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Impact of new functionalized biomaterials on the adhesion and proliferation of human mesenchymal stem cells

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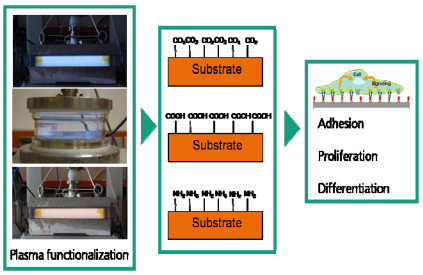
Introduction

Tissue Engineering is an interdisciplinary research field that aims to support or replace diseased human tissues [1]. One prerequisite is the combination of synthetic and living components. A major problem of Tissue Engineering is the terminal differentiation and therefore restrictive *in vitro* proliferation of cells in an adult organism. Alternative cell sources are adult stem cells like human mesenchymal stem cells (h*MSC*s). Their advantages are the high proliferation and differentiation capacity [2]. We designed new biomaterials with amino- or carboxyfunctionalized surfaces to optimize stem cells' proliferation and differentiation conditions.

Material and Methods

Polystyrene surfaces were functionalized by ammonia-, acrylic acid- and CO_2- plasma. A frequency of 13.56 MHz were used to obtain amino and carboxy groups on the surface. As reference surface to commercial available tissue culture served the CO_2-Plasma. Successful functionalization was proved by X-ray photoelectron spectroscopy (XPS) and colorimetric methods like methyl orange and toluidin blue staining. The substrates were analyzed by contact angle measurement. h*MSC*s isolated from bone marrow were seeded on the functionalized substrates. Adhesion and morphology were investigated by light-microscopy.



After 72 h a proliferation assay WST-1 was performed to compare proliferation and functionality of the h*MSC*s on the different substrates. The cell substrate interaction was observed by immunohistochemical staining of Actin-Vinculin. Furthermore the influence of the plasma treated surfaces on the differentiation capacity was measured by the semi quantitative alizarin red staining after 28 days of culturing. The differentiation medium contained specific osteogenic factors like L-Ascorbic acid-2-phosphate, ß-Glycerophosphat and Dexamethason.

Results and Discussion

The functionalization of the plasma treated surfaces was successful and proved by colorimetric methods (data not shown). A significant increase of amino and carboxy groups on the substrates, after the application of low pressure plasma, was demonstrated by XPS-measurements (Fig. 1). h*MSC*s were characterized by the criteria set by the International Society for Cellular Therapy. The proof of plastic-adherence, expression of specific surface antigens and ability to differentiate *in vitro* into the osteogenic, adipogenic and chondrogenic lineage via staining were demonstrated (data not shown). Cell-substrate interaction could be analyzed by fluorescence staining of focal contacts (Fig. 2). All surfaces showed focal contacts proved by Actin-Vinculin staining. The metabolic activity assay WST-1- revealed a different proliferation rate of h*MSC*s on the modified surfaces compared to the tissue culture substrates, which served as a positive control (Fig.3). The influence of chemical modification on the osteogenic differentiation could be shown. Cells cultured on amino functionalized surfaces revealed 30% more mineralization than the control cells (Fig. 4).

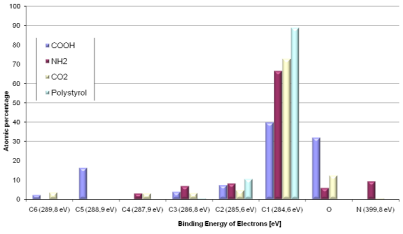


Figure 1: X-ray photoelectron spectroscopy (XPS) measurment. Polystyrene surfaces were functionalized by ammonia-, acrylic acid- and CO_2- plasma. The main component of polystyrol is carbon characterized by the binding enegry of 284,6 eV. In comparison on the ammonia plasma treated surfaces an increase of nitrogen and oxygen compunds could be detected. Acrylic acid plasma treatment evoked a carboxy-specific accumulation of carbon compunds of ca. 15% and oxygen compunds of 30%.

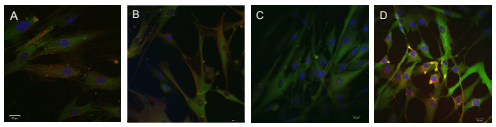


Figure 2: Immunofluorescence staining of h*MSC*s. Cells were grown on cell culture dish (A), carboxy- (B), CO_2- and amino functionalized substrate (B) for 72h. By staining of Actin (red), Vinculin (green) and cell nuclei (blue) the formation of aktin-cytoskeleton and severeal focal adhesion were detected.

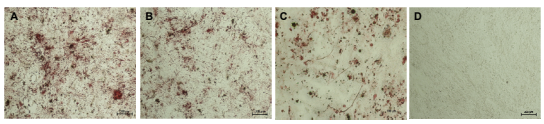


Figure 4: Alizarin red staining of h*MSC*s after 28 days in culture (n=1). Cells were seeded on amino- (A), CO_2- functionalized substrates (B) and Tissue Culture (C). A-C were cultured with osteogenic differentiation medium, D served as negative control (DMEM + 10% FCS (LONZA)).

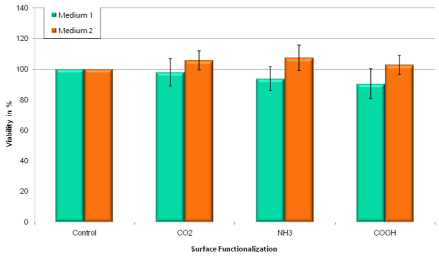


Figure 3: Proliferation assay of h*MSC*s on plasma functionlized surfaces (n=5). WST-1 assay was performed 72 h after seeding. Tissue culture surfaces served as a reference (100%). The viability of the cells depended on the surface modification and the used medimum (Medium 1: MSCBM + 2% FCS (LONZA)). Cells cultured with Medium 2 containing DMEM Ham´s F12, 10% FCS (LONZA) on amino functionalized surfaces showed with 110% an increase of metabolic activity compared to the control cells.

Conclusion

This work verifies an influence of surface modification with amino and carboxy groups on the adhesion and proliferation of h*MSC*s. Furthermore it indicates a positive effect of amino groups on the osteogenic differentiation of h*MSC*s. Therefore further studies concerning the differentiation induction will be performed. Also improvements of the substrates topography as well as further investigations in differentiation conditions are necessary to provide efficient *in vitro* scaffolds for Tissue Engineering.

References

[1] Minuth, W.W., M. Sittinger, and S. Kloth, *Tissue Engineering: Generation of differentiated artificial Tissues for biomedical Applications.* Cell and Tissue Research, 1998 **291**(1): p. 1-11.
[2]. Pittenger, M.F., et al., *Multilineage potential of adult human mesenchymal stem cells.* Science, 1999. **284**(5411): p. 143-7.

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