C10. **Characterisation of mesenchymal stem cells interaction with biomaterials using flow and image cytometry**

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Introduction. Bone regeneration during fracture healing or implant integration is essential for the efficient recovery in orthopaedic pathology. To this end, the efficient direction of proliferating mesenchymal stem cells (MSCs) towards the osteogenic fate is a primary key step. *In vitro*, three differentiation factors are commonly used for osteogenic induction of MSCs: dexamethasone, ascorbic acid, and beta-glycerophospate. Use of growth factors such as bone morphogenic proteins for the facilitation of osteoblast differentiation is well documented. However, few studies reported the use of FGF2 during MSCs differentiation while its effects and mechanism of action are not entirely understood. Its use as component of biomaterial coatings is expected to trigger incresed proliferation[1,2] of osteoprogenitor cells before their commitment to mineralized matrix-producing bone cells. For the design of laser-transferred polymeric thin scaffolds incorporating growth factors, prior investigation of substrates as well as of proteins to be embedded must be thoroughly performed. In the present study, we have screened several polymer combinations for biocompatibility with bone cells using live/dead staining and flow cytometry analysis. To determine the optimal dosage and treatment intervals of FGF2 for enhanced differentiation of bone marrow-derived MSCs, we used a combinatorial approach followed by endpoint mineralization quantification. The effect of FGF2 on MSCs phenotype was characterized using both flow and image cytometry in order to characterize signalling molecules expression during osteogenic induction.

Materials and methods. The readout of MSCs differentiation was the Alizarin Red S staining of mineralized tissue performed at 28 days after induction. We have used the Live/Dead Viability Kit (Invitrogen) to quantify bone cell viability after growth on polymer thin films by flow cytometry using FACSVerse instrument and FACSuite Software (BD Biosciences). Live cells were stained by calcein AM while dead cells were stained by ethidium homodimer-1 following a 15 minutes labelling at room temperature (RT). The expression of signalling proteins was assessed by both flow and image cytometry using Cell Signaling and abcam antibodies. For FACS, cells were fixed and scraped in 1.5% PFA, and incubated for 10 min at RT. Following centrifugation, pellets were permeabilized in cold methanol, washed in 0.5% BSA-PBS staining media and incubated with primary antibodies 30 min at RT. Alexa Fluor conjugated antibodies (Invitrogen) were used for detection. For all experiments, ten thousand cells were acquired using a BD instrument and data were analysed with Cytobank Software. For image quantifications, a dedicated microscope-based TissueFAXSplus cell analysis system was used (TissueGnostics, Austria) for specimen total scanning, image stitching and cytometric measurements on scattergrams. Cells were fixed in 4% PFA in PBS 15 min at RT and permeabilized in cold methanol after a protocol recommended by Cell Signaling Technology. Finally, cells were mounted in Prolog Antifade Reagent (Invitrogen) and subjected to microscopic analysis.

Results. Polyethylene glycol:polycaprolactone (PEG6K:PCL) was chose for further examinations as a potential scaffold for growth factors controlled release. We have characterized the expression of MAPKs during osteogenic differentiation in the presence of FGF2 and determined the optimal conditions to obtain a positive effect on mineralization.

Conclusions. Flow and image cytometry can be used in complementarity to assess cell signaling triggered by specific molecules and to screen for optimal cell growth and differentiation conditions in order to design smart 3D biomaterials.

References: [1]Tsutsumi S *et al.*, Retention of multilineage differentiation potential of mesenchymal cells during proliferation in response to FGF, Biochem Biophys Res Commun. 2001 Oct 26;288 (2):413-9.; [2]Ahn HJ *et al.*, FGF2 stimulates the proliferation of human mesenchymal stem cells through the transient activation of JNK signaling. FEBS Lett. 2009 Sep 3;583 (17):2922-6.