Comparison and Correlation of Synovial Fluid Cytokine Levels and in vitro Cytokine Production by Osteoarthritic Cartilage, Meniscus and Synovium
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INTRODUCTION
Osteoarthritis (OA) is a debilitating disease associated with the loss of functional articular cartilage. Originally, OA was not considered an inflammatory disease, however, it is now understood that inflammation play major roles in the development and progression of the disease. Identifying cytokines that are key players in these processes may help to elucidate key pathways of etiopathogenesis and develop biomarkers for diagnosis, treatment monitoring and prognostication in OA. *In vitro* culture of tissues from OA patients may be useful in identifying specific responses to OA and understanding the tissue metabolism during short term *in vitro* culture. As *in vivo* culture systems will better maintain the *in vivo* metabolism of the tissue during short term *in vitro* culture.

To determine the similarity cultured that positively correlated to the SF cytokine level.

Three cytokines were below the detection limit of the assay. For MCP-1, IL-8 levels in synovial fluid (SF) to cartilage (CART), Meniscus (MEN), and Synovium (SYN)

METHODS
All procedures were performed with IRB (IRB#1042248) approval. The femoral condyles (FC), tibial plateaus (TP), lateral and medial menisci (MEN) with joint capsule containing synovium (SYN) attached, and synovial fluid (SF) were obtained from 5 patients undergoing total knee replacement. For each patient, a tissue explant was made from the body of each lateral and medial MEN and cultured separately in 3ml of supplemented DMEM. The SF was removed from the meniscal explant and cultured separately in 3ml of supplemented DMEM. Three 6 mm cartilage (CART) explants were created from the lateral and medial TP and FC of each patient and cultured separately in 2ml of supplemented DMEM. Tissues were cultured for 7 days and media changed and collected for analysis on days 1 and 4. After 7 days of culture, the explant was digested in papain and the DNA content of the tissue was determined using the Picogreen DNA assay. Media were analyzed on days 1 and 4 for IL-1β, IL-6, IL-8, TNF-α, MIP-1α, MCP-1, and RANTES using the Fluorokine MAP human cytokine multiplex panel (R&D Systems), and standardized to the DNA content of the tissue explant. The results from each patient’s tissue type were averaged to give the mean cytokine production from that patient for each tissue. A log transformation was performed to normalize the data and a Pearson Correlation done to compare the data from each tissue to the SF with significance set at $p<0.05$. Data were compared for each cytokine individually ($n=5$) and with all cytokine data combined ($n=35$).

RESULTS
**Correlation of individual cytokine data (Table 1):** Correlation analysis could not be performed on MIP-1α, IL-1β, and TNF-α because these three cytokines were below the detection limit of the assay. For MCP-1, RANTES, IL-6, and IL-8 there was not a significant correlation between the cytokine levels detected in the synovial fluid and the amount of each cytokine released to the media by CART, MEN, or SYN on day 1 or 4. For each of the cytokines tested, the CART culture was the only tissue culture that positively correlated to the level of cytokines found in the synovial fluid.

**Correlation of total cytokine data (Table 2):** To determine the similarity between the total production of cytokines by joint tissues in *in vitro* and levels found in SF *in vivo*, the data from all cytokine analysis were combined for analysis. When the cytokine data were combined there was a significant ($p<0.001$) and moderately strong ($r=0.600-0.712$) positive correlation between the SF cytokine levels and the production of cytokines by all tissues in *in vitro*. For all cultures the correlation coefficient to the SF increased from day 1 to day 4, and at both day 1 and 4 the cytokine production of the CART culture had the strongest correlation to the cytokine levels of the SF.

**DISCUSSION**
The objective of this study was to determine if the *in vitro* production of cytokines by cartilage, meniscus, and synovial tissue explants correlate to the level of cytokines found in the synovial fluid *in vivo*. The purpose was to determine if *in vitro* culture of joint tissues could be used to study the *in vivo* metabolism of the tissue and potentially identify tissue specific biomarkers for OA. When each cytokine was analyzed individually, there was not a significant correlation between the concentration of the cytokines studied in the synovial fluid and the *in vitro* production of those cytokines by cartilage, meniscus, or synovial tissue explants. This indicates that harvest and processing of the tissues and *in vitro* culture may significantly affect the production of the cytokines tested. However, when the cytokine data was combined, indicating the relative levels of the cytokines in the synovial fluid and tissue cultures, there was a moderately strong and significant correlation between the cytokine levels in the synovial fluid and the media of the tissue cultures. This indicates that while tissue culture did not necessarily maintain the absolute level of cytokine production observed, the relative protein metabolism was maintained. Therefore, using *in vitro* culture of OA affected to tissues to study the *in vivo* metabolism of the tissues may be valid. Further, *in vitro* culture has potential to identify novel protein biomarkers that may be difficult to identify using *in vivo* fluid samples due to low concentration and a high background of non-diagnostic, high abundance molecules like hyaluronic acid and albumin. Currently, proteomic studies are being performed on the media of cultured tissues to identify potential diagnostic, treatment monitoring, and prognostic biomarkers for OA. Additionally, whole organ (meniscal and osteochondral) cultures are being assessed in our laboratory to determine if these culture systems will better maintain the *in vivo* metabolism of the tissue during short term *in vitro* culture.

**Table 1:** Pearson correlation analysis of MCP-1, RANTES, IL-6, and IL-8 levels in synovial fluid (SF) to cartilage (CART), Meniscus (MEN), and Synovium (SYN) *in vitro* culture media levels on days 1 and 4. CC=correlation coefficient

**Table 2:** Pearson correlation analysis of the combined cytokine (MCP-1, MIP-1α, RANTES, TNF-α, IL-1β, IL-6, and IL-8) levels in synovial fluid (SF) to cartilage (CART), Meniscus (MEN), and Synovium (SYN) *in vitro* culture media levels on days 1 and 4. CC=correlation coefficient