Leibniz Universität Hannover
Institut für Technische Chemie, Callinstr. 3, 30167 Hannover
Partner in der Forschung

Development of Biofunctional Scaffolds for Bone Tissue Engineering

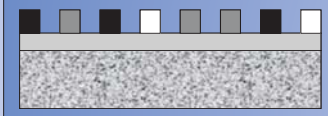
Stefanie Röker[1], Solvig Diederichs[1], Viktor Korzhikov[2], Tatiana Tennikova[2], Cornelia Kasper[1]

[1]Institut für Technische Chemie der Leibniz-Universität Hannover, Callinstr. 3, D-30167 Hannover
[2]Institute of Macromolecular Compounds, Russian Academy of Sciences, Saint-Petersburg, Russia

Introduction

Autologous bone tissue generation for transplantation is a very promising technique in orthopedic surgery and biomedical engineering, since it can eleminate problems of graft scarcity, immune rejection and pathogen transfer. The objective of this work was the development of a novel functionalized biocompatible mineral matrix for bone tissue engineering in order to support and guide the growth of cells. The functionalization was achieved by covering the matrix surface (Sponceram®, a macroporpus ZrO_2 ceramic) 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.

Schematic Approach

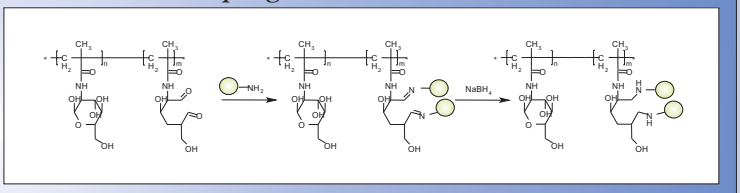


Scaffold: Sponceram®
Polymer: ox.p(MAG)
Non-specific ligand: Poly-L-Lysine
Biospecific ligand: RGD-peptide
Growth factor: BMP-2

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

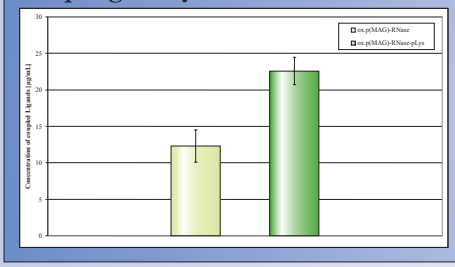
Macroporous structure of Sponceram®

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.

Coupling Analysis

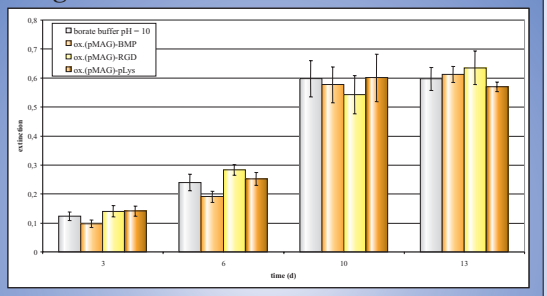


Determination via fluorescence labeling: For the development of the conjugation procedure, two ligands, RNase and pLys, were labeled with FITC. RNase was used as a model protein instead of BMP-2.
First, RNase-FITC was coupled to the polymer. The second ligand, pLys-FITC, was coupled in the second step. The binding of the ligands was investigated via fluorescence measurement at 485/355 nm.

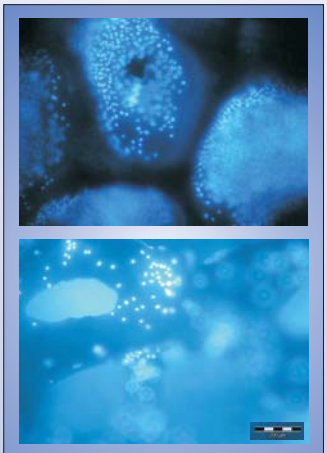
Determination via SDS-Page: The intermediates of all coupling steps as well as RNase and uncoupled ox.p(MAG) were brought into the Gel, which was stained with silver. The increasing staining intensity showed an enhanced protein concentration in the conjugate.

1: Protein ladder, 2: RNase, 3: ox.p(MAG), 4: ox.p(MAG)-RNase, 5: ox.p(MAG)-RNase-pLys, 6: ox.p(MAG)-RNase-pLys-RGD

Long Term Cultivation

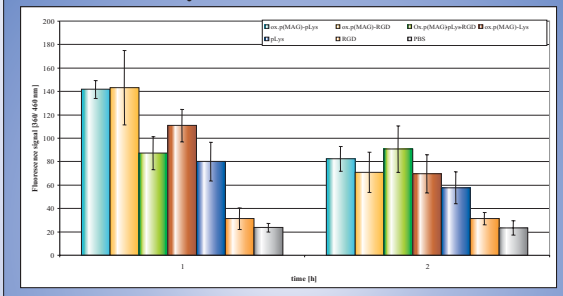


MC3T3-E1 cells were seeded onto composite materials (Sponceram® matrices with adsorbed conjugates). As reference, unmodified Sponceram was used. 3, 6, 11 and 13 days after cell seeding, the MTT assay was performed to determine the influence of the composite materials to the cultivated cells. The results demonstrate that the tested materials did not have a negative influence to the proliferation/ viability of the seeded cells.



DAPI stained cells, seeded onto Sponceram®, viewed 100-times magnified

Adhesion Study



For the adhesion study, $8*10^4$ MT3T3-E1 cells were seeded onto every composite material. As reference, unmodified Sponceram was used, furthermore Sponceram® which was covered with pLys and RGD. After 1, 2 and 24 hours, the cells were fixed with Ethanol and then stained with DAPI. The amount of attached cells were determined with the fluorescence reader at 360/460 nm. It is obvious, that the amount of attached cells on the composite materials is increased compared to the cells on Sponceram®.

Discussion and Outlook

The experiments show, that the coupling of different desired ligands to the polymer ox.p(MAG) is possible and can be performed in reproducible quantity. Methods for qualitative and quantitative analyses were found and established. The composite materials were tested in cell culture and showed no cytotoxic effect or any negative influence to the viability of the cultivated cells. Furthermore, the composites with adhesion enhancing ligands (RGD peptide and poly-L-lysine) were tested in an adhesion assay. Their desired effect was approved.
In future, the BMP-2 containing composite materials will be seeded with mesenchymal stem cells. The influence of the materials towards the differentiation of the MSCs to osteoblasts will be studied and compared with BMP-2 diluted in cell culture medium. Furthermore, all composite materials will be tested in a special bioreactor for dynamic cell cultivation.

Acknowledgement

BMP-2 was kindly donated by Professor Sebald, Würzburg, Germany. Sponceram® was provided by Zellwerk, Oberkrämer, Germany.