MECHANICAL STRENGTH AND MINERALISATION OF THE SUBCHONDRAL BONE PLATE OF THE HUMAN PATELLA

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Purpose: The subchondral bone plate is a dynamic component that shows a functional adaptation to long-term loading history. This can be seen in the density distribution of the subchondral bone plate. In the human patella findings obtained with the photoelastic model show stress maxima in the lateral facet, which agrees with results showing the region of maximum bone density to be constantly found on the lateral facet. The visualization of the density distribution patterns has been generated with the help of CT-osteosorptiometry (CT-OAM), a method for densitometric evaluation of CT-scans according to the Hounsfield units (HU). Recent tests on human humeral heads showed that the density distribution of the subchondral bone plate correlates to the mechanical strength of it. As for the human patella, structural studies on subchondral bone strength only focussed on the subchondral trabecular bone, the subchondral bone plate has not been addressed.

The aim of this study was to look at the density distribution of the human patella in correlation to the mechanical strength of the subchondral bone plate. We hypothesise that the mineralisation shown with CT-OAM and the mechanical strength of the subchondral bone plate correlate.

Methods: 20 patellae were collected from human cadavers, and measurements were performed at 34 coordinate points for each.

To visualize the density distribution patterns, the CT data of the patellae were evaluated with the help of ANALYSE 8.1 (Mayo Foundation, Rochester, MN, USA). Using a “maximum intention projection”, the maximal dense value of the subchondral bone plate was projected to the surface and presented in a false-colour diagram assigning false colours to every 100 HU (Fig. 1A). Density measurements were taken at the defined coordinate points and recorded in a standardized grid system.

To determine the mechanical strength, an indentation test machine (Synenergy 100, MTS Systems, Eden Prairie, MN; 2 kN loadcell) was used. A steel needle (ø 1.3 mm) created a standardised hole of 7 mm depth (1 mm/sec) at the same coordinate point the density measurement was made. The penetration forces as well as the maximum force were recorded in a standardized grid system with its corresponding coordinates and visualised (Fig. 1B). Linear regression was used to evaluate statistical correlations (Fig. 2).

Results: We show that neither the mineralisation nor the mechanical strength of the subchondral bone plate is distributed homogeneously on the patella. The maximum values consistently showed to be on the lateral facet (Fig. 1). A linear correlation was found between the density distribution and the mechanical subchondral bone plate strength (Fig. 2). The coefficient of correlation (range: 0.89 to 0.97; mean 0.92) was significant.

Conclusions: A direct relationship between the subchondral bone plate density and the mechanical strength could be expected. Since the subchondral bone plate adapts to its mechanical needs, areas of high load transmission increase the strength of the subchondral bone plate by osteoblastic calcium deposition. This increase in calcium is presented in the density distribution patterns. The correlation of mineralisation and mechanical strength makes CT-OAM a valuable tool to determine the strength of the subchondral bone plate in vivo.

MODULATION OF GENE EXPRESSION IN HUMAN SUBCHONDRAL BONE CELLS CO-CULTURED WITH HUMAN ARTICULAR CHONDROCYTES.

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Purpose: To investigate modulation effects in gene expression in a coculture model of human bone cells and human condrocytes from osteoarthritic (OA) and normal (N) joint tissue.

Methods: Human bone cells from the subchondral plate (hSBCs) and human articular chondrocytes (hCHs) were used. Cells were isolated from knee tissue discarded during joint replacement surgeries using standard procedures. For the co-culture experiments hCHs were cultured on the membrane of BD Falcon culture inserts while hSBCs were cultured in the wells of companion plates. In this way both cells types are co-cultured without physical contact but allowing paracrine interactions. Three different combinations were tested, namely, normal (N) hSBCs co-cultured with N hCHs, N hSBCs co-cultured with OA-hCHs and OA-hSBCs with OA-hCHs.

After 7, 14 and 21 days of co-culture, total RNA was extracted using Trizol® following the manufacturer instructions. qRT-PCR analyses were performed to study the effects on gene expression in the hSBCs due to the co-culture with hCHs. In particular, we looked at the expression of alkaline phosphatase (ALP), osteocalcin (OCN), collagen type I (COLI), osteoprotegerin (OPG) and RANKL in the hSBCs.

Results: hSBCs monocultures: Differences were found in gene expression between the N and OA hSBCs. ALP expression increased progressively from day 7 to day 21 in both normal and OA hSBCs monocultures up to about 4 fold with respect to the basal cells expression. OCN gene was similarly expressed in normal and OA cells in monoculture at 7 days, but its expression was markedly increased in the OA cells at both 14 and 21 days with respect to the normal cells (15.5 and 16 fold increase, compared to 4.5 and 2.8 fold increase, respectively). Conversely, COLI gene expression was reduced in the OA cells with respect to the normal cells (78 vs. 14.9) at 21 days of culture. Additionally, while in the N hSBCs OPG increased up to 22 fold at 14 d and was moderately reduced to 18 fold at 21 d, in the OA hSBCs, OPG increased only to 8 fold at 14 d and was further reduced to 5 fold at 21 d. Finally, both N and OA hSBCs RANKL gene expression was downregulated at 7, 14 and 21 d with respect to the basal cells expression.

Co-cultures with normal or OA chondrocytes: ALP, OCN and COLI gene expression in both normal and OA hSBCs was reduced at 21 d when these cells were co-cultured with OA hCHs compared to the expression in N hSBCs co-cultured with N hCHs. OPG gene expression was higher in hSBCs co-cultured with OA hCHs at 14 d but this effect was inverted at 21 d (with respect to the co-culture with normal hCHs). In the case of RANKL, while in N hSBCs co-cultured with N hCHs expression was downregulated for all the time points investigated as was the case in the N hSBCs monoculture, in the hSBCs cultured with OA hCHs RANKL expression was increased in both N and OA cells at 14 d and in the OA hSBCs this effect was also seen at 21 d.

Conclusions: Co-culture of hSBCs from either normal or OA tissue with hCHs from OA tissue modulated the expression of ALP, OCN, COLI, OPG and RANKL genes. These results suggest that paracrine signals from condrocytes in OA might be capable of regulating gene expression of relevant
osteoblastic genes involved in bone matrix formation and remodelling in both normal and OA hSBCs cells.

EVALUATION OF BONE REGENERATION FOLLOWING CONCENTRATED AND NON-CONCENTRATED AUTOLOGOUS BONE MARROW TRANSPLANTATION IN A RABBIT OSTEONECROSIS MODEL

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Purpose: Osteonecrosis of the femoral head is also known as ischemic necrosis. We used concentrated autologous bone marrow aspirate transplantation (CABMAT) to treat this disease, obtaining good clinical results. Using autologous bone marrow transplantation, Sakai S et al reported that the concentrations of fibroblastic colony-forming-unit (CFU-F) were approximately 5-fold higher than in the concentrated bone-marrow aspirates. Ogawa T et al observed bone regeneration following drilling in a necrosis model using the fourth tarsal bone of rabbits. Using a femoral head necrosis model, Zuoqin Yan et al reported differentiation of osteoblasts following mesenchymal stem cell transplantation, contributing to bone repair. The CABMAT has become a new treatment option for previously difficult to treat cases of femoral head necrosis. However, it has not been confirmed if it leads to restoration of the biological and biomechanical properties of necrotic osteochondral tissue. We therefore conducted this experiment to examine the histological changes following transplantation with concentrated and non-concentrated bone marrow aspirate in necrotic bone tissue.

Methods: Using 36 12 week-old adult female (bones=72) Japanese white rabbits, we compared the effects of concentrated bone marrow transplantation (CM), bone marrow transplantation (M), and drilling (D). In Group D, a hole was drilled in three places along the cortical bone, and a 2 mm diameter Kirschner wire was inserted under the skin following immersion in liquid nitrogen. In Group M, bone marrow (3 ml) was obtained from the iliac and injected into the holes. In Group CM, concentrated marrow was obtained from bone marrow (20 ml) collected by a syringe containing acid citrate dextrose (ACD) solution (2 ml) from the iliac, which was then injected into the holes. We sacrificed three rabbits (bones=6) in each group 2, 4, 8, and 12 weeks after transplantation and their bones (the fourth tarsal bone) were evaluated for temporal changes. A calcine fluorochrome label (20 mg/kg body weight) was injected subcutaneously 1 week and 2 days before the animals were killed. The bones were embedded in resin to create hard tissue specimens and staining (toluidine blue, tartrate-resistant acid phosphate (TRAP), and alkaline phosphatase (ALP)) was applied. By measuring the fluorescence-labeled trabecular surface, newly formed bone was calculated as a percentage of the surface area of all trabecular bone. We measured the number of colony forming units, platelet count (PLT), and the number of bone marrow cells to evaluate the concentration of bone marrow.

Results: We observed an increase in bone formation at 4 and 8 weeks, but decrease at 12 weeks (Fig. 2). TRAP staining showed there was an increase in the number of multinucleated osteoclasts after 4, 8, and 12 weeks (Fig. 3). The concentration rate of transplanted bone marrow was more than double that of the average. Conclusions: In our experiment, concentrating the bone marrow increased the number of transplanted mesenchymal stem cells, which enhanced the bone regeneration capacity. We also confirmed that early bone formation and resorption was greater in bones of rabbits in the concentrated bone marrow transplantation group than in those in the non-concentrated group.