Introduction: Autologous chondrocyte implantation (ACI) has been used to successfully repair defects in articular cartilage. More than 13,000 patients have been treated with Carticel autologous chondrocytes, which was the first FDA-approved cell therapy. In addition, over 5,000 European patients have been treated with Matrix-induced Autologous Chondrocyte Implantation (MACI implant), which consists of chondrocytes seeded onto a collagen membrane. Both of these procedures involve harvesting primary chondrocytes from a cartilage biopsy and expanding the cells in culture. Current growth media contains fetal bovine serum (FBS). While FBS is effective as a growth promoting agent, it introduces risk associated with the possibility of bovine to human disease transmission. In addition, there is considerable lot-to-lot variability in terms of growth promotion in FBS. A serum-free media (SFM) for chondrocyte expansion would eliminate these problems. Here we describe our approach to the development of a SFM for expansion of autologous human chondrocytes.

Materials and Methods: Cell Culture: Primary human chondrocytes were grown in DMEM with 9.1% FBS or a SFM containing insulin, PDGF and bFGF, known as cDRF/P/L. During growth phase in the second passage, cells were harvested and RNA was prepared using Trizol reagent.

Microarray analysis: cDNA and labeled cRNA were prepared using standard techniques. Samples were then subjected to microarray analysis using the Affymetrix U133 microarray chip. Of the 47,000 transcripts interrogated, statistical analysis revealed differential expression of 3,784 transcripts. We then queried gene chip annotation to identify 178 potential candidate receptors, hormones and growth factors from the group of 3,784 differentially expressed transcripts.

Testing candidate growth factors: Candidate peptides were tested for growth promotion activity by addition to the cDRF/P/L SFM. Peptides were solubilized according to the manufacturer's instructions and stocks were stored at -20°C. All peptides were made using recombinant DNA technology and did not contain bovine carrier protein. Stock peptides were diluted to the desired final concentration in cDRF/P/L. Chondrocytes isolated from human cartilage biopsies were plated directly in cDRF/P/L with test peptides. DMEM/FBS and cDRF/P/L served as controls. Cells were grown for three passages in T-flasks, with subculturing occurring when cells reached 50% to 80% confluent. At each passage, cell number was determined and population doublings per day were calculated.

Agarose Culture: Third passage chondrocytes were maintained until confluence, then harvested from T-flasks and tested for the ability to redifferentiate by initiating agarose cultures. Approximately 1 x 10^6 cells were seeded into 2% agarose. Strains were tested in triplicate. The agarose cultures were maintained for 21 days, feeding every 2-3 days with DMEM with 9.1% FBS. Redifferentiation was evaluated by staining the proteoglycan matrix that forms around cell colonies with 2 mg/mL safranin O. The cultures were evaluated by determining the number of colonies ≥ 50 microns as a percentage of the total colony number.

Results: Testing identified the cytokines IL-6 (interleukin 6) and OSM (oncostatin M) as potent stimulators of chondrocyte growth in the absence of serum. A serum-free medium containing IL-6, OSM, PDGF and bFGF (named E93) was compared directly to medium with FBS for the ability to stimulate growth of primary human chondrocytes through three passages, and the potential of the cells to redifferentiate was examined. Cells were split when cultures reached 50% to 80% confluent. Days in culture were nearly the same for each condition (Figure 1). However, the growth rate (not shown) and total cell yield (Figure 1) for cells grown in SFM, at both high and low seeding densities, were greater than that for cells cultured in medium with FBS for all three passages. It was observed that cells grown in E93 did not spread out as much as those grown in serum, allowing the growth of more cells per unit surface area in the same amount of time.

Discussion: We employed microarray analysis to identify factors that might stimulate chondrocyte growth in the absence of serum. Our strategy was to compare expression of genes in the same strains of primary cells grown in parallel in DMEM with FBS or a serum-free media that was known to support growth at a low rate for a few passages. Analysis of differentially expressed genes suggested candidate growth factors for follow-up testing. Using this approach, IL-6 and OSM were found to stimulate growth rate and cell yield while maintaining chondrogenic potential, as measured by colony formation in agarose. It is interesting to note that IL6 and OSM belong to the same cytokine family, and OSM has been shown to regulate IL-6 production in endothelial cells. Additional characterization of chondrogenesis by cells grown with E93 is underway.