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Development of "Smart" Scaffolds for Bone Tissue Engineering

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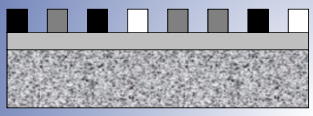
Introduction

The major aim of this work was the development of "smart" multifunctional scaffolds for bone tissue engineering, which may support and guide the growth of cells. The ideal scaffold has to mimic ECM features and is supposed to be made of a biomaterial that provides all the necessary signals for the cells to grow, differentiate and interact, forming the desired structure.
For this application, we used a ZrO_2-doped macroporous ceramic, polymers of vinylsaccharid N-methacrylamidoglucose and non-specific ligands as well as biospecific ligands.

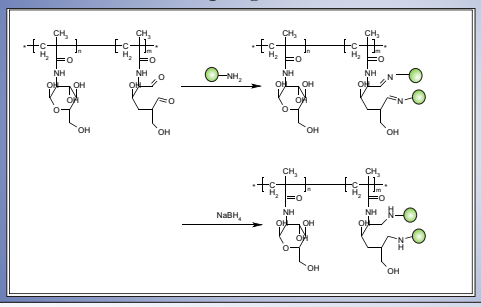
Schematic Approach



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

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

Mechanism of Coupling Procedure



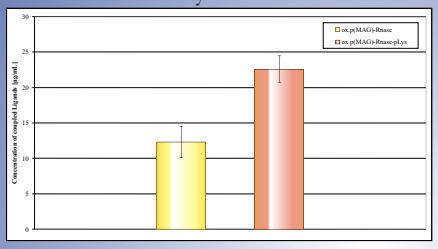
The desired ligands were coupled to the polymer via aldehyde chemistry. The advantage of this method is the absence of toxic byproducts. In order to achieve a stable connection between polymer and ligands, the Schiff's bond was reduced by sodium borhydride.

Materials and Methods: Conjugation

Scaffold: Sponceram®, a macroporous, ZrO_2-doped ceramic
Polymer: oxidized polymer of vinylsaccharide N-methacrylamidoglucose (ox.p(MAG))
Non-specific ligand: Poly-L-lysine Hydrobromide (pLys)
Model protein: Ribonuclease A
Fluorescence marker: Fluorescein isothiocyanate (FITC)

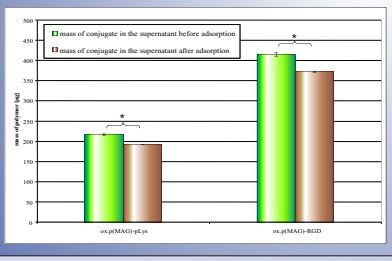
For the development of the conjugation procedure, two ligands, RNase and pLys, were labeled to FITC. RNase was used as a model protein instead of BMP-2.
Both ligands were dissolved in borate buffer, pH 10.
First, RNase-FITC was coupled to the polymer. The reaction took place at room temperature for 1 h at slight stirring. The second ligand, pLys-FITC, was coupled in the same manner. The binding of the ligands was investigated via fluorescence measurement.
After the procedure was finished, the remaining Aldehyde groups were reduced with sodium borhydride.
For the adsorption studies, Sponceram® scaffolds were incubated in solution of the conjugates for 24 hours at slight stirring. The mass of the adsorbed conjugate was determined by UV absorption measurement of the supernatent.

Fluorescence Assay



The fluorescence assay shows the amount of the ligands, which bound covalently to the polymer. Obviously, we succesfully coupled both ligands, step by step, to the polymer.

Adsorption study



The adsorption study was performed with two single conjugates: ox.p(MAG)-pLys and ox.p(MAG)-RGD. It is apparently, that the adsorption took place after 24 hours.

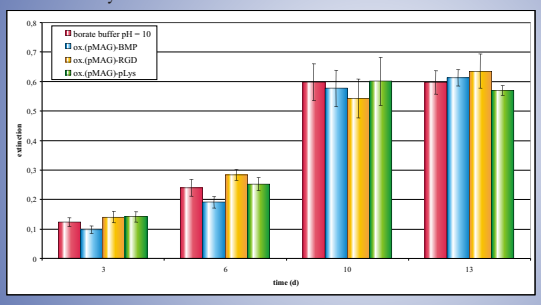
Conjugate	mass of adsorbed conjugate (per matrix) [µg]
ox.p(MAG)-pLys	2,459 ± 0,228
ox.p(MAG)-RGD	3,222 ± 0,545

Materials and Methods: Cell Culture

Composites: Sponceram®-ox.p(MAG)-BMP, Sponceram-ox.p(MAG)-pLys, Sponceram-ox.p(MAG)-RGD
Cells: MC3T3-E1

For the cell culture testing of the composites, the latter were incubated for 24 h in cell culture medium (DMEM + 10 % FCS + 1 % Penicillin/Streptomycine) at 37°C, 5 % CO_2.
$2*10^4$ cells were seeded on each scaffold in 96 well plates, and attached in 2 h while gently stirring.
For the assay of the cell metabolism, an MTT-test was performed. Therefor, the scaffolds were transferred into a second 96 well plate. The assay was carried out 3, 6, 11 and 13 days after cell seeding.

MTT assay



The MTT assay was performed to examine the influence of the composites to the viability of the cells.
The results demonstrate that the composite materials do not have a disadvangeous influence to the proliferation/ viability of the seeded cells.

Discussion and Outlook

Sponceram®-based conjugates were succesfully produced with different desired ligands. No cytotoxic effect of any part of the conjugates was figured out. In future, a triple conjugate with poly-L-lysin, RGD peptide and BMP-2 will be prepared. Furthermore, the bioactivity of BMP-2 and the effect of RGD and pLys to the cell adhesion will be assayed in static and dynamic cell cultivation .

Acknowledgement

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