Simple Approach to Micropattern animal Cells on Common Culture Substrates : Fluorescent Microscopy and AFM Characterization

The cytoskeleton is a cellular "skeleton" made of proteins that plays a crucial role in a multitude of cellular processes: it maintains the cell shape and cell mechanic, ensures the intracellular transport, cell migration and even cell division.
Eukaryotic cells contain three main kinds of cytoskeletal filaments, which are microfilaments, intermediate filaments, and microtubules.

Cultivation of eukaryotic cells is a complex process by which cells are grown under controlled conditions outside of their natural environment. For the duration of ex vivo culture, cells are maintained at an appropriate temperature and gas mixture (typically, 37°C, 5% CO_2 for mammalian cells) in a cell incubator.

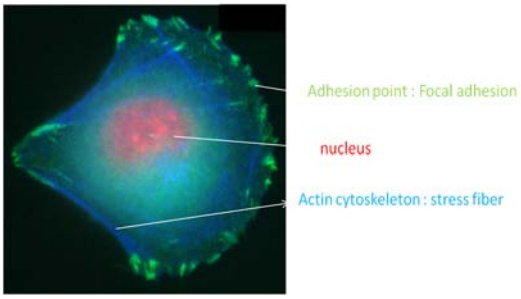
During the practical work, you will get acquainted with the bases of cell culture: sterile technique, subculturing anchorage-dependent cells, counting the cells in haemocytometer and cell seeding.

Cell culture procedures are performed in sterile conditions. Sterile technique prevents from introducing contaminating microorganisms that can destroy the cells. All cell culture material (dishes, media, pipette tips etc.) are sterile, and all procedures are done in a laminar flow hood (or cell culture hood) that provides an aseptic work area.

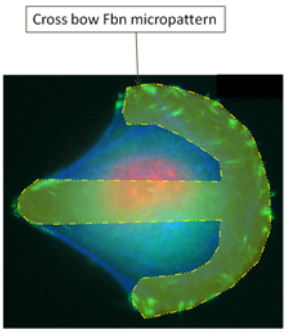
The goal of this practical work is to visualize cytoskeleton (actin filament bundles), nucleus and cell adhesion structures called Focal adhesion of purposely shaped osteoblasts cells using techniques of fluorescent microscopy and AFM. For this, you will seed the cells over fibronectin micropatterns (Cytoo Chip), allow them to adhere and spread, and then, their actin cytoskeleton and nucleus will be labeled using the method of fluorescent staining.
You will observe β3 integrin clusters, actin bundles, nuclei and compare:
i) cell adhesion structures formed during the spreading process
ii) actin cytoskeleton remodelling
ii) location of nuclei inside the cells
at least, on 2 different shapes of micropatterns.

Mouse osteoblasts will be used as a cellular model. Cells can be observed by bright field inverted phase contrast microscopy to qualitatively visualize their adhesion and spreading onto different micropatterns.

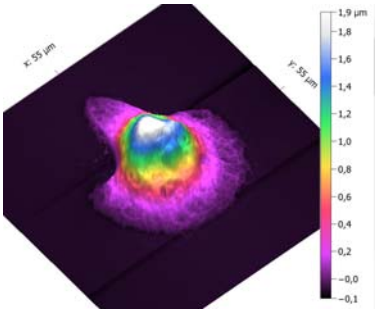
During this **PW** you should prepare two samples, one will be used for characterization in epi-fluorescence microscopy and the other for the Atomic Force Microscopy (AFM) characterization.



Fluorescent image of osteoblast cell spread over a micropatterned Fbn substrate



Same showing the underlying micropatterned Fbn substrate



AFM image of osteoblast cell spread over a micropatterned Fbn substrate