



Bone Tissue Engineering using functionalized biomaterials and an innovative rotating bed bioreactor system

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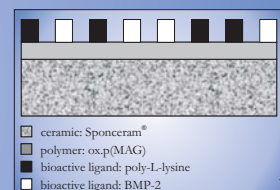
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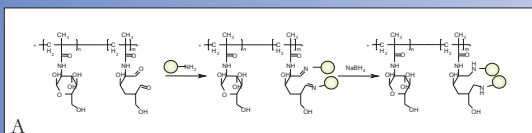
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Introduction

The aim of this study was the development of functional composite biomaterials based on a macroporous zirconium dioxide ceramic (Sponceram[®]) for the application in Bone Tissue Engineering. Two bioactive ligands, poly-L-lysine and BMP-2 were used to enhance the adhesion of seeded cells and to induce their differentiation. In order to avoid loosing of bioactivity caused by high proximity of the ligands to the ceramic surface, a polysaccharide based polymer (oxidized version of N-methacrylamidoglucose; ox.p(MAG)) was used as spacer between ligands and Sponceram[®]. The composite materials consisting of Sponceram[®], polymer and ligands (fig. 1) were tested under static as well as under dynamic cultivation conditions. Therefore, the innovative rotating bed bioreactor platform ZRP[®] was used.

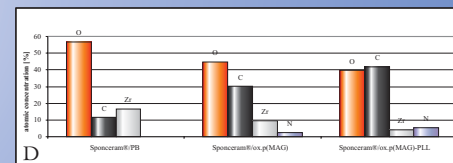
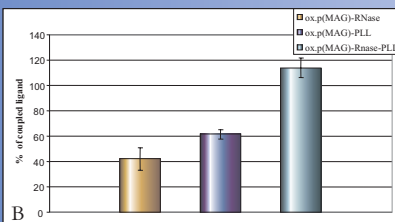
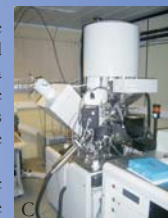


Preparation of composite materials

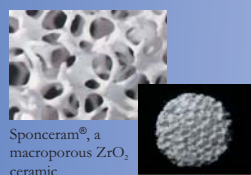


The first step of the composite production was the synthesis of conjugates containing the polymer ox.p(MAG) and one or two bioactive ligands. The used ligands were poly-L-lysine and due to economic reasons RNase as model protein instead of BMP-2. The ligands were coupled via their amino group to the aldehyde group of the polymer at pH 10. In order to get a stable bond the resulting imine group was reduced with sodium borhydrid (fig. A). The coupling procedure was analyzed by labeling the ligands with the fluorescence marker FITC (fig. B) and measuring the fluorescence signal at 485/ 535 nm.

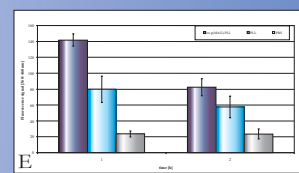
The adsorption of the conjugates onto the ceramic surface was performed at pH 7. The ceramic material Sponceram[®] was incubated into conjugate solution for 24 hours at room temperature. The analysis of the adsorption process was performed using a XPS spectrometer (Axis Ultra DLD, Kratos, Manchester, UK; fig. C). Untreated, in phosphate buffer incubated Sponceram[®] (Sponceram[®]/PB) was measured in comparison to Sponceram[®] incubated into polymer solution (Sponceram[®]/ox.p(MAG)) and Sponceram[®] incubated into conjugate solution (Sponceram[®]/ox.p(MAG)-PLL). The nitrogen peak present at both coated Sponceram[®] samples detects the adsorption of the polymer/conjugate (fig. D).



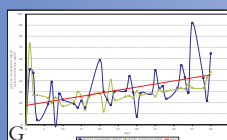
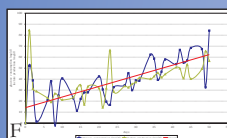
Static cultivation



For the static cultivation, MC3T3 cells were seeded onto small Sponceram[®]/ composite discs (diameter 10 mm, 80.000 cells each discs). 1, 3, 5, 7 and 24 hours after cell seeding, the discs were washed twice with PBS and then incubated in ice cold ethanol for at least 1 hour in order to fix the adhered cells. After completed cultivation, the cells were stained with DAPI. The fluorescence signal of the stained cells were measured in the fluorescence reader at 360/ 460 nm. Untreated Sponceram[®] was compared to Sponceram[®] incubated into PLL solution and Sponceram[®]/ox.p(MAG)-PLL. Obviously, the cell count on Sponceram[®]/ox.p(MAG)-PLL is increased compared to untreated Sponceram[®] and Sponceram[®]/PLL (fig. E).



Dynamic cultivation



The ZRP[®] system used in this study is a rotating bed reactor with a perfusion mode (fig. J). The rotating bed is activated by a magnetic drive without any contact to the scaffolds. The scaffolds were moved through the cell culture medium and the gaseous overlay atmosphere alternately. The reactor is placed into a breeder, which provides a sterile and tempered working space. For the cultivation Sponceram[®] discs with a diameter of 60 mm were used. One disc was incubated into BMP-2 solution and one disc was incubated into solution of ox.p(MAG)-BMP-2. The concentration of BMP-2 was 50 mg/mL in both cases. 2.0×10^6 cells were seeded onto every disc which were then cultivated into two separate ZRP systems. The cultivation was performed for 50 days using standard culture medium (DMEM + 10% FCS + 1% L-glutamine + 1% penicillin/ streptomycin). Glucose and lactate concentration in both reactors were measured every day. After the cultivation, the cell count and the differentiation status were determined using histological staining procedures.

Regarding the glucose/lactat balances of both cultivations it is seen that the cell count on the composite material Sponceram[®]/ox.p(MAG)-BMP-2 (fig. F) is increased compared to the cell count on Sponceram[®]/BMP-2 (fig. G). The histological stainings show that the cells on Sponceram[®]/ox.p(MAG) expressed more alkaline phosphatase (alkaline phosphatase staining, fig. H, left side) and secreted more calcium (alizarin red staining, fig. I left side) than the cells on Sponceram[®]/BMP-2 (right side, reference beneath).



Conclusions

The preparation of composite materials consisting of Sponceram[®], the polymer ox.p(MAG) and the ligands poly-L-lysine and BMP-2 was successful. We showed in static cultivation an enhanced cell count on the composite material compared to untreated Sponceram. Performing a dynamic cultivation procedure in the ZRP system we could determine the positiv effect of the spacer between BMP-2 and the ceramic material which increased the proliferation and differentiation of seeded cells.

Acknowledgement

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