Osteochondral chips out of predifferentiated human BMSC stimulated by mechanoperfusion techniques

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INTRODUCTION

In tissue engineering of osteochondral constructs biomaterials composed of biological or synthetic matrices and bone marrow stromal cells (BMSC) are promising approaches (2). Homogenous cell distribution and sufficient initial scaffold stability remain key issues next to a proper cell phenotype in a successful multidisciplinary strategy. The purpose of this study was to examine whether a chondrogenic medium and/or mechanical stress is necessary or not to differentiate human BMSC in a 3-D osteochondral matrix stimulated in a bioreactor system.

METHODS

Human BMSC were harvested from the iliac crest during routine trauma surgery of vertebral fractures (18 healthy donors, age 19-43 y). All procedures were approved by the institutional ethical committee, and informed consent was obtained from all donors. Briefly, density centrifugation was used to obtain a cell pellet that was resuspended in culture media (DMEM/Ham’s F12 1:1, Biochrom) supplemented with 10% fetal calf serum, 200 U/ml penicillin/streptomycin (Gibco), 2.5 µg/ml amphotericin B (Biochrom), supplemented with FGF-2 (3 ng/ml, Pepro Tech) buffered with Hepes buffer (Roth, pH 7.0), and subsequently plated in 175 cm² culture flasks and incubated at 37°C and 5% CO2 in humidified atmosphere. The medium was changed 3 times a week. After reaching confluence at day 14-21, the cells were released with 0.25% trypsin (Gibco), counted and subcultured. Cells of the third passage were used for the experiments. For 7 days a 2-dimensional predifferentiation culture was initiated by replacing FGF-2 with 100 ng/ml IGF-1 and 5 ng/ml TGF-b2. After expansion cells were then seeded into the biologic hybrid scaffold with a concentration of 1x10⁶ cells per ml. The osteochondral matrices consisted of commercially available products: CaReS® (rat collagen I, Ars Arthro, Esslingen, Germany) and Tutobone® (bovine spongiosa, Tutogen Medical GmbH, Neunkirchen a. Br., Germany). Continuous pressure and vacuum forces were applied in a specially developed glass kit. The constructs were exposed to a cyclic compression protocol (10 % compression at 0.5 Hz) under continuous perfusion in a mechnano-bioreactor for 14, 21 and 28 days (fig.2). Controls were uncompressed constructs and static group. Effects were evaluated using light microscopy after standard staining (HE, toluidine blue) to identify matrix penetration as well as collagen 2, 3, and 10 expression. GAG (Blyscan Glycosaminoglycan, Biocolor, Newtonabbey, UK) and DNA (DNA Quantification Kit, Sigma-Aldrich, Taufkirchen, Germany) were quantified using standard kits for photometry. Preliminary biomechanical characterization was conducted using a confined compression quasi-static loading setup (at 0.5 mm/sec). Size (length/diameter) of the harvested cylinders (nominal diameter 6 mm) was measured using a contactless laser micrometer. Load take-up (toe-in region) and modulus of the constructs were evaluated for the matrix constructs. At least six experiments were performed in each group on each day. Statistical analysis was performed by the unpaired t-test and the Wilcoxon test using SPSS (Version 11.0, SPSS Inc., Chicago, IL, USA).

RESULTS

Penetration and cell distribution was demonstrated homogenous and vital over time (83±6.3%). DNA quantification showed no significant differences over time and different stress patterns. For GAG quantification significant differences were observed after three and four weeks compared to the static control (p<0.05). The different stress patterns had only little influence on differences between groups. Mechanical tests showed no significant difference over four weeks, but the mechanical group was stiffer compared to the static control (p<0.05). The quality of tissue was not improved by IGF and TGF in our system focusing histology, biochemistry and mechanical properties of these tissues. The tissue was demonstrated to look cartilage-like in histology.

DISCUSSION

There have been numerous attempts to repair osteochondral injuries and tissue engineering may provide a breakthrough to treat those injuries (1). The aim of this project was to investigate the response and cell distribution of predifferentiated human bone marrow stromal cells seeded on a 3-dimensional biologic hybrid scaffold using hydrostatic compression and vacuum forces. The integration of mechanical stimulation in the tissue engineering process lead to a progress in the structural and biomechanical properties of these tissues. However media supplements to foster the quality of the tissue showed no progress in our system although it is well known that those are important to induce a chondrogenic phenotype. It could be postulated that those could be left out in such a system which would be an advantage with a view on clinical applications and certification process. To minimize assay variations caused by cell heterogeneity, we combined cells derived from several subjects, but the small sample size is a limitation in this study. However, at least tendencies were demonstrated. Further studies need to clarify influence of stress patterns. The system could offer new possibilities in the management of articular injuries and degenerative diseases.

REFERENCES
