

**OSTEOBLASTS RESPONSE TO BONE SUBSTITUTES *IN VITRO***

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**INTRODUCTION:**

The repair of large osseous defects still represents an unsolved problem in bone surgery. The use of autogenous bone grafts is widely accepted and considered to be the “golden standard” in the treatment of bone defects. However, the disadvantages of autogenous bone grafts are limited availability, harvesting morbidity, and insufficient biomechanical properties. These problems with autografts have initiated the development of several allogenic, xenogenic, and synthetic bone graft alternatives. Their complication rate due to interaction between biomaterials and host tissues could be reduced. Still cell-mediated immune responses, as well as synthesis and resorption processes by osteoblasts and osteoclasts respectively are not yet fully controllable. The main problem in clinical use remains osteoclastic resorption and remodelling, representing a crucial issue of long term mechanical stability. Furthermore, vascularisation and involvement of neuronal fibres and neuropeptides are very important for the development, growth, and differentiation of bone cells and matrix.

The present investigation has focused on the biocompatibility of allogenic and xenogenic solvent dehydrated cancellous bone (SDCB) with phenotypic osteoblasts *in vitro*.

**MATERIAL AND METHODS:**

**Allogenic and Xenogenic Solvent Dehydrated Cancellous Bone (SDCB)** from human and bovine femoral necks were prepared, 14 mm diameter and 3 mm thickness to fit into the 24 well plates, for our experiment *in vitro*.

**Human osteoblasts** were harvested from the cancellous bone (iliac crest), obtained under sterile conditions from patients undergoing ORIF with bone grafting. Specificity of the primary human osteoblast was controlled by biochemical marker of osteoblasts **alkaline phosphatase (ALP)** and **Osteocalcin (OC)**. All patients provided informed consent.

**Cell proliferation:** Cytotoxicity and cell proliferation were determined by the **MTT-Test**.

The MTT [3-(4,5 dimethyl-thiazol-2-yl)2,5-diphenyltetrazolium bromide]- based on colorimetric assay as described by Mosmann.

**Osteocalcin:** OC was measured by a non-radioactive ELISA kit (Dako, Switzerland) in conditioned medium, which was collected on day 1, 3, and 7 and frozen at minus 80°C.

**Reverse Transcription Polymerase Chain Reaction (RT-PCR):**

RNA for Osteocalcin (OC) and Alkaline Phosphatase (ALP) was extracted from confluent primary human osteoblasts cultured on human and bovine SDCB and as control the cells without SDCB discs with an Qiagen RNase Kit (Hilden, Germany). As control we used the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The amplification primer sets for PCR were from the NCBI nucleotide database.

**Cell Morphology:** For SEM cells seeded discs were fixed with 2.5% glutaraldehyde (pH 7.3) on day 7, 14, and 21.

**RESULTS:** There was no significant difference by the MTT-Test between the human and bovine discs compared to control without discs. Osteocalcin level was significantly lower with human discs on day 3, and increased on day 7. In RT-PCR, **ALP** and **OC** gene expression did not show difference compared to control. SEM showed completely homogenous distribution of the cells and were growing into the pores to integrate throughout the scaffold.

**CONCLUSION:**

In conclusion, our *in vitro* observations suggest that SDCB may be a suitable bone substitute which provides a well structured and biocompatible scaffold for ingrowing human osteoblasts.