that CM is able to increase subchondral bone mineral density (BMD) in an experimental model of osteoporosis.

**Objectives:** To evaluate if CM could prevent the subchondral BMD alterations induced by OA, in association to an improvement in synovial membrane inflammation and cartilage damage in an OA model in rabbits.

**Methods:** Ten male New Zealand rabbits were submitted to knee surgery to induce OA by transection of anterior cruciate ligament. CM was performed using the chiropractic adjusting instrument ActivatorV 3 times a week during 8 weeks as follows: Force 2 setting was applied onto the tibial tubercle of the rabbit right hind limb (CM-OA group), at an angle of 90°, whereas the corresponding left hind limb received a false manipulation (FM-OA group) consisting of ActivatorV firing in the air and touching the tibial tubercle. Three healthy animals were used as controls. Following sacrifice, tibiae and femora were removed for mCT and histological analysis.

**Results:** In the OA rabbits, subchondral BMD decreased in relation to control animals as measured by western blot. Following sacrifice, tibiae and femora were removed for mCT and histological examination. Synovial inflammation was evaluated by Krenn’s score and the protein presence of VEGF, MMP3 and CollagenVI in the synovial membrane was evaluated by western blot. In the OA rabbits, subchondral BMD decreased in relation to control animals.

**Conclusions:** These results support the hypothesis that CM may ameliorate subchondral BMD alterations induced by OA, in association to an improvement on synovioapathy and cartilage degradation.

**REFERENCES:**


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**AB0095**

**ATTEMPT TO DEVELOP A CHIMERIC CO-CULTURE SYSTEM TO DIFFERENTIATE MOUSE OSTEOCLASTS BY CULTURING MOUSE PRECURSOR CELLS WITH HUMAN SYNOVIAL FIBROBLASTS**

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**Background:** In rheumatoid arthritis (RA), inflammatory synovial tissue called the pannus proliferates and erodes the articular cartilage and bone in the affected joints. Osteoclasts, multinucleated cells of monocyte/macrophage lineage, are implicated in the bone destruction in RA. Thus, osteoclasts are considered an important therapeutic target in the prevention of the joint destruction. Mouse bone marrow cells differentiate into osteoclasts when co-cultured with osteoblasts or stromal cells in the presence of reagents such as 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) and prostaglandin E2 (PGE2). In contrast, that of osteoclast differentiation factor, RANKL. We then cultured mouse osteoclast precursors with human synovial fibroblasts in the presence of 1,25(OH)2D3 and PGE2. The murine cells seemed to disappear in the course of the co-culture, whereas they survived when exogenous human M-CSF was added to the system. Interestingly, however, they did not become TRAP-positive multinuclear cells, suggesting that synovial fibroblasts do not provide a sufficient amount of the osteoclast differentiation factor, RANKL. Following these results, we used ELISA to quantify the level of human RANKL and OPG in the culture supernatant of synovial fibroblasts. Predictably, the level of RANKL was below the detection limit with or without the presence of 1,25(OH)2D3 and/or PGE2, in contrast, that of OPG was very high, irrespective of the reagents added. We also quantified the levels of mouse RANKL and OPG in the culture supernatant of mouse osteoclasts. As expected, RANKL was detectable in this case. Interestingly, the level of OPG was very high and comparable to that of human OPG produced from synovial fibroblasts.

**Results:** We confirmed that mouse osteoclasts could be differentiated in vitro by culturing bone marrow cells in the presence of human M-CSF and RANKL. We then cultured mouse osteoclast precursors with human synovial fibroblasts in the presence of 1,25(OH)2D3 and PGE2. The murine cells seemed to disappear in the course of the co-culture, whereas they survived when exogenous human M-CSF was added to the system. Interestingly, however, they did not become TRAP-positive multinuclear cells, suggesting that synovial fibroblasts do not provide a sufficient amount of the osteoclast differentiation factor, RANKL. Following these results, we used ELISA to quantify the level of human RANKL and OPG in the culture supernatant of synovial fibroblasts. Predictably, the level of RANKL was below the detection limit with or without the presence of 1,25(OH)2D3 and/or PGE2, in contrast, that of OPG was very high, irrespective of the reagents added. We also quantified the levels of mouse RANKL and OPG in the culture supernatant of mouse osteoclasts. As expected, RANKL was detectable in this case. Interestingly, the level of OPG was very high and comparable to that of human OPG produced from synovial fibroblasts.

**Abstract AB0095 – Figure 1**

**Conclusions:** Synovial fibroblasts cannot be substituted for osteoblasts in a co-culture system of osteoclast differentiation. This is probably because synovial fibroblasts do not provide sufficient RANKL and M-CSF. Instead, they produce a large amount of OPG. This may be a mechanism by which ectopic osteoclastogenesis is inhibited. Thus, the osteoclasts observed in the pannus may be dependent on membrane-bound RANKL from other sources, like lymphocytes, or may be differentiated by stimulation with cytokines other than RANKL, such as TNF-α and IL-6.1, 2

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**Disclosure of Interest:** None declared

Individual Functions of the Histone-}

1. K. Klein1.

Results: Immunoreactivity for Y1, Y2, and Y5 NPY receptors was observed in C28/I2 cells. In human cartilage, a positive signal was found for the Y2 receptor in all samples while Y1 receptor immunoreactivity was undetectable, regardless of disease state, gender, and age of the donors. Y2 receptor immunoreactivity was observed in male and female OA cartilage samples, as well as in those from non-OA females, but not in those from non-OA males. 50 mM NPY was sufficient to significantly increase the levels of phospho-JNK, -p38, -ERK1/2, -PKA and PKC with similar kinetics, but much slower than IL-1β. A 6 hour stimulation with 50 or 100 mM NPY decreased LC3B-I and II levels in comparison with untreated cells. In the presence of chloroquine (ChQ), NPY increased LC3B-II levels relative to those found in cells treated with ChQ alone, indicating an increased delivery of LC3B-II to the lysosome consistent with autophagy activation by NPY.

Conclusions: This study shows that distinct NPY receptor subtypes are present and functional in C28/I2 cells and in human chondrocytes in situ in the articular cartilage, strongly suggesting that non-neuronal cells and tissues of the joints, namely chondrocytes, are relevant as NPY targets. The presence of each receptor subtype seems to be determined by gender and, in males, also by the disease state. The role of age is unclear as most cartilage donors were aged >55 years old. Future studies will be addressed at further elucidating the role of NPY and its receptors in modulating male and female chondrocyte functions, both in health, ageing and osteoarthritis.

References:

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