

Bone Engineering: Allogenic and Alloplastic Bone Transplants vitalized by Osteoblast-like CellsM. Hinze¹, S. Sauerbier¹, M. Wiedmann-Al-Ahmad¹, U. Hübner¹, R. Schmelzeisen¹, R. Gutwald¹¹ Department of Oral and Craniomaxillofacial Surgery, University Hospital Freiburg, Freiburg, Germany**Background and 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.

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. 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. Osteocalcin was analysed using a competitive EIA kit and an ELISA-Reader. For cell proliferation analysis, the nonradioactive assay EZ4U 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.

Results:

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%). Immunocytochemistry 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®.

Discussion and Conclusion:

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 mitogenic 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, Germany) 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.

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