Introduction:
In osteoarthritis (OA), articular chondrocytes release multiple inflammatory factors and degradative enzymes within the joint space and into the serum. Animal models of OA and human clinical observations implicate ligamentous injury, limb malalignment, joint overload (obesity) and trauma together with genetic predisposition as risk factors for the onset of OA. These risk factors are associated with changes in the distribution of joint loads on the cartilage and as a result may contribute to progressive matrix destruction. Daily activity generates a compression force across diarthrodial joint surfaces that is accompanied by varying degrees of sliding, rolling, spinning and fluid exudation. These phenomena create two types of fundamental stresses, shear stress and hydrostatic stress (pressure) within the extracellular matrix. Both types of stress modulate chondrocyte metabolism. However, in spite of data implicating mechanical effects in OA, little information is available detailing the precise levels of shear stress required to activate gene expression and produce proinflammatory mediators by OA chondrocytes. The purpose of this study was to examine the time course for the release of the proinflammatory cytokine, interleukin-6, and nitric oxide in response to shear stress applied to human OA chondrocytes. In addition, the expression levels of the mRNA for IL-6 and nitric oxide synthase were examined to determine how production followed regulation of gene expression.

Materials and Methods:
OA chondrocytes were isolated from cartilage samples obtained in accordance with an approved IRB under the exempt tissue protocol (Stanford University). The cartilage matrix was dissociated in culture medium (DMEM) containing gentamicin (25 μg/ml) and bacterial collagenase (Life Technologies) at a final concentration of 1.2 mg/ml (overnight at 37 °C in 7.5% CO2 and 100% humidity). Isolated chondrocytes were filtered through a nylon mesh filter and collected in a PBS to remove any traces of collagenase. The final cell pellet was suspended in the serum-free medium and the cell number determined by hemacytometer together with assessment of viability using Trypan Blue exclusion. Viability of osteoarthritic chondrocytes exceeded 96%.

Fluid-induced shear stress (ShS) was applied using the cone viscometer system. The loading conditions ensure that the chondrocytes are subjected to a uniform distribution of shear stress without turbulence. The maximum level of shear stress attained by this system reaches 1.6 Pa if operated at a rotation speed of 200 rpm. In these studies, the level of shear stress was determined by a rotational speed of the cone viscometer of 100 rpm for a periods of 6, 12 and 18 hours. The culture medium was at the termination of the loading period. For loading, the viscometer of 100 rpm for a periods of 6, 12 and 18 hours. The culture incubator at 37 °C and 100% humidity. Shear stress was applied to chondrocytes in high density monolayer culture in 100 mm culture plates in serum-free conditions in a HEPES buffered medium to maintain pH. The serum-free medium consisted of a 1:1 mixture of Ham's F12/DMEM supplemented with selenium, and a liposome supplement.

NO was measured using the Griess reaction. Matrix macromolecule mRNA signal levels were determined using reverse transcriptase polymerase chain reaction and quantified by imaging analysis software and by real-time PCR (Applied Biosystems).

Results:
The effects of shear stress on the OA chondrocyte release of IL-6 exhibited variable responses with levels ranging from a 10 to 20-fold increase depending on the sample. This variability was independent of ELISA plate functionality and may represent interference by factors influencing cytokine detection in the culture medium samples. It remains possible that some OA chondrocytes express increased proteolytic activity or an IL-6 binding protein. In sharp contrast to the variability in IL-6 protein levels, IL-6 mRNA was clearly increased in the OA chondrocytes in response to application of shear stress and showed a time dependent up-regulation (Fig. 1). The data show the results for three independent patient samples tested after exposure to shear stress for 6, 12 and 18 hours, where the culture medium and total RNA were collected at the end of the testing period. IL-6 mRNA expression in cultures exposed to shear stress for only 6 hours was increased by 12-fold when compared to culture not exposed to shear stress, when normalized to 18S RNA as a reference signal for real time PCR. Signal levels for 18S RNA remain unaffected by application of shear stress but did vary with individual patient samples. Inducible nitric oxide synthase levels were clearly up-regulated maximally after 18 hours of shear stress (Fig 2). Interestingly, nitric oxide levels increased after only 6 hours of applied shear stress (Fig 3).

Discussion: The results of this study demonstrate that the reactivity of OA chondrocytes to deformational stress may contribute significantly to the progressive deterioration of the cartilage extracellular matrix. Increased level of IL-6 may significantly reduce the normal replacement of aggrecans through inhibitory effects on synthetic rates. Increased level of nitric oxide in response to elevated production of nitric oxide synthase may contribute to a low grade but persistent inflammatory state, also leading to matrix degeneration.

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