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Development of functionalized Scaffolds for Bone Tissue Engineering

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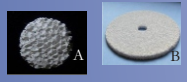
Introduction

Presently, one basic research in Tissue Engineering is the development of "smart" multifunctional scaffolds for supporting and guiding the growth of cells. In order to develop such a scaffold, matrices can be modified with various bioactive ligands enhancing cell adhesion, cell growth and differentiation. In this study, polymers and copolymers of vinylsaccharide N-methacrylamidoglucose (MAG) were used as spacer arms between scaffold and ligands. They were immobilized via adsorption on the matrix material Sponceram®, a macroporous doped ZrO_2 ceramic.
The desired ligands like growth factors and cell adhesion promoting molecules were conjugated to the polymers by aldehyde chemistry.

Schematic Approach

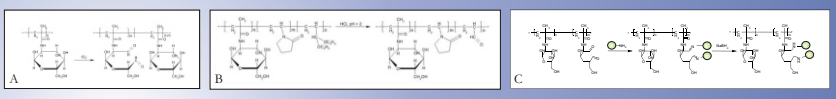
Scaffold: Sponceram®
Polymer: ox.p(MAG)/ p(MVA)
Non-specific ligand: Poly-L-Lysine
Growth factor: BMP-2



Sponceram®, a doped macroporous ZrO2 ceramic.
A: Sponceram minidisc (diameter 10 mm, thickness 3 mm);
B: Sponceram disc (diameter 65 mm, thickness 3 mm)

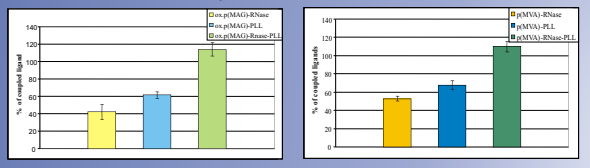
The main idea is the development of scaffolds with different surface ligands to enhance the adhesion and proliferation of cells.

Mechanism of Coupling Procedure

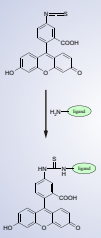


In order to achieve aldehyde groups in the polymer, both of them had to be activated. The polymer of N-Methacrylamidoglucose p(MAG) was oxidized to ox.p(MAG) by using sodiumperiodate (A). The acetales of the polymer Methacrylamidoglucose-vinylpyrrolidone-diethylacetale p(MVDAAc) were converted into aldehyde groups in acid environment (B).The resulting polymer is called p(MVA). The ligands poly-L-lysine and RNase (as model protein) were coupled to the polymer via aldehyde chemistry. The advantage of this method is the absence of toxic byproducts. In order to achieve a stable connection between polymer and ligands, the Schiff's bond was reduced by sodium borhydride (C).

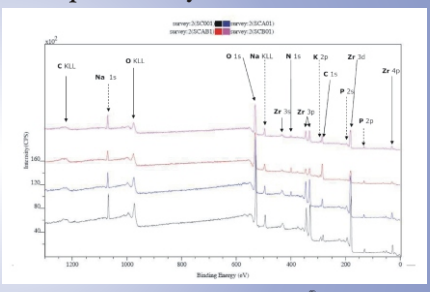
Fluorescence Assay



For the development of the conjugation procedure, two ligands, RNase and poly-L-lysine (PLL), were labeled with FITC. RNase was used as a model protein instead of BMP-2.
First, RNase-FITC was coupled to the polymer. The second ligand, PLL-FITC, was coupled in the second step as well as alone in the single conjugate. The binding of the ligands was investigated via fluorescence measurement.

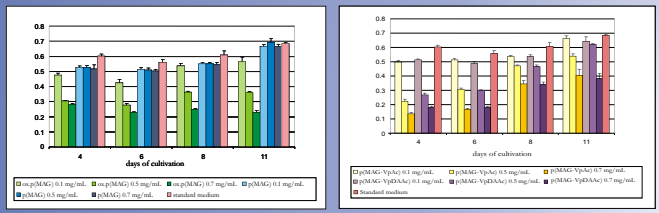


Adsorption Study



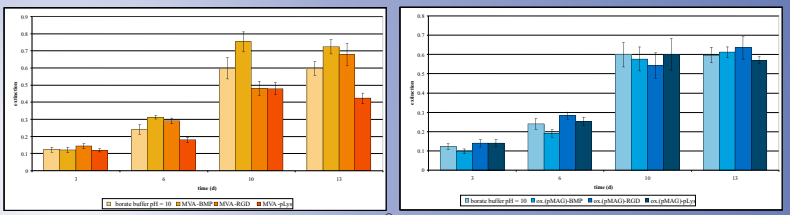
For the adsorption procedure, the Sponceram® discs were incubated into ox.p(MAG)-PLL solution (in phosphate buffer, pH = 7) over night at room temperature and slight stirring. After the incubation time, the discs were washed thrice with Phosphate buffer and dried at room temperature. For the qualitative analysis, the samples were measured by XPS measurement (Al monochromatic, 12 kV/ 5 mA), compared to unmodified Sponceram® (incubated into phosphate buffer), PLL coated Sponceram® and polymer coated Sponceram®.

Cytotoxicity assay



MC3T3-E1 cells were cultivated with medium, in which p(MAG), ox.p(MAG), p(MVDAAc) and p(MVA) were dissolved in different concentrations. p(MAG) had no significant influence to the viability of the cells. The proliferation of the cells decreased with increasing concentration of ox.p(MAG), p(MVDAAc) and p(MVA).

Longterm cultivation



MC3T3-E1 cells were cultivated on Sponceram® matrices with different immobilized conjugates. MTT assay was performed to determine their influence to the cultivated cells. The results demonstrate that the materials containing ox.p(MAG) as spacer do not have a negative influence to the proliferation/ viability of the seeded cells.
p(MVA) containing composite materials show a different trend. Cells seeded on p(MVA)-PLL and p(MVA)-RGD coated Sponceram showed a slighter viability than on pure Sponceram.

Discussion and Outlook

The experiments demonstrated, that the presence of ox.p(MAG), p(MVA) and p(MVDAAc) in cell culture medium shows a negative influence to the cell viability/ proliferation. But in case ox.p(MAG) was used as a spacer for the coupling of ligands to the mineral matrix, no negative effect was found. Furthermore, the single coupling of the desired ligands was possible as well as the developmant of a double conjugate. Besides that, the expected adsorption of the conjugates to the matrix took place.
In future, the differentiation inducing potential of BMP-2 containing composite materials will be studied by RT-PCR and special osteogenic protein arrays. Besides, the developed composite materials will be seeded with mesenchymal stem cells derived from fat or umbilical cord tissue and cultivated in a special rotating bed bioreactor system.

Acknowledgement

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