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Bone Engineering: Allogenic and Alloplastic Bone Transplants vitalized by Osteoblast-like Cells

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Introduction

The search for suitable techniques and materials for the reconstruction of bone defects is a primary goal in many clinical disciplines. Implants made of synthetic polymers, ceramics or metals as well as allogenic materials like collagen or cartilage are used for bone grafting. Up to now no grafting material exists with the quality of the original tissue. These artificial materials show problems in anchoring and mechanical stability or induce immunological reactions. A new approach in therapy is the application of tissue engineered bone grafts. The possibility of cell culturing in vitro and the exclusive use of endogenous cells opens the way for a "self cell therapy" and thus avoids problems like limited resources. Additionally, the risk of donor site morbidity is decreased because only small biopsies have to be harvested. In this study, we focused on the search for a biomaterial which represents a suitable matrix for three-dimensional growth of human osteoblast-like cells in vitro and for the surgical management of intraoral applications.

Material and Methods

Human osteoblast-like cells were cultured on two different biomaterials: a human demineralised bone matrix (DBX® Mix, Musculoskeletal Transplant Foundation, NJ, USA, distributed by symthes) and a non-sintered, nanocrystalline, phase-pure hydroxylapatite (Ostim® Paste, Heraeus Kulzer, Hanau, Germany). Cortico-lamellar bone was obtained during dental surgery. Opti-minimal-essential-medium (Opti-MEM, Gibco Life Technologies, NY, USA) was used for primary culture with 10% fetal calf serum (FCS, PAA Laboratories, Linz, Austria), 2% HEPES (Gibco Life Technologies, NY, USA) and the antibiotics penicillin (1%) and streptomycin (1%, PAA Laboratories, Linz, Austria). The confluent primary osteoblasts were detached from the culture flask by incubation with 0.5% trypsin (PAA Laboratories, Linz, Austria) in phosphate buffered saline (PBS) for 5 min at 37°C. The cells were filtered through a 100µm cell-strainer (Falcon, NJ, USA) in a 50ml tube (Falcon, NJ, USA), centrifuged and resuspended in 1ml fresh medium RPMI 1640 (Gibco Life Technologies, NY, USA), supplemented with 10% FCS, 2% HEPES, penicillin (1%) and streptomycin (1%). The osteoblasts were transferred into a 75cm2 culture flask (Falcon, NJ, USA), filled up with 30ml culture medium. After 2-3 weeks, the cells were trypsinized again from the culture flask, centrifuged and resuspended in 1ml medium. The cells from the first passage were seeded on the two different biomaterials. An aliquot of the same passage was seeded in cell culture plates and served as control of the cell proliferation. Additionally, plates with 1x 105 cells/ml were incubated for 1 week for the detection of alkaline phosphatase and collagen. Cell cultures were kept in a humified atmosphere of 5% CO2 at 37°C. For the staining of osteoblast-like cells an alkaline phosphatase assay kit (Sigma, Deisenhofen, Germany) was used. The evaluation of collagen type-I was done by light microscopy and the computer program Analysis 3.1 after immuno-staining with anti-collagen I antibody (Sigma, Deisenhofen, Germany). Osteocalcin was analysed using a competitive EIA kit (Osteomedical, Bünde, Germany) and an ELISA-Reader (Anthos Labtech, Salzburg, Austria). For cell proliferation analysis, the nonradioactive assay EZ4U (Biomedica, Wien, Austria) was used. The cell vitality was evaluated by fluorescence microscopy and a dichromogenic PI/FDA-staining. For the cell colonization analysis the samples were examined by scanning electron microscopy at 15 kV.

Results

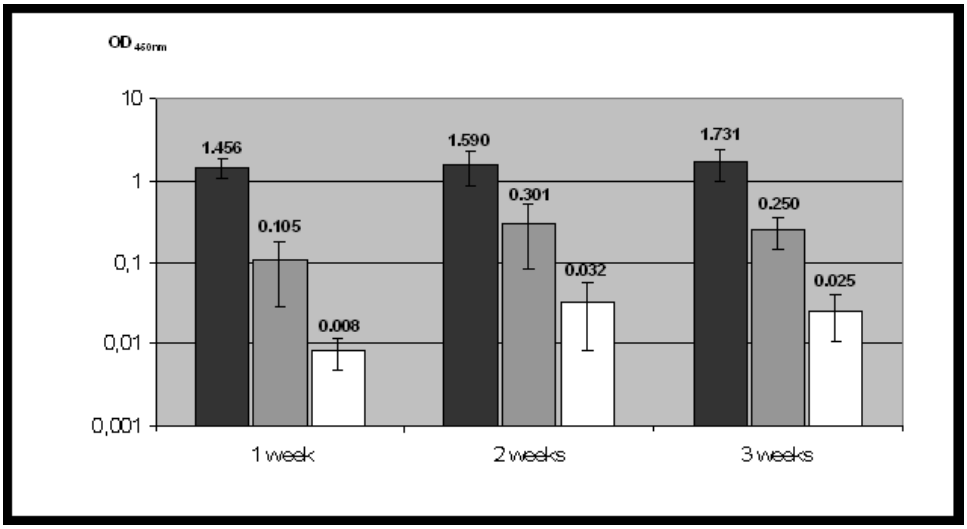


Fig. 1. Cell proliferation analysis of human osteoblast-like cells in cell culture (black) and seeded onto DBX® (gray) respectively onto Ostim® (white).

All cell culture supernatants of human osteoblast-like cells examined were osteocalcin positive with approximately 10 ng/ml osteocalcin and the alkaline staining of these cells typically resulted intensively positive (about 36.9%). Immuncytochemistry of the fixed cells showed the presence of collagen type-I in about 10.5% of the cells. Osteoblast-like cells seeded onto the human demineralised bone matrix (DBX®) showed a ten times higher rate of proliferation capacity than the cells cultivated on hydroxyapatite Ostim® (Fig.1). After 3 weeks of cultivation the vital cells migrated over the biomaterial and a beginning vitalization could be observed on DBX® (Fig.2). The surface of Ostim® was sparsely covered by human osteoblast-like cells after 3 weeks of cultivation indicating that there is no vitalization in vitro (Fig.3). Thin sections of the demineralised bone matrix (DBX®) showed a multilayered growth of human osteoblast-like cells already after 2 weeks of cultivation (Fig. 4). In comparison, Fig. 5 shows thin section of osteoblasts after a period of two weeks grown on Ostim®. Scanning electron microscopy after 3 weeks of cultivation on DBX® a dense network of multilayered polygonal shaped cells could be observed (Fig. 6). Fig. 7 shows an isolated and scattered growth of osteoblast-like cells upon Ostim®.

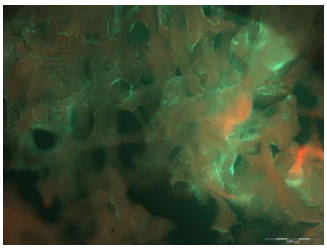


Fig. 2. Fluorescence microscopy after PI/FDA-staining of human osteoblast-like cells after cultivation for three weeks on DBX® (magnification 25x).

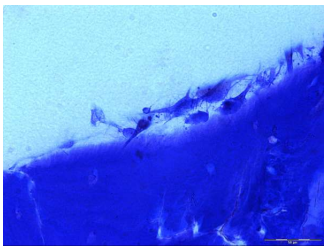


Fig. 4. Thin section of human osteoblast-like cells after a cultivation period of two weeks on DBX® (magnification 500x). (Toulidinblue)

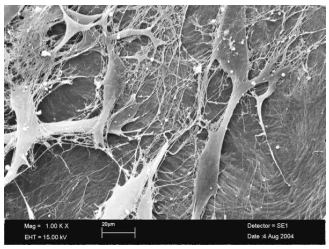
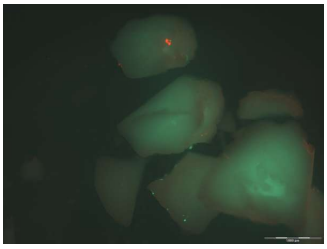
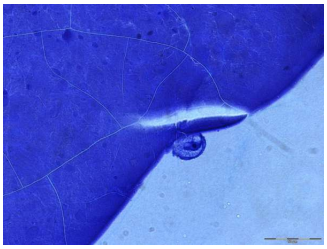


Fig. 6. Scanning electron microscopy of human osteoblast-like cells cultivated three weeks on DBX® (magnification 1000x).





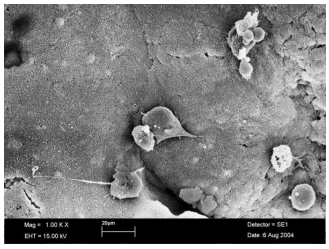


Fig. 3. Fluorescence microscopy after PI/FDA-staining of human osteoblast-like cells after cultivation for three weeks on Ostim® (magnification 25x).

Fig. 5. Thin section of human osteoblast-like cells after a cultivation period of two weeks on Ostim® (magnification 500x).

Fig. 7. Scanning electron microscopy of human osteoblast-like cells cultivated three weeks on Ostim® (magnification 1000x).

Conclusions

The topographic structure of the biomaterial surface could be a reason for different proliferation rates. Anselme (2000) described the decisive role of surface roughness, chemistry or surface energy regarding cell adhesion, cell migration or cell proliferation upon biomaterials. The mitogene effect of demineralised bone matrix can be attributed to the existence of various growth factors in the bone matrix, such as BMP's (Urist 1965). Wozney et al. (1992) showed that BMP's, belonging to the TGF-superfamily, are activated by the process of demineralization. Furthermore, Zhang et al. (1997) described that BMP's are directly bound to the bone mineral and the demineralization process release them, indicating a proportional connection between the demineralization level, the accessible BMP's and the osteoinductive effect. Further in vivo studies are necessary to examine if the present in vitro results correspond with the in vivo conditions. In future, it appears conceivable to produce made-to-measure and biological integrative biomaterials in combination with autologous cells. Pradel et al. (2006) clinically applied demineralized bone matrix (Osteovit, Braun, Melsungen, Gremany) cultured with osteoblasts in mandibular cysts. Nonetheless, further research with regard to the clinical application of such biomaterial/cell constructs are of essential importance for the further development of bone engineering.

Literature

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Poster Faksimile:

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[1] Department of Oral and Craniomaxillofacial Surgery, University Hospital Freiburg, Freiburg, Germany

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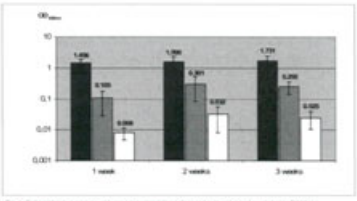

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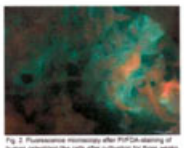

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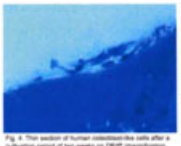

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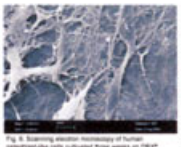

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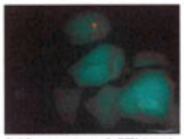

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