Introduction: Articular cartilage degeneration is the hallmark characteristic of osteoarthritis (OA). Cartilage destruction primarily results from an imbalance between synthesis and degradation of the extracellular matrix, particularly type II collagen and aggrecan. The expression and activity of matrix catabolic enzymes such as matrix metalloproteinases (MMPs) and aggrecanases are up-regulated during OA. Type II collagen is degraded by collagenases found in the articular cartilage and synovial fluid during OA. Cytokines such as interleukin(IL)-1β and TNF-α are elevated in OA. We have previously shown that a peptide fragment of type II collagen can act as a matrikine to human normal and OA chondrocytes by a feedback mechanism in chondrocytes mediating cartilage degradation (1). Synovial fluid has been shown to contain collagen fragments released by the turnover of the articular cartilage. The aim of this study was to determine the effect of this peptide on the expression, activation and production of collagenase, MMP-1 and MMP-13 on human OA synoviocytes cultures and determine the presence of this epitope in OA synovial fluid.

Materials and Methods: Peptide synthesis: A 24 amino acid synthetic peptide (CB12II) lacking RGD sequence derived from the helical region of type II collagen was synthesized by FMOC chemistry.

Cell Culture: Human OA synoviocytes (age range: 33-75) were isolated from cartilage obtained from knee arthroscopy. Synoviocytes were grown to confluency cultured in the presence of DMEM plus 10% FBS for 48h. The synoviocytes were then serum starved for 24h followed by stimulation with 0.25, 2.5 or 25 μg/ml CB12II for 48h in serum-free media. 5 ng/ml human recombinant IL-1β and TNF-α was used as positive control for upregulation of OA mediators. CB12II, IL-1β or TNF-α was not added to negative controls (Ctrl).

PCR: Semi-quantitative relative gene expression for MMP-1, MMP-13 and GAPDH were determined using total RNA extracted from the synoviocytes. Proteins of MMP-1 and MMP-13 secreted into the media were measured with Western blotting.

Western blot of synovial fluid: Synovial fluid from human OA patients (ages 60-82) were spun through an albumin removing column and then separated by SDS-PAGE. Proteins were transferred onto nylon membrane and blotted with anti-CB12II antibody.

Results: The addition of CB12II peptide stimulated the gene expression, production and secretion of collagenases MMP-1, and MMP-13. Protein expression of MMP-1 and MMP-13 was in a dose-dependent manner (Fig 1). MMP-1 was also secreted in the pro and active forms while only pro-MMP-13 was seen secreted. Also, 25 μg/ml CB12II was able to stimulate MMP-1 and MMP-13 to levels comparable to the addition of 5ng/ml of IL-1β and TNF-α.

Western blot of synovial fluid from human OA patients with anti-CB12II antibody (Fig 2) also showed the presence of high levels of type II collagen fragments with the CB12II epitope.

Discussion: MMPs have been implicated in the turnover of the ECM in OA. Collagenases MMP-1 and MMP-13 have been shown to be involved in type II collagen turnover and found both in the cartilage and the synovial fluid. The turnover of type II collagen in the articular cartilage can generate type II collagen degradation fragments that are found in the synovial fluid. This cleavage results in production of collagen degradation fragments that can feedback and further activate MMPs. The cleavage products of matrix proteins have been recently termed matrikines. In this present study, we have shown that CB12II, a peptide in the helical region of type II collagen can act as a matrikine by stimulating the expression, production and activation of MMPs including MMP-1 and MMP-13 in human OA synoviocytes similar to that of IL-1β and TNF-α. This finding suggests that the release of the matrikine can act as a potent stimulator of collagenases in OA synoviocytes and contribute to increase collagenases in the synovial fluid which then can degrade the articular cartilage and contribute to the pathology of OA.