Introduction: Osteoarthritis (OA) is the most common joint disorder in the aging population and a significant health challenge. Despite its prevalence, treatment of OA is symptomatic and restricted to a few classes of drugs that have not been shown to inhibit the progression of OA [1]. Pro-inflammatory cytokine IL-1β plays a critical role in cartilage degradation and elicits its catabolic response via enhanced production of nitric oxide (NO) in human chondrocytes. NO has been implicated in cartilage matrix degradation and apoptotic chondrocytes death (2). High levels of NO are produced by the action of inducible nitric oxide synthase (NOS-Ⅱ, iNOS) and studies have shown that IL-1β is a potent inducer of iNOS expression and NO production in human chondrocytes. Polyphenols present in green tea (Camellia sinensis) are anti-inflammatory and have been shown to inhibit the production of NO in cancer cell lines (reviewed in 3). In this study we determined the effect of green tea polyphenol epigallocatechin-3-gallate (EGCG) on IL-1β-induced expression of iNOS and production of NO in human chondrocytes.

Methods:
Preparation of Chondrocytes. Human chondrocytes were prepared by enzymatic digestion of femoral head cartilage obtained at the time of joint replacement surgery (3 samples, mean age 68 years, 2 female, 1 male). Chondrocytes were plated (1 x 10^6) in 35 mm culture dishes in DMEM containing 10% FBS, antibiotics and antimycotics and incubated for 3 days in a tissue culture incubator with 5% CO₂ and 95% air. Chondrocytes were serum starved and then treated with recombinant human IL-1β (2 ng/ml), EGCG (200 µM), or IL-1β + EGCG.

I. Chondrocyte Viability Assay. Viability of human chondrocytes was assayed by MTT assay using a commercially available kit (R & D Systems, MN). II. Quantification of Nitrites. Chondrocytes were treated as described above and the production of NO was determined as NO₂ concentration in culture supernatants using a commercially available kit (R & D Systems). III. Western Blot Analysis. After the treatment, chondrocytes were lysed and total protein (25 µg/lane) was resolved by gradient SDS-PAGE and transferred to nitrocellulose membranes by electroblotting. Membranes were blocked and incubated with the anti-iNOS antibody sc-651 (Santa Cruz Biotechnology) for > 2 hr at room temperature with shaking, washed with fresh TBST and incubated with a 1∶5,000 dilution of horse radish peroxidase (HRP) conjugated anti-rabbit IgG (Southern Biotechnology Associates, AL). After washing with TBST, protein bands were visualized by enhanced ECL (Amersham, IL). Blots were scanned and the digitized image was analyzed using the Un-Scan-it gel software v. 5.1 (Silk Scientific Corp., UT). IV. NF-κB ELISA. Cellular levels of active NF-κB p65/Rel proteins in human chondrocytes treated with IL-1β and EGCG were determined using a highly specific ELISA method (4). V. IL-6 ELISA. Human IL-6 was measured in the supernatants of chondrocytes cultures treated as described above by a sandwich ELISA (R & D Systems) according to the instructions of the manufacturer. VI. Statistical Analysis. Experiments were repeated using age and sex matched samples. Data was analyzed using student’s ‘t’ test (Sigma Plot) and p<0.05 was considered significant. Values shown are Mean ± SEM.

Discussion: Results of our studies showed that the antioxidative polyphenol EGCG was non-toxic to human chondrocytes, at least for the duration and the doses tested. Suppression of IL-1β-induced NO production (Figure-1) in human chondrocyte cultures suggests that EGCG may be useful for inhibiting the excessive production of NO in arthritic joints. These studies further showed that the inhibition of NO production correlated with the inhibition of iNOS expression in human chondrocytes. As most of the inflammatory effects of IL-1β are mediated via the activation of transcription factor NF-κB in the target cell, including the expression of iNOS, we also analyzed the effect of EGCG on the levels of active NF-κB in human chondrocytes. Our results (Figure-2) indicate that EGCG inhibited the IL-1β-induced activation of NF-κB in human chondrocytes. Thus, the inhibition of iNOS expression and the production of NO may be related to the inhibitory effect of EGCG on the IL-1β-induced activation of NF-κB in human chondrocytes. Although this remains to be confirmed. Results of additional studies (not shown) demonstrated that EGCG was highly effective in inhibiting IL-1β-induced production of pro-inflammatory cytokine IL-6 in human chondrocytes. Taken together, these results indicate that the dietary antioxidant EGCG possesses unique properties that may be of value in inhibiting inflammatory, and possibly degenerative, processes in arthritic joints.

References.