37.P07
Superhydrophobic platforms for the combinatorial analysis of biomaterials-cells interactions using arrays of 3D scaffolds with distinct mechanical and morphological properties

MB Oliveira, CL Salgado and JF Mano
3B's Research Group–Biomaterials, Biodegradables & Biomimetics, Univ. of Minho, Headquarters of the European Institute of Excellence on Tiss. Eng.&Regen. Med., ICVS/3B's-PT Government Assoc. Laboratory, Portugal

High-throughput studies of cells mechanotransduction are usually performed using 2D biomaterials. However, cells-extracellular matrix interactions in the body occur in 3D environment. By using cells entrapped in hydrogels, it is not easy to isolate the mechanical effect from the chemical cues of biomaterials. We used a cytocompatible and non-expensive platform based in the patterning of wettable spots in superhydrophobic surfaces to deposit porous biomaterials in these regions. Freeze-dried alginate/chitosan scaffolds were used to create an array of mechanical properties and porosities. Adaptation of dynamic mechanic analysis equipment allowed performing on-chip single scaffold analysis. Micro-computed tomography allowed acquiring data for whole chips simultaneously. Results were validated using individual scaffolds and single-formulation chips. A sub-array with combined modulus/porosity properties was selected. Fibronectin (Fn) in different concentration was adsorbed in the scaffolds, and fibroblasts and osteoblast-like cells were seeded. The independent study of variables influence in cell response was performed by image-based methods. In the absence of Fn fibroblasts did not respond to mechanical properties in the chip range. Osteoblast-like cells showed higher cell adhesion in stiffer substrates. The adsorption of Fn was studied qualitatively by image methods. Results related with Fn amount and scaffolds' mechanical properties were analyzed for each cell type.

37.P08
Extrinsic biophysical stimulus can override the influence of local substrate in determining stem cell fate

SD Thorpe, CT Buckley, AJ Steward, DJ Kelly
Trinity Centre for Bioengineering, Trinity College Dublin, Ireland; Department of Mechanical Engineering, University of Notre Dame, Ireland

The aim of this study was to explore how cell-matrix interactions and extrinsic mechanical signals interact to determine stem cell fate in response to transforming growth factor-β3 (TGF-β3). Bone marrow derived mesenchymal stem cells (MSCs) were seeded in agarose and fibrin hydrogels and subjected to dynamic compression in the presence of TGF-β3. Markers of chondrogenic, myogenic and endochondral differentiation were assessed. Free-swelling agarose constructs stained positively for chondrogenic markers, and appeared to be progressing towards terminal differentiation as indicated by collagen type X and mineral staining. Dynamic compression suppressed differentiation along this endochondral pathway. In contrast, fibrin constructs supported differentiation along an alternative myogenic pathway in long-term free-swelling culture. Given that fibrin clots support a chondrogenic phenotype in vivo within mechanically loaded joint defect environments, the influence of long term dynamic compression on MSC differentiation was investigated. Mechanical signals generated by this extrinsic loading ultimately governed MSC fate, directing MSCs along a chondrogenic pathway as opposed to the default myogenic phenotype supported within unloaded fibrin clots. In conclusion, this study demonstrates that external cues such as the mechanical environment can override the influence of specific substrates, scaffolds or hydrogels on determining mesenchymal stem cell fate.

37.P09
Evaluation of cell response on permanent and pulsed atmospheric pressure stressed cells

B Rieder, A Weihs, A Teuschl, G Knebl, J Kollmitzer, H Redl and D Rünzler
University of Applied Sciences Technikum Wien, Austria; Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, TGM - Die Schule der Technik, Austria

Introduction: Many studies display that mechanical stimuli already fulfill the needs for cell differentiation, without any need for additional growth factors. Thus, we concentrated on mechanical stimuli of atmospheric pressure on cells using a tailor-made pressure chamber. Aim of the study was to determine pressure limits of cells and possible side effects to find supporting conditions for cell proliferation or differentiation.
Materials and methods: Cells of the cell lines U937 and MG63 were permanently kept at 37°C and buffered with HEPES during the experiments. The atmospheric pressure was applied on 1- respectively 2-well-chamberslides in the pressure chamber. For static pressure experiments, cells were permanently stimulated at 200, 300 or 400 kPa for 1 respectively 12 h and cultured for 2 weeks afterwards. For dynamic pressure experiments, pressures between 300 and 400 kPa were applied at frequencies of 0.25–4 Hz. Cell growth was observed for 5 days and evaluated by trypan blue staining and MTT assay.
Results: We could not observe any negative or positive effects on cell proliferation after the static pressure mode experiments. In the dynamical pressure experiment, 24 h after last pressure exposure, cells restored the original conditions and gave the same read-out compared to the untreated control.
Discussion: Thus, our tailor-made pressure chamber seems to be appropriate to find more specific parameters that induce cell differentiation of adult stem cells.

37.P10
Epigenetic modification of beta-catenin in 3D Titania mesenchymal cell cultured scaffolds under variable flux conditions in bioreactor chambers

R Couceiro, A Gil, C Garcia, P Diaz, J Couceiro, M Freire-Garabal and F Guitian
Institute of Ceramcis, USC, Spain; School Of Medicin, Dept of Pharmacolog, USC, Spain; School of Pharmacy, USC, Spain; Royal Academy of Medicine, Spain

Mesenchymal stem cell fate determination is heavily regulated by the WNT pathway through β-Catenin.Biomaterial topography triggers cytoskeleton biochemical and physical responses, which will ultimately modify gene expression leading to an osteoblastic phenotype. It is already known that several cytoskeleton related genes are mechanoregulated. In this study as a result of exploring epigenetic regulations by methylation levels of Beta-Catenin, a more accurate insight of osteogenic control could be achieved. Engineering a stem cell niche is a task that considers not just a 3D scaffold which will mimic a cellular microenvironment, but also the dynamic forces exerted on cultured stem cells.A functional tissue engineering (FTE) method could be completed by identifying methylation patterns within dynamic culture models.Titania (TiO2) scaffolds were synthesized with pore sizes ranging from 300–400 μm by Direct Ink Writing and cultured with human mesenchymal stem cells. In order to evaluate the existence of potential differential methylation rates, scaffolds were placed in a bioreactor chamber under pulsed flux conditions (2, 6, and 12 h). A non flux control group was used. Methylation specific polymerase chain reaction (MSP) and direct sequencing showed how CpG islands related to Beta Catenin are being directly modified under variable flux conditions

DOI: 10.1002/term.1586