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EFFECT OF HIGH HYDROSTATIC PRESSURE ON BONE TISSUE – A NEW WAY OF PROCESSING ALLOGENIC GRAFTS

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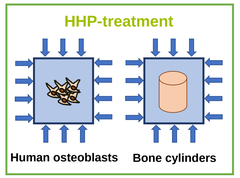
INTRODUCTION

Autologous bone still remains the gold standard to reconstruct large bone defects. However, limitations are harvest morbidities and tissue capacity. Other therapeutic approaches such as allogenic tissue are not limited and the low potential of immunological response is achieved by conventional chemical/physical processing methods. Though, these are associated with a significant reduction in biomechanics. Therefore, the use of High Hydrostatic Pressure (HHP) could be a gentle alternative to achieve good biocompatibility while preserving matrix integrity. In order to estimate the effect of HHP on bone, a specific pressure protocol was established by treating human osteoblasts (hOBs) with different HHPs. Then, this protocol was applied on human bone cylinders.

AIM

The aim was to determine the devitalization potential of HHP on hOBs. Afterwards, an optimized HHP protocol was applied on bone cylinders to determine mechanical properties and tissue morphology.



RESULTS

Cell Experiments with Human Osteoblasts

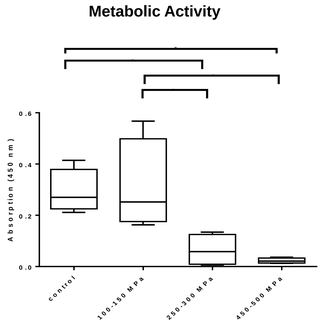


Figure 1. Metabolic activity of human osteoblasts (n = 4) following treatment with different HHPs ranging from 100-150 MPa, 250-300 MPa and 450-500 MPa. After HHP exposure, cells were incubated at 37 °C and 5 % CO_2 over a period of 24 h. Subsequently, metabolic activity was determined with water-soluble tetrazolium salt (WST-) 1 assay. Data are presented as boxplots. Significance between stimulation groups was determined via One Way Anova: * p ≤ 0.05.

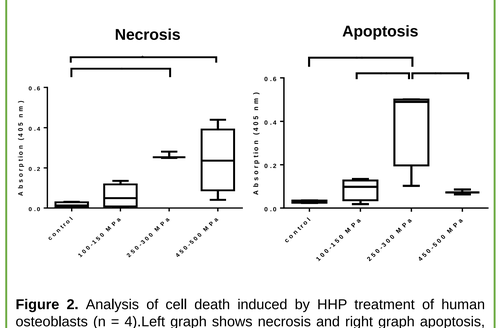


Figure 2. Analysis of cell death induced by HHP treatment of human osteoblasts (n = 4).Left graph shows necrosis and right graph apoptosis, both detected via Cell Death Detection ELISA. After HHP treatment, the cells were centrifuged at 118 x g for 8 min and the supernatants were collected for necrosis detection. The residual cells were lysed 30 min with 200 µl lysis buffer provided by the kit. After centrifugation at 118 x g for 8 minutes, supernatants were collected for apoptosis detection. All data are presented as boxplots. Significance between groups was determined via One Way Anova. * p ≤ 0,05; ** p ≤ 0,01.

Experiments with Human Trabecular Bone Cylinders

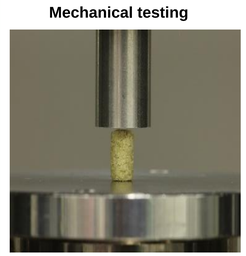


Figure 3. Test setup for the uniaxial unconfined compression test for trabecular bone cylinders.

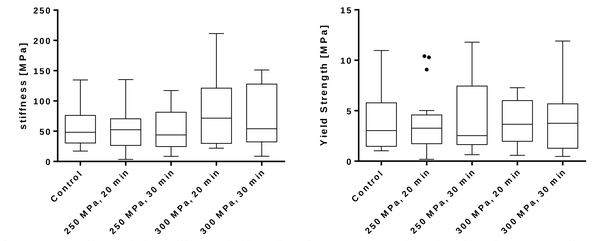


Figure 4. Analysis of the stiffness (left) and Yield Strength (right) of trabecular bone cylinders treated with HHP. Mechanical properties were tested by a uniaxial compression test. Data are shown as boxplots with median and interquartile ranges from 25-75 %. Statistical analyses were performed by a One Way Anova. Control group (n = 20); 250 MPa, 20 min (n = 18); 250 MPa, 30 min (n = 19); 300 MPa, 20 min (n = 16); 300 MPa, 30 min (n = 14).

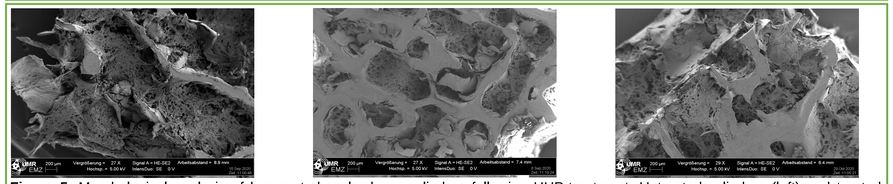
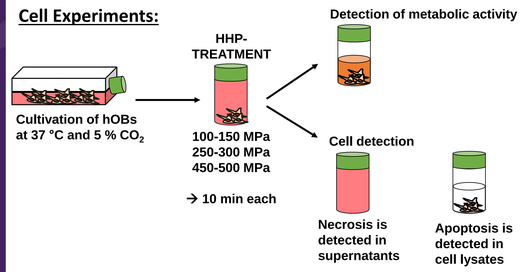


Figure 5. Morphological analysis of human trabecular bone cylinders following HHP-treatment. Untreated cylinders (left) and treated cylinders (centre: 250 MPa, 20 min; right 300 MPa, 30 min were fixed to carry out field emission electron microscopy. Scale bar: 200 µm.

METHODS

Cell Experiments:



Tissue experiments: Human trabecular bone cylinders (Ø = 6 mm, length = 10 mm), harvested from distal epiphysis of femora were treated with HHP (250-300 MPa) for 20 min and 30 min, respectively. An uniaxial compression test was performed to analyze stiffness and yield strength. Morphology of the extracellular matrix was evaluated by field emission electron microscopy.

CONCLUSIONS

With increasing HHP the metabolic activity of hOBs decreased significantly. However, the range of 100-150 MPa had only little effect on the cells compared to the control group. The highest HHP induced high levels of necrosis, whereas HHP of 250-300 MPa induced both apoptosis and necrosis. This result indicated that the risk of necrosis-associated inflammatory responses can be influenced by differing HHP ranges. An optimal devitalization of bone cells could thus be determined at 250-300 MPa. Applying this HHP on bone cylinders and performing an uniaxial compression test, neither the stiffness nor the yield strength was reduced in comparison to the control group. It can be assumed that ECM-proteins, important for biomechanics, are not damaged by HHP-treatment which is supported by electron microscopic images. **HHP-treatment of bone tissue appears to be a convenient alternative to existing chemical or physical methods to provide tissue replacement materials.**

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