Surface properties of calcium phosphate silica composite material and their relation to biocompatibility

M. Teller[1,a], U. Bulnheim[2,b], H.-G. Neumann[1,c] J. Rychly[2,d]
[1]DOT GmbH Charles-Darwin-Ring 1a, D-18059 Rostock, Germany
[2]Dept. of Internal Medicine, University of Rostock, D-18055 Rostock, Germany
[a]teller@dot-coating.de , [b]ulrike.bulnheim@med.uni-rostock.de,
[c]neumann@dot-coating.de, [d]joachim.rychly@med.uni-rostock.de

Introduction: Surface properties of bioactive materials are reflected in their resorption behaviours. It is well known that negative and positive surface charges have an influence on adhesion of osteoblasts. In literature experiments are described which demonstrate that the variation of surface charge by manipulation with electrically polarized hydroxyapatite can influence cell proliferation. In vitro cell attachment, cell expansion and differentiation on biomaterials are indispensable for further in vivo application of cell material constructs for bone replacement and regeneration of bone defects. We investigated surface charge and the adhesion and growth of hMSCs on the BONITmatrix® (BM) and hydroxyapatite (HA) surfaces and their ability of producing some osteogenic markers as a signal for promoting osteogenic differentiation by the material. Studies with titanium powder were included for comparison of surface charges.

Materials and methods: BM represents a bone regeneration material which is synthesised by a sol gel process. It is structured as a composite material consisting of two calcium phosphate components (60% hydroxyapatite and 40% ß-tricalcium phosphate (w:w)) and containing a silica portion of 13%. Zeta potential measurements were carried out for BM, hydroxyapatite and titanium powder with Zetasizer 3000 (Malvern UK). Human mesenchymal stem cells (hMSC) derived from bone marrow were proliferated up to 3 passages in expansion medium. $2x10^4$ cells were seeded onto each BM disc and cultivated for 22 d in expansion medium. Cell adhesion and growth were visualized by SEM after 24 h, 3 d, 9 d and 22 d.
Determination of osteogenic markers at the mRNA level was performed by RT-PCR. Therefor cells were harvested after 14 d of cultivation on BM. Total RNA extraction and RT-PCR were carried out as described elsewhere.

Results and discussion: Zeta potential measurements are a suitable possibility to investigate surfaces of implants and use the results for interpretation of physicochemical interactions between surface and environs.

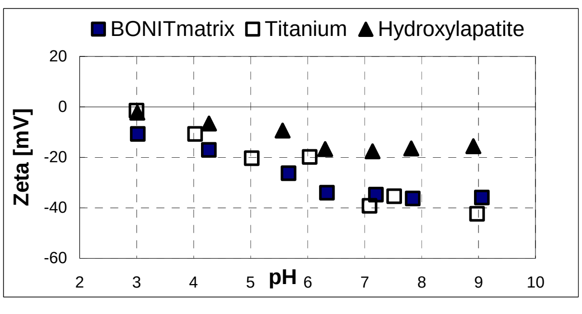

Fig.1: Comparison of Zeta potential measurements of BONITmatrix®, titanium and hydroxyapatite powder

In the neutral pH range BM showed a Zeta potential of –34.1 mV, likewise 45 µm sized titanium particles with –39.2 mV. By way of contrast for HA powder a Zeta potential of –17.6 mV was measured (figure 1). The measurement values in figure 1 presents no significant difference between titanium powder and BM for the complete pH range from 2 to 10.
Also charge density measurements (PCD) were carried out. Thus, BM possesses a negative surface charge of –6.31 µeq/g. In this connection also the components of BM were analysed by PCD measurements. These results show significantly that a large part of negative charge in the composite is caused by embedded hydroxyapatite particles (–3.59 µeq/g).
In adhesion and growth experiments with hMSCs on BM surfaces the adhered cells displayed a flat and wide spread phenotype with extensive formation of filipodia and covered approximately 50 % of the surface area (fig.2) recorded 24 h after seeding the MSCs onto BM discs. Between days 3 and 9 the disc surfaces were completely covered with cells and after day 22 they formed a dense layer with signals of formation of extra cellular matrix.

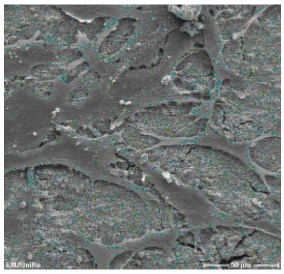

Fig. 2: hMSCs 24 h after seeding onto BONIT-matrix® compact surface in expansion medium, magnification:500x

After 14 d cultivation the expression indicates levels of some osteogenic markers in MSCs at BM discs and at the control surfaces (tissue culture polystyrol (TC) and HA were used as controls). There was an increased expression of alkaline phosphatase and bone sialoproteine as well as the transcription factor Runx2 both on HA and BM in comparison to TC. But the effects were stronger at HA. At most samples there was not detectable any effect of expression of the transcription factor Osterix (Osx).

Conclusion: Zeta potential measurements of BONITmatrix® at neutral pH present distinct negative charges comparable to those of titanium powder. This composite material possesses a higher negative charge density in comparison to hydroxyapatite, its major component. The results of adhesion and growth experiments with hMSCs indicate that the surfaces of BONITmatrix® and other calcium phosphate materials can stimulate the mRNA expression of markers which are representative for osteogenic differentiation.