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MULTIFUNCTIONAL POLYMER-MINERAL COMPOSITE MATERIALS AS BIOACTIVE SCAFFOLDS FOR BONE TISSUE ENGINEERING

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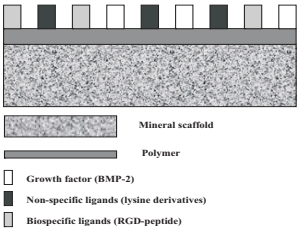
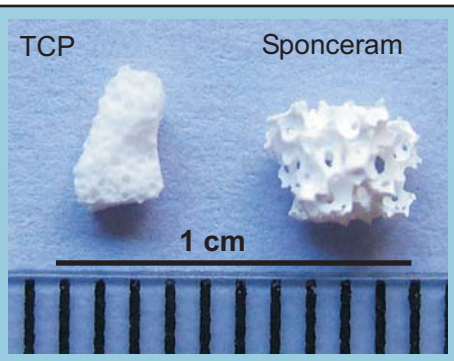
1 Introduction

The main goal of this work is the development of an "intelligent" approach to construct multifunctional composite scaffolds for bone cell growth and differentiation. For this purpose, different kinds of mineral macroporous supports, water soluble aldehyde-containing copolymers of N-vinylpyrrolidone as well as different non-specific and biospecific ligands governing cell adhesion and growth have been used. The composite materials were tested initially in cell culture experiments.

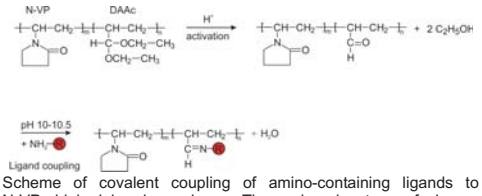
2 Schematic approach



The main idea is to develop polymer-mineral composite materials with different surface ligands.



3 Scheme of coupling



Scheme of covalent coupling of amino-containing ligands to N-VP-aldehyd bearing polymer. The main advantage of chosen chemistry is the absence of toxic or non-metabolic byproducts. Moreover, the resulting Schiff´s bonds are stable at physiological conditions which provide the stability of the ligand in its immobilized form.

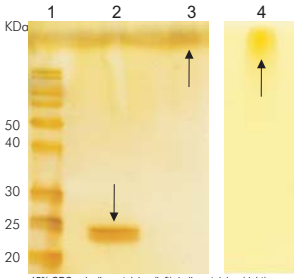
4a Material and methods

Copolymer of N-vinylpyrrolidone with acroleyn diethyl acetal (N-VP-DAAc) was used as functional vector. The activation was carried out in aqueous solution at pH 2, 70-80°C, 60 min. The solution was then adjusted to pH 7. For **covalent binding**, BMP-2 or RNase (as model protein) + polymer were dissolved in sodium borate buffer, pH 10. The reaction took place at room temperature for 1 h at slight stirring. The total binding of BMP-2 to polymer was investigated by SDS-PAGE. For **adsorption**, TCP and Sponceram were incubated in polymer solution at room temperature for 2 h at slight stirring. The concentration of adsorbed polymer was determined by analysis of the supernatent in comparison to the same non adsorbed solution.The concentration of polymer was determined using a specific **iodine test**.

4b Material and methods

Scaffolds: granulated tricalcium phosphate (TCP); monolithic macroporous Sponceram
Cell seeding: After adsorption of polymer, the scaffolds were washed with PBS and incubated for 24 h in cell culture medium (DMEM containing 10% FCS, L-glutamine, antibiotics) at 37°C, 5% CO_2. An excess of osteoblast-like Saos-2 cells were seeded on each scaffold in 96-well plates for 2 h at gentle stirring. Before each of the following tests the scaffolds wereplaced into a new 96-well plate. Since it was not possible to estimate the attached number of cells on the scaffolds, the first measurements were performed directly after cell seeding. Cell metabolism was assayed using **MTT-test**. Total protein content was measured using Micro **BCA** assay. **Alkaline phosphatase (AP)** was determined by an assay based on the hydrolysis of p-nitro-phenyl phosphate to p-nitrophenol.

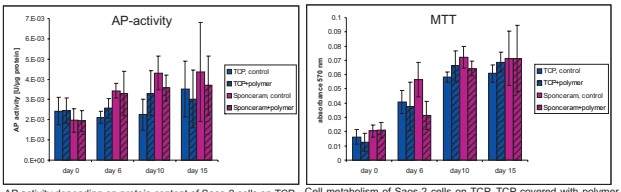
5 Gel electrophoresis



15% SDS gel, silver staining (left); iodine staining (right)
Lane 1: Marker; lane 2: BMP-2; lane 3 BMP-2 + polymer
lane 4: BMP-2 + polymer

The quality control of covalent binding was based on detection and comparison of migration distance of coupled and non-coupled BMP-2. While the charged protein migrates across the gel (lane 2), the uncharged complex does not move (lane 3). Lane 4 shows the iodine staining of the polymer (same sample as in lane 3).

6 Cell culture



AP activity depending on protein content of Saos-2 cells on TCP, TCP covered with polymer, HA, HA covered with polymer over a time period of 15 days. Values represent the mean of 5 samples of cultured scaffolds +/- SD.

Cell metabolism of Saos-2 cells on TCP, TCP covered with polymer, Sponceram, Sponceram covered with polymer over a time period of 15 days. Vaues represent the mean of 5 samples of cultured scaffolds +/- SD.

One goal was to test the modified scaffolds for their cytotoxity. It is obvious that covering TCP and Sponceram by polymer did not influence the amount of vital cells. Also the AP activity showed a congruent course over a time period of 15 days.

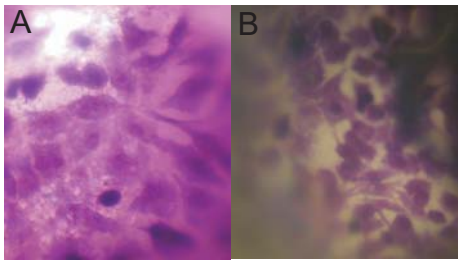
7 Adsorption

Copolymer*	Adsorption	Desorption		
	µg/g sorb.	2 days,	6 days,	14 days
		µg/g sorb.	µg/g sorb.	µg/g sorb.
Sponceram				
N-VP-DAAc	205	10	5	0
N-VP-Ac – RNAse (10:1)	205	0	—	0
TCP				
N-VP-DAAc	690	1	2	0
N-VP-Ac-RNase (10:1)	495	7	5	0

* **Reactive ratios of coupled polymer and ligands are pointed in the brackets.**

A sonication step was added to the washing procedure to extract non-adsorbed polymer from the inner porous space of the monolithic material. There was no significant desorption of polymer measured within 14 days at physiological conditions.

8 Microscopy



Toluidin-Blue staining of Saos-2 cells cultured on TCP (A) and Sponceram (B) for 7 days.

Conclusion

The results obtained in this research clearly showed that water soluble biocompatible polymers based on N-vinylpyrrolidone are worthwhile perspectives for the development of composite scaffolds for bone tissue engineering. The copolymer of N-VP with aldehyde bearing monomer seems to be appropriate for the purpose of generating multifunctional solid surfaces triggering intensive bone cell growth.

Acknowledgement

BMP-2 was kindly donated by Professor Sebald, Würzburg (Germany). Saos-2 cells were a gift of CCS, Hamburg (Germany). TCP was kindly provided by Mathys (Switzerland) and Sponceram by Zellwerk, Oberkrämer (Germany).