Influence of Environmental Factors on Cell Behaviour and Functional State

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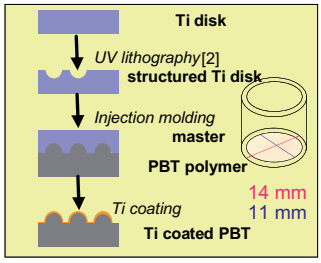
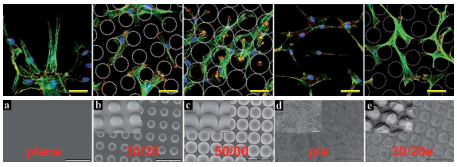
INTRODUCTION

The functionality of cells, i.e., state of differentiation, (re)activity, viability is directly determined by its environment. Latter is defined by the substratum (structure and chemistry) including cell-cell contacts, forces and extracellular fluid composition including (nano)particles. So far the mechanism how each of these factors affect cells and in how far each factor is able to modify the effect of the other factor is largely unknown. In our lab cell-surface interactions are investigated taking into account each of these factors in order to define parameters that are able to steer cell reactions. Latter is a premise to obtain new tools to define cell reactions, to optimize existing and to define new implants and clinical therapies.

SURFACE STRUCTURE



HB(M)Cs were cultured on different surface topographies (plane, hemispheres diameter and intersphere distance in µm (30/20, 50/0), etched (e) or not)



Cell morphology

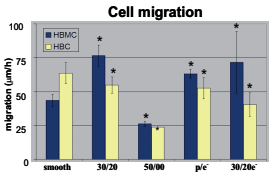
TEM (a-e) and SEM images (top): cultures stained for F-actin (green), vinculin (red) + nuclei (blue) stained after 7 days in culture.

bars: 50 mm, 20 mm, 100 mm.

30/20: cells esp. around hemispheres, focal contacts (FC) in plane area

50/00: actin filament span from one hemisphere to neighbouring hemi-sphere, dense focal contacts on top of hemispheres

p/e- and 30/20e-: cells in a spindle-shaped, condensed morphology with only diffuse focal contacts



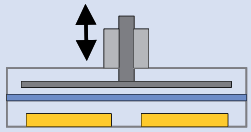
Cell morphology

Time-lapse microscopy of Dil-labeled HBMCs and HBCs on the different topographies for 6 h to measure cell migration

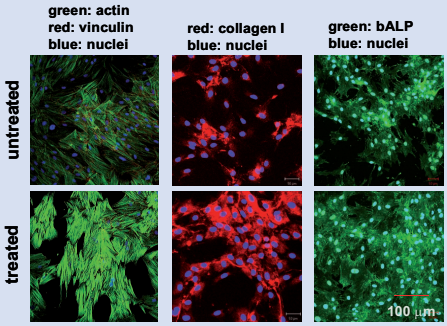
HBC and HBMC exhibited lowest migration on the 50/00 structure, where they became locked in the hemispheres

HBC and HBMC were differently affected

EFFECTS OF MECHANICAL FORCES (DYNAMIC PRESSURE)

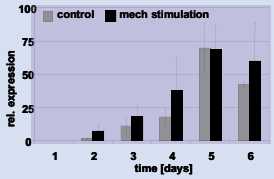


HBCs cultivated in osteogenic medium with 30', 3 times a day for 1-7 days with max. pressure of 10 kPa, 20 Hz.



Triple (or double) staining of HBCs cultivated after application of cyclic pressure for 7 days.

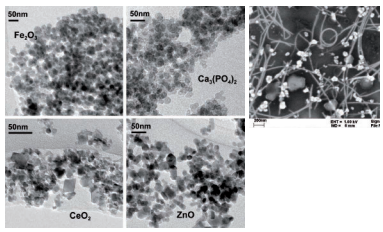
→Treated HBC expressed more actin filaments, collagen-I and bone-specific alkaline phosphatase (bALP) compared to the untreated cells



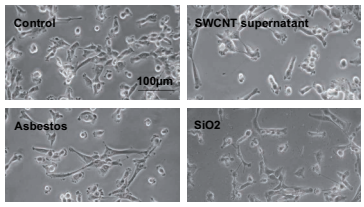
Quantitative RT-PCR analysis of osteocalcin mRNA in HBC after application of cyclic pressure for 1 to 6 days. Mean ± SEM of 3 experiments relative to day 1

→ Treated HBC showed higher levels of osteocalcin mRNA

MEDIUM COMPONENTS



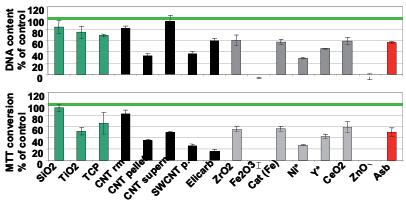
Treatment of different cells with dispersed nanosized materials and evaluation of the effects 3 days later



Effects of 7.5 µg/ml various nanosized materials on MSTO211H morphology

→ Cell density and cell shape are affected by the particles

Proliferation, cell activity



Effects of 15 µg/ml various nanosized materials on MSTO211H cell proliferation (DNA) and cell energy state (MTT)

→ Effects are cell type specific (not shown)

→ Parameters are differently and particle dependently affected

CONCLUSION AND ACKNOWLEDGEMENT

In our lab primary human trabecular bone (marrow) cells (HBMC, resp. HBC), fibroblasts, cell line cells as well as primary embryonic chicken nerve cells are cultured under various conditions. Cell are partly transfected with gene constructs in order to fluorescently label the whole or only parts of the cell (e.g. only specific cytoskeletal elements like vinculin or paxillin) or in order to report for the activity of a gene of interest (e.g. osteocalcin). During the cultivation time in a part of the experiments the migration of the total cell or in case of nerve cells solely the growth cone is on-line monitored using modified CLSM. At the end of the experiment various biochemical parameters are measured (e.g. functional state, specific proteins, specific mRNA levels). In future in addition population dynamics will be investigated using FACS. Compound/particle effects are analyzed according new toxico-/ pharma+codynamic models

LOGOs