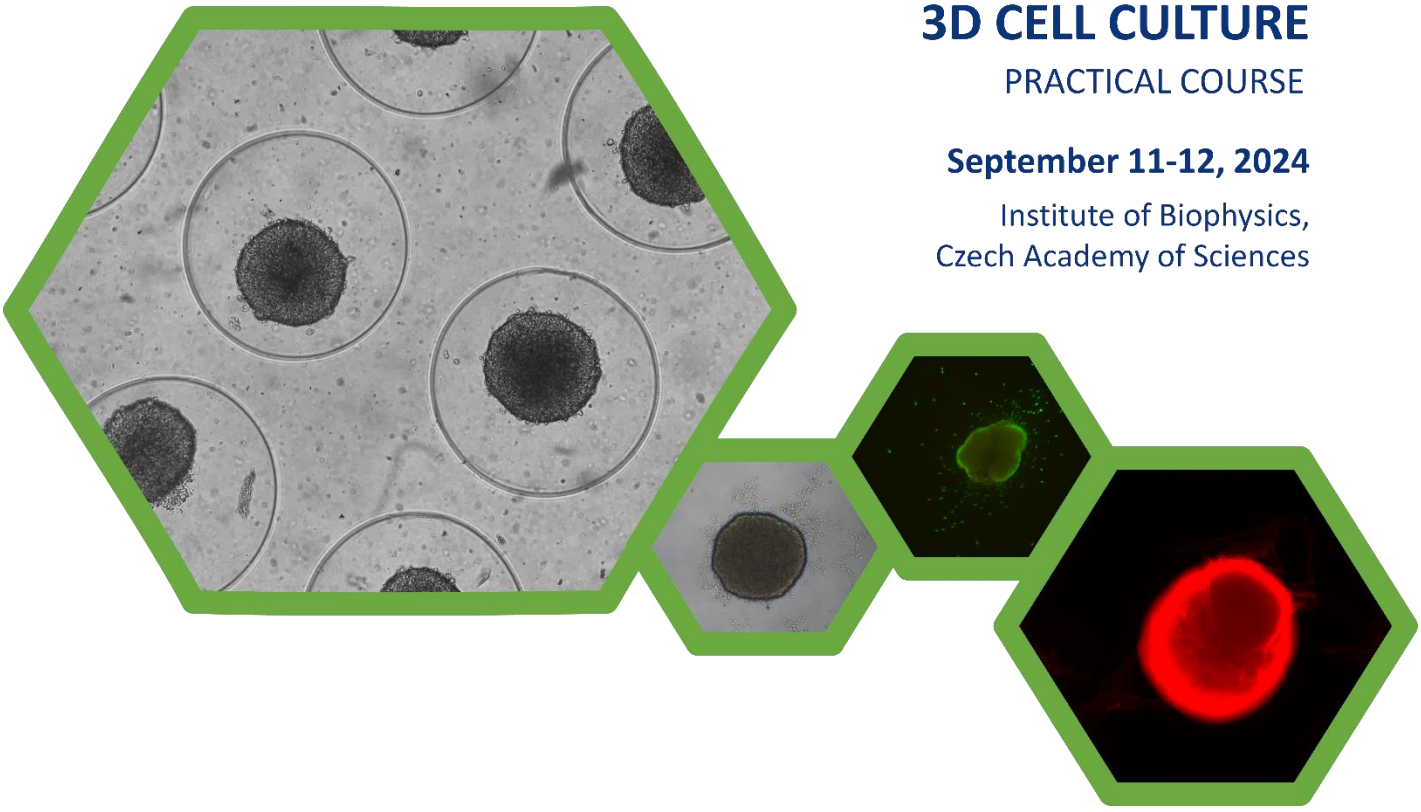

3D CELL CULTURE

PRACTICAL COURSE

September 11-12, 2024

Institute of Biophysics,
Czech Academy of Sciences

INSTRUCTOR

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IBP Institute of Biophysics

HR EXCELLENCE IN RESEARCH

SEDLACKOVA LAB

OVERVIEW

Course objective

3D cell culture models such as microtissues or organoids are widely used in life science research and offer insights into cell behavior in a three-dimensional environment. Participants of this practical course will learn about potentials and challenges of currently available 3D culture and will have access to hands-on training on different 3D culturing methods and simple assessments.

3D culture model approaches in the course

- 3D Petri Dish®
- U-bottom plates
- Agarose-coated plates
- Hanging-drop culture

Schedule

Day 1

09:00 – 09:15 Welcome and introductory words

09:15 – 10:00 Lecture: 3D Cell Culture Systems

10:00 – 10:45 Hands-on: Preparations for 3D cell cultures (Group I)

11:00 – 11:45 Hands-on: Preparations for 3D cell cultures (Group II)

12:00 – 13:00 Lunch break

13:00 – 14:30 Hands-on: Cell seeding (Group I)

14:30 – 16:00 Hands-on: Cell seeding (Group II)

Day 2

10:00 – 11:00 Hands-on: Viability testing & microscopy (Group I)

11:00 – 12:00 Hands-on: Viability testing & microscopy (Group II)

12:00 – 13:00 Lunch break

13:00 – 15:00 Discussion

LAB & COURSE RULES

The course is performed at the Sedlackova Lab, which kindly provides rooms and equipment for the course. **It is of utter importance to follow the rules listed below.**

1. **Use protective equipment** (e.g. gloves, lab coat etc.) when working in a laboratory. Wear suitable **laboratory shoes** in all labs. Outdoor shoes are not allowed, particularly in the cell culture laboratory.
2. Work in a way that is as **sustainable** as possible but do not compromise the quality of your experiment.
3. **Keep the cell culture lab clean and tidy**. Use gloves only for the cell culture lab. Never use gloves from the core lab in the cell culture as there is a high risk of bacterial or fungal contamination of cell cultures. Before your cell culture work, clean your gloves with EtOH, clean all surfaces you are working on (laminar box, tables etc.) and clean your bottles with media, PBS and trypsin before placing them into the laminar box. Once you finish your work, clean everything with EtOH again (also aspirator and pipettes if needed).
4. Keep the **door** of the cell culture lab **closed** always. Never switch off the **air conditioning** in the room.
5. **Follow instructions** of instructors and protocols. If any part of the protocol or the practical work is not clear, please ask the instructors for advice and clarification.
6. Please **arrive on time** to each part of the practical course. Every part of the practical course starts sharp at the time points given in the agenda.
7. **Be respectful** with other course participants and colleagues from the institute (*e.g.* do not play loud music, do not talk on the phone in the labs and seminar rooms during the course etc.).
8. The course will be held in **English**. Please stick to English during all course activities.
9. Any form of violence, bullying, discrimination or harassment is not tolerated.
10. Please **respect the ongoing experiments and work** of the Sedlackova lab members. Thus, do not use or remove any of the equipment, materials or workspace, if not specifically advised by the instructors. Only use materials and equipment as indicated by the instructors. If you are not sure what and how equipment, materials or workspace should be used, ask the instructors for advice. Also, do not enter any rooms of the institute that are not part of the course.

Thank you!

PREPARATIONS FOR 3D CELL CULTURES

Hanging-drop culture

Hanging-drop cultures can be prepared in sterile petri dishes of any size, depending on how many spheroids you want to produce. Before you seed your cells to form spheroids in a hanging-drop culture, practice the following procedure. Use culture medium without cells for the practice procedure.

- take the lid of a petri dish
- pipette drops in a range of different volumes onto the inside surface of the lid
- reverse the lid in a single motion to have the drops hanging downwards from the lid
- note down which volume is the largest that still generates stable drops and do not fall down or merge with other drops
- note down how close you can place two of the largest droplets to each other before they merge when you move/reverse the lid
- fill the bottom part of the petri dish with water and place the lid with the drops back on the bottom part
- observe how much distance you need to keep between the boarder of the lid and the droplets to be able to close the petri dish without losing any droplets
- How much liquid can you add to the bottom of the petri dish so that you are still able to carry and move the petri dish without losing any drops or spilling the liquid?

Agarose

2% agarose in PBS is provided for the course and stored at 4°C. For use, agarose has to be heated in a (*e.g.* in a 50°C incubator or microwave) until it becomes entirely liquid.

- make sure that the bottle cap is only loosely placed on the bottle containing agarose but not closed
- when heating agarose in a microwave, stop the microwave immediately as soon as the agarose starts to boil and only start the microwave again when the boiling stopped
- repeat until you receive a homogeneous solution of agarose

Attention: the bottle will be hot, handle with care!

Agarose plates

- take a 96-well plate and pipette 80µl per well of hot liquid agarose
- make sure that the agarose covers the bottom of each well completely
- make sure that there are no air bubbles in the agarose
- Wait for the agarose to turn solid. You can place the plates into the fridge to speed up the process. (Seal plates with agarose for longer storage in the fridge.)

3D Petri Dish®

- fill 3D Petri Dish® for large spheroids with hot liquid agarose with a 1ml pipette without touching the 3D Petri Dish® with the pipette tip
- avoid producing air bubbles
- wait for the agarose to turn solid in the 3D Petri Dish®
- take out the solid agarose molds and soak them with culture medium
- transfer the agarose molds to a 24-well plate and cover them with culture medium

CELL SEEDING

Cells

In this practical course we are using primary mesenchymal stromal cells from the human gingiva. The cells were isolated from gingiva tissue attached to teeth extracted at the University Clinic of Dentistry, Medical University of Vienna, Vienna, Austria. To isolate cells, collected gingiva was cut into small pieces which were then placed into petri dishes containing medium for cell outgrowth and expansion. The harvested cells are adherent display a fibroblast-like morphology. They fulfill the minimal criteria of mesenchymal stromal cells and possess osteogenic, chondrogenic and adipogenic differentiation potential to different extents, depending on the cell donor. Also, doubling time strongly varies between donors. Cells will display these characteristics for approximately 6 - 7 passages, if cultured in α-minimal essential medium or Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum and antibiotics and kept at 37 °C, 5% CO_2 and 95% humidity.

- ➔ For this practical course, cells will be provided to the participants in suspension after trypsinization from culture flasks together with respective cell concentrations.

Hanging-drop culture

- prepare the following three cell concentrations in separate tubes:
    - 3 x 10^4 cells/ml
    - 5 x 10^4 cells/ml
    - 10 x 10^4 cells/ml
- calculate the volume based on the most suitable drop size tested during the practice
- calculate a final volume that will be enough to place at least two drops per cell concentration
- place the drops containing your cells on the lid of a petri dish as practiced in the morning and place the petri dishes into an incubator until the next day

Agarose and U-bottom plates

- prepare the following three cell concentrations in separate Eppendorf tubes:
    - 3 x 10^4 cells/ml
    - 5 x 10^4 cells/ml
    - 10 x 10^4 cells/ml
- calculate a final volume that will be sufficient for at least two wells per cell concentration
- pipette 100µl of the respective cell suspension per well
- place the culture plate into an incubator until the next day

3D Petri Dish®

- prepare a concentration of 730 x 10^4 cells/ml in a total volume of 75µl for each agarose mold
- pipette the 75µl cell suspension into the agarose mold without touching it
- let the cells sink to bottom of the recesses of agarose mold for approximately 15 minutes
- when the cells arrive to the bottom of the agarose mold, cover the molds carefully with 500µl culture medium per well without washing the cells out from the mold again
- place the culture plate into an incubator until the next day

MICROSCOPIC EVALUATION

Inspect the different 3D cell culture models under a brightfield microscope and answer the following questions for each model:

- Did cells form solid spheroids in all models?
- Which models show spheroids witch a clear round shape?
- Are spheroid sizes reproducible within the models?
- Do spheroid sizes correlate to the initially seeded cell numbers?

VIABILITY TESTING

Viability of cell spheroids will be tested with a Live-Dead staining kit for Live-Dye™ (a cell-permeable green fluorescent dye to stain live cells) and propidium iododide (a cell non-permeable red fluorescent dye to stain dead cells) staining. After staining, viability is assessed by fluorescence microscopy. Choose one 3D cell culture model from the course for viability testing.

- calculate the total required staining solution volume for the chosen type of culture model
- for 1ml of Staining Buffer add 1µl of Staining Solution A and 1µl of Staining Solution B
- remove culture medium from spheroids and carefully add the staining solution
- incubate for 15-30 minutes at 37°C
- observe cells under a fluorescence microscope

EVALUATION

Rate each 3D cell culture model based on your experience from the course.

3D CELL CULTURE MODEL	HANDLING	OUTCOME
Hanging-drop culture		
Agarose-coated plates		
U-bottom plates		
3D Petri Dish®		

NOTES