Early OA Stage Like Response of synovial fibroblasts

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As events triggering early osteoarthritis onset can be related to mechanical stress and proinflammatory signaling, the effect of different mechanical strain protocols on the expression of proinflammatory genes, as well as extracellular matrix remodelling in human synovial fibroblasts are of interest. Three distinct models of tensile stretching were analyzed: static isotropic tensile strain at 0 Hz, 16% tension for 48 hours; short-term high-frequency cyclic tension at 1 Hz, 10% tension for 4 hours; and dynamic tensile stretching for 48 hours, consisting of two blocks of moderate stretching at 0.2 Hz, 2%, advanced stretching at 0.5 Hz, 15%, or a combination of both. General signs of inflammation were present after static isotropic tension, whereas short-term high-frequency cyclic tension showed increased levels of IL-6 paired with diminished levels of IL-1. Reduced inflammatory effects of TNF-, IL-6, and IL-1 were observed when exposed to advanced stretching. Long-term tensile strain induced extracellular matrix remodelling at the gene and protein levels. While hyaluronan acid synthesis was increased with static tensile strain, dynamic tensile stretching had a reducing effect.
It is suggested that proinflammatory markers are activated by mechanical strain as seen in static isotropic tension and short-term high-frequency tensile strain, whereas long-term exposure induced extracellular matrix remodelling processes.

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Synovial fibroblasts (SF) express various recognition receptors, making them capable to sense joint damage as well as invading pathogens [29]. Several stimuli (e.g., cytokines, growth factors, adipokines) are able to activate SF and promote inflammation and joint destruction [30,31].

In order to retrace the effect of mechanical strain and understand OA-related processes, different studies analysed the effect of tensile strain and cyclic mechanical strain on gene expression in the context of OA pathology. We chose two published cyclic tensile strain protocols characterised by a single session of short high-frequency tensile strain and compared it to prolonging static tensile strain and varying cyclic tensile strain. Based on the comparison of the different set-ups, insight into inflammation and extracellular matrix deposition was obtained.

First, analysis of cell proliferation and viability were done and showed no effects of short-term high-frequency cyclic and dynamic stretching on synovial fibroblasts. This served as proof that observed findings in gene expression and extracellular matrix deposition were not caused by a reaction towards apoptotic or cytotoxic effects. Only in static isotropic stretching an increase in cell number was observed. As adherent synovial fibroblasts were used in the experiments, the persistently increased surface by static isotropic stretching most likely offered more space for increased cell proliferation leading to higher cell numbers.

In macrophages cyclic tensile strain increased the expression of mechanotransduction-related NK1R (neurokinin receptor 1) gene, which mediates reduction of cell adhesion presumably by altered SP (substance P) gene and protein expression [22], whereas in chondrocytes mechanical stimulation altered the integrin FAK-MAPK mechanotransduction cascade and activated integrins [23]. Like chondrocytes and macrophages, synovial fibroblasts showed an altered gene expression of *interleukin-6* (*IL-6*), when triggered with mechanical strain. When bruises occur, synovial fluid comprises higher levels of IL-6 and activates an inflammatory response, climaxing in subchondral bone layer changes by dampened type II collagen production [32,33,34]. TNF-α acts synergistically with IL-6 and IL-1β in osteoclast activation [35]. When activated, it diminishes type II collagen synthesis in chondrocytes. Beside this, it induces COX-2, PGE2, and IL-6- synthase, playing an important role in the course of OA [36,37,38]. During OA, the cartilage releases increased amounts of IL-1β, stimulating chondrocytes and promoting inflammation and cartilage destruction [39,40]. In OA pathology increased levels of IL-1β can be detected in the synovial fluid and joint tissue [41,42,43,44]. The proinflammatory marker IL-6 plays a role in the pathophysiology of OA [45]. Increased IL-6 levels can be found in osteoarthritic groups [46], while synovial fluid levels of IL-6 seem not to correlate with OA severity [47]. The application of pressure as mechanical strain has a major impact on gene expression in synovial fibroblasts [48]. Mechanical strain can induce inflammation and can alter extracellular matrix composition. In contrast, application of static tensile strain on fibroblasts of periodontal origin caused an anti-inflammatory reaction [21]. We saw an arthritis-associated inflammation in case of static isotropic tensile strain, as the proinflammatory markers (TNF-α, IL-1β, IL-6, COX-2) were significantly upregulated. By the application of short-term high-frequency tensile strain *IL-6* indicated an inflammatory status similar to macrophages [22]. In contrast, observed downregulation of *IL-1β* indicated absence and damping of inflammation. Beside inflammation induction, IL-6 also acts in innate immune response acquisition, mononuclear cell recruitment and T-cell apoptosis inhibition. Furthermore, IL-6 functions in an anti-inflammatory manner, while activated STAT3-guided signalling supports epithelial cell proliferation and inhibits cell apoptosis [49]. As TNF-α shows no altered gene expression, while IL-1β is downregulated, the induced IL-6 gene expression in the short-term high-frequency tensile strain set-up may be due to activation of regenerative processes in an anti-inflammatory manner [49,50,51]. Contrary to this, beneficial anti-inflammatory effects were achieved when synovial fibroblasts were exposed to dynamic stretching. A correlation with *TNF-α*, which is involved in IL-6 regulation [39,52], was observed for static isotropic stretching and advanced stretching. Our results indicate that short-term high-frequency cyclic tensile strain induced a mechanical stress response, as a significant increase in the inflammatory and innate immune response activating marker *IL-6* could be detected. This concurs with previous findings applying pressure on fibroblasts [24,48]. Interestingly, the application of the dynamic stretching protocol had beneficial effects on *IL-6* expression in synovial fibroblasts, contrary to chondrocytes [23]. Based on findings on chondrocytes exposed to shear stress, an activation of proinflammatory cytokines by COX-2 was assumed [17]. In contrast, we detected no significant changes in *COX-2* gene expression while expression of several proinflammatory genes were altered.

In ongoing OA inflammation processes, chondrocytes switch to a degradative phenotype and activate matrix-degrading proteinase secretion, inducing articular cartilage destruction by secretion of MMPs and ADAMTS [53,54]. Beside these events, catabolic cytokines and chemokines will activate a positive feedback loop, enhancing articular cartilage damage and promoting synthesis of cartilage inflammation, as well as enhancing chondrocyte apoptosis,

matrix protein, and collagen degradation. Type I collagen is present in almost all connective tissues and able to stabilize structures with its enormous tensile strength [55]. In the case of cartilage fissure, local tensile stress can build up, promoting chondrocyte phenotype change and type I collagen formation [56]. In late-stage OA, increased levels of type I collagen are present, whereas type II collagen decreases [57]. Our findings indicate that also synovial fibroblasts, originating from the synovium, change gene expression of collagens due to tensile stress. We see a decrease in *COL1A2* expression suggesting extracellular matrix remodelling induced by isotropic and dynamic stretching. In line with these findings, the presence of COL fractions was also diminished by tensile strain.

Hyaluronic acid synthase 1 (HAS-1) is involved in hyaluronic acid (HA) synthesis and plays a major role in joint lubrication [11]. Altered levels of HA are associated with inflammation and degeneration occurring in arthritis [58,59]. A deficiency of HAS-1 is associated with chronic joint inflammation and intra-articular fibrosis [60]. When static isotropic tension was applied to the synovial fibroblasts, increased levels of HA GAGs and HAS-1 were present, similar to observations done in a compressive setup [48]. In contrast, dynamic tensile strain reduced HA GAGs in the cell supernatant and *HAS-1* expression, favouring inflammatory processes.

As the synovium and the infrapatellar fat pad experiences a close proximity to each other, tissue innervation and close vascularization [6,7] enable tissue cross-talks, when molecular imbalances occur. Therefore, when analysing the effect of mechanical strain on synovial fibroblast monoculture metabolism, it is possible that no adequate picture of inflammation induction or changes in extracellular matrix deposition can be observed, as synergistic effects induced by the crosstalk of infrapatellar fat pad are prevented.