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Biofunctional Polymer-Mineral Composites as Scaffolds for Bone Tissue Engineering

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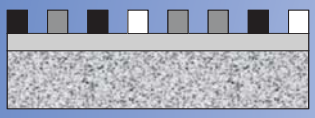
Introduction

The objective of this work was the development of a novel functionalized biocompatible mineral matrix for bone tissue engineering, which shall support and guide the growth of cells. The functionalization was achieved by covering the matrix surface with water soluble biocompatible but non-degradable polymers. These were modified with different bioactive ligands enhancing cell adhesion, cell growth and differentiation.
In this study, oxidized polymer of vinylsaccharide N-methacrylamidoglucose (ox.p(MAG)) was used as "spacer". Poly-L-lysine (pLys) and RGD peptide were used as ligands increasing cell adhesion, BMP-2 was used as a ligand enhancing cell differentiation.
The applied matrix material was Sponceram®, a doped, ZrO_2 macroporous ceramic. The modified materials were tested in cell culture using osteoblastic precursor cells MC3T3-E1 with regard to cell viability/ proliferation.

Schematic Approach

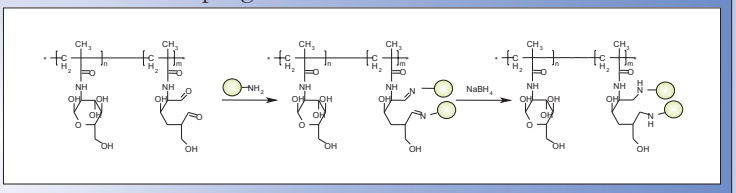


- Scaffold: Sponceram®
- Polymer: ox.p(MAG)
- Non-specific ligand: Poly-L-Lysine
- Biospecific ligand: RGD-peptide
- Growth factor: BMP-2

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

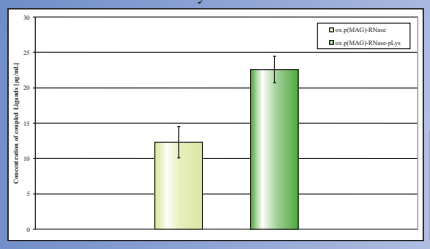
Sponceram®, picture from Zellwerk

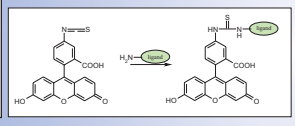
Mechanism of Coupling Procedure



The ligands 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.

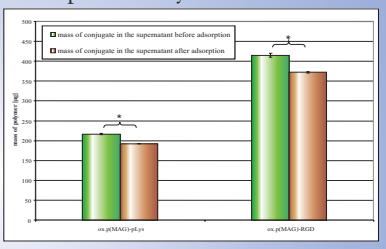
Fluorescence Assay





For the development of the conjugation procedure, two ligands, RNase and pLys, 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, pLys-FITC, was coupled in the second step. The binding of the ligands was investigated via fluorescence measurement.

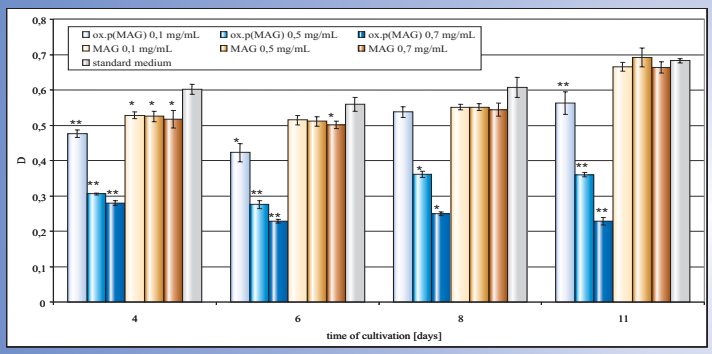
Adsorption Study



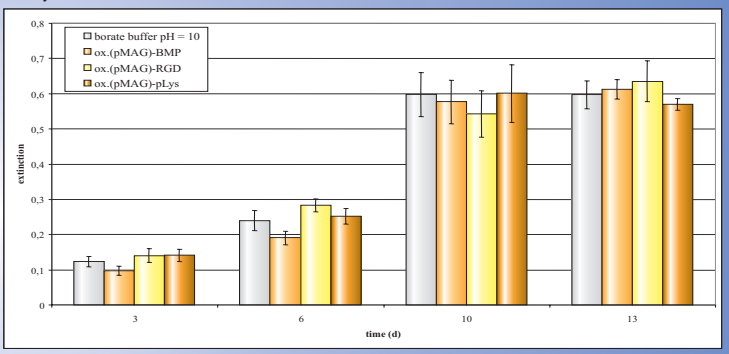
Conjugate	mass of adsorbed conjugate (per matrix) [µg]
ox.p(MAG)-pLys	2,459 ± 0,228
ox.p(MAG)-RGD	3,222 ± 0,545

The adsorption study was performed with two single conjugates: ox.p(MAG)-pLys and ox.p(MAG)-RGD. For the adsorption studies, Sponceram® scaffolds were incubated in conjugate solution for 24 hours at slight stirring. The quantity of the adsorbed conjugate was determined by UV absorption measurement of the supernatent. It is apparently, that the adsorption took place after 24 hours.

MTT assay



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



MC3T3-E1 cells were cultivated on Sponceram® matrices with different immobilized conjugates. The MTT assay was performed to determine their influence to the cultivated cells. The results demonstrate that the tested materials do not have a negative influence to the proliferation/ viability of the seeded cells.

Discussion and Outlook

The experiments demonstrated, that the presence of ox.p(MAG) in cell culture medium shows a negative influence on the cell viability/ proliferation. But in case it is 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 development of a double conjugate. Besides that, the expected adsorption of the conjugates to the matrix took place.
In future, a triple conjugate with all three ligands will be developed to enhance cell proliferation/ viability and differentiation with one scaffold. Therfor, the differentiation status will be studied by RT-PCR.

Acknowledgement

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