

Phenotypic Osteoblasts for *in vitro* Testing of Bone Implants and Substitutes

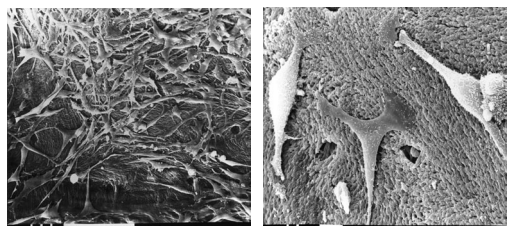
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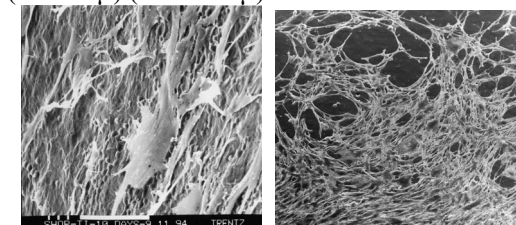
INTRODUCTION: The treatment of osseous defects remains an unsolved problem. Autogenic bone grafts represent the state-of-art treatment of bone defects. However, disadvantages of autogenic bone grafts include limited availability, harvesting morbidity, and insufficient biomechanical properties. These problems have initiated the development of several allogenic, xenogenic, and synthetic bone graft alternatives. Still, related cell-mediated immune responses, as well as synthesis and resorption processes by osteoblasts and osteoclasts are not yet fully controllable. Bone remodelling is a result of the balance between osteoclastic resorption and osteoblastic synthesis, clinically representing a crucial issue as to 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 [1]. In the present *in vitro* study we investigated the feasibility to culture osteoblasts of various origins on synthetic, xenogenic, and metallic materials.

METHODS: Cells: Mouse embryonic cells (MC3T3-E1), Primary Human Osteoblasts (hOB)

Implant Materials: Metals: Titanium (c.p. Titanium ISO TC150 3832/2), Stainless steel (ISO TC150 3832/1). Bone Substitutes: Xenogenic Solvent Dehydrated Cancellous Bone, Synthetic Bone Substitutes Hydroxylapatite. Methods of Analysis: MTT-Test [2], Alkaline phosphatase (ALP) and Osteocalcin (OC) level were measured on day 1, 3, and 7 after culture. Additionally Scanning Electron Microscopy (SEM) was performed on day 7.



MC3T3-E1+Hydroxylapatite (SEM10 μ) hOB + Hydroxylapatite (SEM 100 μ)



hOB + Titanium (SEM 100 μ) hOB + stainless steel (SEM 100 μ)

Fig 1: SEM of *in-vitro* osteoblasts on different biomaterials.

RESULTS: MTT-Test: Cytotoxicity and cell proliferation did not show significant differences between the various cells and implant materials. ALP

level was significantly lower regarding human osteoblasts cultured on stainless steel and Hydroxylapatite (Table 1). OC production of hOB was significantly lower on Hydroxylapatite [3]. MC3T3-E1 cells showed comparable growth on all investigated materials. Primary human osteoblasts (hOB) showed different growth on all biomaterials. Best results were obtained on titanium.

MC3T3-E1	24 hours	72 hours
Titanium	89.7 \pm 2.7	82.0 \pm 3.8
Steel	*77.7 \pm 1.5	*58.3 \pm 2.0
HA	81.7 \pm 4.1	*27.7 \pm 1.2
hOB	24 hours	72 hours
Titanium	104.7 \pm 7.7	108.7 \pm 9.8
Steel	*77.7 \pm 1.5	*75.7 \pm 1.8
HA	*80.3 \pm 2.3	*40.3 \pm 3.2

Table I: ALP 24 & 72 hrs after culture * p < 0.05

CONCLUSION: Transplantation of cultured bone cells grown on biomaterials represents a promising concept for trauma and orthopaedic surgery. However, appropriate materials are necessary for efficient cell scaffolding. The present results suggest that primary human phenotypic osteoblasts represent the most appropriate model system to evaluate the biocompatibility of bone substitutes *in vitro* [4]. Moreover, the related osteoblast markers (Osteocalcin, ALP, ProCollagen Type I) are expressed more adequately compared to human osteoblast cell lines.

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