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How to optimize growing techniques of osteoblasts on bone substitute of bovine origin

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Introduction

In reconstructive surgery of craniomaxillofacial defects the application of tissue engineered osteoblasts seeded on 3D-matrices to replace autologous bone grafts is needed. There is still a variety of indications for bone substitutes providing enough stability versus support to reconstruct e.g. calvarial defects or to elevate the maxillary sinus floor. The materials differ in pore size, composition, permeability, durability and pore density. Industrial and individual methods exist to produce such materials. The individual technique of rapid prototyping allows a three dimensional growth.

Material and Methods

Two different materials we used: one native calf spongiosa and the calf spongiosa with collagen. In the first experiment the materials were put into a culture medium over night without any prehandling. After 24 hours 1 x 105 cells were seeded on the materials. Parallel in the second experiment the materials were put in a perfusion culture for 48 hours. 1 x 105 osteoblasts were seeded. We divided into two groups and put one of it into a vacuum exicator for 1 minute. Both groups were put into the CO2 cell incubator like in the other experiment.
After one week the evaluation was made with cell proliferation tests (EZ4U) and the cell colonization was investigated with scanning electron microscope. Additionally we compared sheep and human osteoblasts for these experiments.

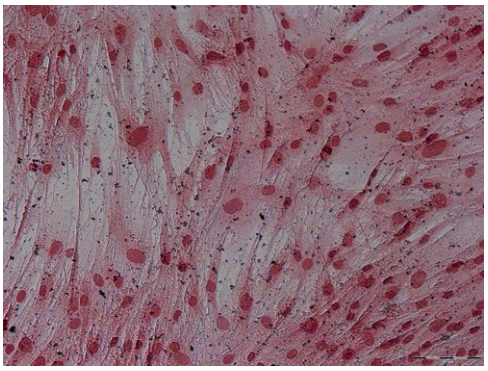
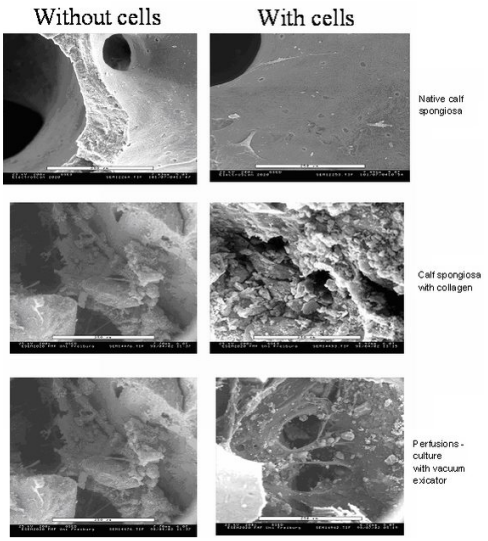


Fig 1: Osteoblasts characterized with Alkaline phosphatase, Collagen Type 1 and osteocalcin.



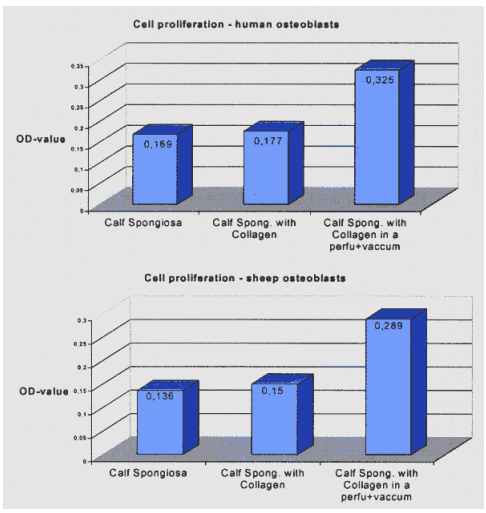


Fig 2: Scanning electron microscopy study of industrial and individually plotted materials: on the left side (Fig A,C) are the collagen matrices and on the right side (Fig B,D) are the PLGA scaffolds produced by rapid prototyping. Only human osteoblasts are shown. They were cultivated and seeded with 1x105 osteoblasts/ml on each material.

Fig 3: Cell proliferation analysis of human (n=10) and sheep (n=10) osteoblasts seeded on different materials. Little difference in cell proliferation between native calf spongiosa and calf spongiosa with collagen was seen. Optimal growth conditions are given on the material with perfusion chamber and vacuum.

Results

This study reveals that the cell proliferation of the human osteoblasts is higher than the cell proliferation of sheep cells. Therefore the growing is nearly similar between the native calf spongiosa and the calf spongiosa with collagen. In both cases there is a big difference (human and sheep) in the use of the perfusion chamber plus vacuum.

Conclusions

As conclusion there was different adhesion and growing pattern of the osteoblasts. The best results were found in the perfusion chamber combined with the vacuum treatment and worst results without prehandling.

Literature

- How to optimise seeding and culturing of human osteoblast-like cells on various biomaterials, Wiedmann-Al-Ahmad M, Gutwald R, Lauer G, Hübner U, Schmelzeisen R, Biomaterials 23(2002) 3319-3328
- Qualitative and quantitative study of human osteoblast adhesion on materials with different surface roughness, Anselme K., Bigerelle M., Noel B. et al., J Biomed Mater Res 2000; 49: 155-66

This Poster was submitted by Dr. Dr. Bettina Hohlweg-Majert.

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Poster Faksimile:

How to optimize growing techniques of osteoblasts on bone substitute of bovine origin

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Introduction:

In reconstructive surgery of craniomaxillofacial defects the application of tissue engineered osteoblasts seeded on 3D-matrices to replace autologous bone grafts is needed. There is still a variety of indications for bone substitutes providing enough stability versus support to reconstruct e.g. calvarial defects or to elevate the maxillary sinus floor. The materials differ in pore size, composition, permeability, durability and pore density. Industrial and individual methods exist to produce such materials. The individual technique of rapid prototyping allows a three dimensional growth.

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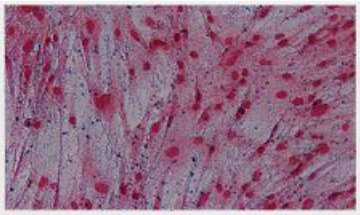

Fig 1:
Osteoblasts characterized with Alkaline phosphatase, Collagen Type 1 and osteocalcin.

Materials and methods

Two different materials we used: one native calf spongiosa (Bio-Oss®; Geistlich, Wolhusen, CH) and calf spongiosa with collagen (Bio-Oss® collagen; Geistlich, Wolhusen, CH).

In the first experiment the materials were put into a culture medium over night without any prehandling. After 24 hours 1 x 10^5 cells were seeded on the materials.

According to the results of the first experiment in a second experiment only calf spongiosa with collagen was prehandled. First the matrix was put in a perfusion chamber for 48 hours.1 x 10^5 osteoblasts were seeded and afterwards put into a vacuum exicator for 1 minute and into the CO_2 cell incubator like the first experiment.

After one week evaluation was made with cell proliferation tests (EZ4U) and cell colonization was investigated with scanning electron microscope. Additionally sheep and human osteoblasts were compared.

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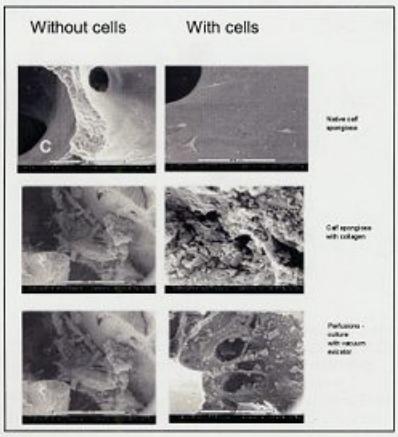

Fig 2:
Scanning electron microscopy study of calf spongiosa is shown. In the first row calf spongiosa, the second calf spongiosa with collagen and the third the prehandling with perfusion chamber and the vacuum is seen. On the right side 1x10^5 osteoblasts/ml were seeded in each case. Only human osteoblasts are shown.

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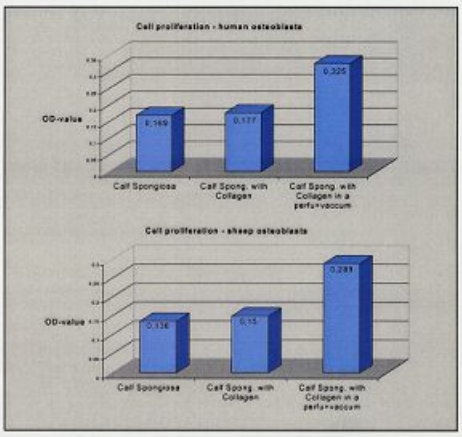

Fig 3:
Cell proliferation analysis of human (n=10) and sheep (n=10) osteoblasts seeded on different materials. Small difference in cell proliferation between native calf spongiosa and calf spongiosa with collagen was seen. Optimal growth conditions are given on calf spongiosa with collagen prehandled in a perfusion chamber and vacuum.

Results:

Different adhesion and growing patterns of osteoblasts could be observed. The growing is nearly similar between native calf spongiosa and calf spongiosa with collagen. This study reveals optimal growing conditions with nearly double cell proliferation (OD-Value = 0,325) by using perfusion chamber combined with vacuum treatment. Minor values were observed without prehandling (OD-Value = 0,177). In addition human osteoblasts showed higher cell proliferation as sheep osteoblasts.

Conclusion:

Using biomaterials the growing conditions should be tested with different preconditions to obtain better results in vivo.

Literature:

How to optimise seeding and culturing of human osteoblast-like cells on various biomaterials. Wiedmann-Al-Ahmad M, Gutwald R, Lauer G, Hübner U, Schmelzeisen R. Biomaterials 23: 3319-3328, 2002

Qualitative and quantitative study of human osteoblast adhesion on materials with different surface roughness. Anselme K., Bigerelle M., Noel B. et al. J Biomed Mater Res. 49: 155-166, 2000