that CM is able to increase subchondral bone mineral density (BMD) in an experimental model of osteoporosis¹.

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 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 evaluation. 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.

Results: In the OA rabbits, subchondral BMD decreased in relation to control animals (OA 4729±193 vs Control 5181±209 mg/cc), been partially reversed in the tibiae of OA rabbits with CM (TM-OA 5055±216 vs FM-OA 4404±170 mg/cc). Subchondral trabecular bone structural parameters were analysed by microCT and a significant decrease of bone volume/trabecular volume (BV/TV), trabecular number (TbN) and trabecular thickness (TbTh) was observed in the OA rabbits, while trabecular separation (TbS) increased compared to control animals. TM-OA group showed a significant improvement of these parameters compare to FM-OA group. TM-OA had lower cartilage damage compare to FM-OA (TM-OA 4±0.67 AU vs FM-OA 8±1.25 AU). TM-OA synovial membranes presented a total Krenn score lower than FM-OA joints (TM-OA 3±0.35 vs FM-OA 4.5±0.38 AU). OA synovial membranes showed higher levels of CollagenVI respect to control ones; TM-OA synovial membranes presented less expression of CollagenVI than FM-OA group (TM-OA 1.4±0.13 vs FM-OA 2.2±0.3 AU), been this associated with a decrease of both MMP3 (TM-OA 1.2±0.1 vs FM-OA 1.7±0.2 AU) and VEGF (TM-OA 1.2±0.14 vs FM-OA 1.9±0.26 AU).

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

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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). There are no osteoblasts in the RA synovium, but there are fibroblasts and various inflammatory cells such as macrophages and lymphocytes. Thus, synovial fibroblasts may function as supporting cells for osteoclastogenesis in place of osteoblasts.

Objectives: The aim of this study was to establish a chimeric co-culture system of osteoclast differentiation using human synovial fibroblasts and mouse monocyte/macrophage lineage cells.

Methods: Synovial tissues were obtained from RA patients who underwent joint replacement surgery. Mouse osteoblasts were obtained from the calvariae of 2- or 3-day-old newborn C57BL/6 (B6) mice. Mouse bone marrow cells were prepared from femoral bones. Osteoclasts were visualised with tartrate-resistant acid phosphatase (TRAP) staining. The protein levels of RANKL and its decoy receptor, osteoprotegerin (OPG), in the culture supernatant were quantified using ELISA.

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 osteoblasts. 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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AB0096

EXPRESSION AND FUNCTION OF NEUROPEPTIDE Y RECEPTORS IN HUMAN ARTICULAR CARTILAGE: INFLUENCE OF GENDER AND OSTEOARTHRITIS

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Background: Elevated levels of neuropeptide Y (NPY) were reported in osteoarthritic (OA) joints¹. No information exists regarding the role of NPY in OA joints, besides mediating or potentiating nociceptive transmission.

Objectives: To determine whether NPY receptors are present and functional in human chondrocytes, by evaluating the ability of NPY to activate important signalling pathways and to modulate autophagy, which was shown to be induced by this neuropeptide in hypothalamic neurons² and to be a crucial homeostatic mechanism in chondrocytes, whose reduction contributes to OA pathogenesis³.

Methods: Immunofluorescence for the NPY receptor subtypes, Y₁, Y₂ and Y₅, was performed in the human chondrocytic cell line, C28/I2, and in human cartilage sections obtained from multi-organ donors (4 males, 55–75 years old, mean=67.25, 3 females, 33–68 years old, mean=55.33) at the Bone and Tissue