Introduction:
Cartilage metabolism depends in part on a cellular response to mechanical forces, including shear stress and hydrostatic pressure, that occur during joint loading. Models of tissue loading histories propose that a tissue differentiation process occurs in which degeneration and ossification of cartilage are accelerated by shear stress, but remain inhibited by intermittently applied hydrostatic pressure (1). In vitro studies confirm that chondrocytes in culture respond to a variety of loading conditions (2,3). Other factors influencing cartilage metabolism depend on the biochemical state of the joint environment. Application of mechanical loads to chondrocytes retrieved from osteoarthritic joints alters the release of cytokines, chemokines, and matrix metalloproteinasises (4,5).

One potential mechanism influencing cartilage degeneration is the secretion of the angiogenic factor VEGF, that creates an environment in which bone remodeling occurs and loss of cartilage form and function is experienced. This study tested the hypothesis that shear stress increases VEGF expression through activation of the transcription factor HIF-1 and MAPK.

Methods:
Articular chondrocytes were isolated from cartilage obtained as autopsy specimens from patients (n=6) undergoing primary total knee arthroplasty using enzymatic digestion. The chondrocytes were plated at a density of 2x10^5 cells/plate in DMEM/F12 supplemented with 10% FBS, ascorbate and gentamicin. Shear stress was applied using a cone viscometer at a rotational velocity of 100 rpm (0.81 Pa) for four or 24 hours for VEGF protein or transcription factor analysis, respectively. Control cultures were maintained in the absence of shear stress. VEGF levels in the culture medium were measured via ELISA. For transcription factor analysis, Hypoxia-Inducible Factor 1 (HIF-1) levels were measured in nuclear protein extracts using a commercially available ELISA based kit. For analysis of MAPK involvement, the MEK-1 inhibitor PD 98059 was added to test cultures one hour prior to mechanical stimulation (n=4) and phosphorylated p44/42 was detected by western blotting. Statistical analysis was carried out using ANOVA and t-tests with Bonferroni’s correction and values of p<0.05 were considered significant.

Essential Results:
Application of shear stress for 24 hours increased human osteoarthritic chondrocyte VEGF release by 630%, relative to unsheared control cultures (p<0.05). Exposure of the osteoarthritic chondrocytes to shear stress for four hours increased HIF-1 levels in nuclear extracts by 195%, relative to unsheared control cultures.

Western blot analysis of cell extracts demonstrated increased levels of MAPK in chondrocytes exposed to shear stress, relative to chondrocytes maintained in the absence of mechanical stimulation. Addition of the MEK1 inhibitor PD 98059 to chondrocytes inhibited both P44 and P42 isoforms in a dose dependent manner. Addition of PD 98059 to chondrocytes exposed to shear stress also resulted in a dose dependent decrease in VEGF release, when compared to chondrocytes exposed to shear stress in the absence of the inhibitor (Figure 1). Levels of VEGF release from chondrocytes exposed to shear stress in the presence of PD 98059 (50 uM) at 6, 12, and 24 hours were similar to the levels of VEGF release from chondrocytes maintained in the absence of shear stress.

Discussion:
It is generally recognized that mechanical loading of normal joints contributes to the maintenance of the articular cartilage extracellular matrix. However, the precise relationship between mechanical loading and chondrocyte metabolism remains undefined. Areas of high shear stress may lead to cartilage degeneration and loss of joint function. This study showed that shear stress regulates the expression of the angiogenic factor VEGF through activation of MAPK and the transcription factor HIF. Increased in vivo production of VEGF in response to shear stress may influence cartilage homeostasis and accelerate endochondral ossification in diseased joints.

References:

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