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Measuring clinically relevant endpoints in a serum-free, three-dimensional, primary cell culture system of human osteoarthritic articular chondrocytes

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ABSTRACT

Osteoarthritis (OA) is characterized by degeneration of articular cartilage within the joint, inflammation and pain. The purpose of this study was to develop a primary, serum free cell culture system of human osteoarthritic articular chondrocytes (HOACs) with which to study manifestations of the disease process. Joint tissues were obtained from OA patients undergoing total knee arthroplasty (TKA). HOACs isolated from the femoral condyles and tibial plateau of the same side were combined, plated in three-dimensional, alginate beads and cultured for five days in serum, hormone and protein free medium. More living cells were obtained from the femoral condyles than the tibial plateau. The optimal plating density was 2.5×10^6 cells/ml of alginate. The amounts of DNA, RNA, proteoglycans and total collagen were similar in cultures prepared from the sides of least and greatest pathology. More type 1 than type 2 collagen was detected in the medium on days 2 and 5. A greater percentage of type 1 than type 2 collagen was degraded. The inflammatory cytokine interleukin-1 beta was present in the medium and alginate associated matrix. Although variation in the metabolic profiles between subjects was observed, HOACs from all patients continued to reflect the OA phenotype for five days in culture. This serum free, three-dimensional primary culture system of HOACs provides a platform with which to measure clinically relevant endpoints of OA and screen potential disease modifying OA therapeutics.

1. Introduction

Osteoarthritis (OA) is characterized by a loss of articular hyaline cartilage, bone remodeling and muscle weakness surrounding the joint. The degenerative process eventually results in pain and joint instability. Cartilage damage and excessive joint loading are the most common causes of injury-related OA [1]. Approximately 27 million people in the United States are affected by OA, accounting for 25% of the visits to primary care physicians and half of all non-steroidal anti-inflammatory drug (NSAIDs) prescriptions [2]. The prevalence of OA is expected to increase from 47.8 million in 2005 to 67 million by 2030 [3,4]. In addition to the impact of OA on quality of life, the treatment and management of OA places a heavy burden on the U.S. health care system [4–6].

The current standard of care for OA subjects is directed towards later stages of disease, with the goals of pain relief and improvement in joint function. Treatments include NSAIDs and intra-articular injections of hyaluronans or steroids [7]. These interventions do not target the progression of articular tissue damage, and therefore, they have little effect on cartilage degeneration, the primary pathology seen with

Abbreviations: OA, osteoarthritis; HOACs, primary human osteoarthritic articular chondrocytes obtained from subjects with severe OA that underwent total knee arthroplasty; OARSI, Osteoarthritis Research Society International standard; ECM, extracellular matrix; MMP, matrix metalloproteinases; TNF, Tumor Necrosis Factor; IL, Interleukin; ITS, insulin, transferrin, selenium; TKA, total knee arthroplasty; AKA, above knee amputation; HBSS, Hanks buffered saline solution; DMEM, Dulbecco's modified essential medium; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; IL1β, interleukin-1 beta; EDTA, ethylene diamine tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; CM, conditioned medium; AAM, alginate-associated matrix; DMOADs, disease modifying osteoarthritis drugs; NSAID, non-steroidal anti-inflammatory drug

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OA. Ultimately, patients are faced with joint replacement as the only effective means to improve their quality of life.

The hallmarks of OA at the tissue level are progressive destruction of the cartilage extracellular matrix (ECM), inflammation, chondrocyte hypertrophy and replacement of cartilage with bone [8,9]. A balance between production and degradation of ECM molecules is crucial for maintaining articular cartilage architecture and function [9–11]. Chondrocytes are responsible for production of the ECM that consists primarily of type 2 collagen, proteoglycans and glycoproteins [12]. As OA progresses, articular chondrocytes shift from synthesizing type 2 to type 1 collagen [9,13,14]. The enhanced ECM breakdown typical of OA is mediated, in part, by aberrant activity of matrix metalloproteinases (MMPs) that have multiple substrates, including types 1 and 2 collagens, proteoglycan core protein and members of the fibrillin family [15–21]. In addition, MMPs' degradation of the ECM stimulates chondrocytes to release inflammatory cytokines of the interleukin (IL) and tumor necrosis factor (TNF) families which further induce MMP production and matrix degradation [22–24].

Biochemical analyses of cartilage tissue have been instrumental in identifying potential targets for therapeutic intervention. *Ex-vivo* systems of human osteoarthritic articular chondrocytes are particularly useful for screening the efficacy and toxicity of potential disease modifying drugs; however, there are challenges that come with culturing primary human osteoarthritic articular chondrocytes (HOACs) that limit their utility for measuring clinically relevant endpoints. Most studies with human articular chondrocytes have involved culturing cartilage tissue first as an explant and then collecting and re-plating the chondrocytes in tissue culture dishes [25–28]. Once chondrocytes are isolated from their ECM and plated as a monolayer, they dedifferentiate and phenotypically resemble fibroblasts [29,30]. Furthermore, expansion of primary articular chondrocytes or cell lines *in vitro* has required growth in serum containing medium that complicates measurements of metabolism and promotes the maturation and hypertrophy of chondrocytes [26,27,31,32].

Several limitations of chondrocyte cell culture have been overcome with the development of a three-dimensional system consisting of alginate beads that more closely resembles the architecture of cartilage *in vivo* than monolayer culture. Alginate is a polysaccharide harvested from brown seaweed algae that forms a gel in the presence of calcium. Alginate beads have been used to culture chondrocytes from adult rabbits [33,34], cows [35], and humans [26,27,35,36] in serum or supplement containing medium. Hauselmann and Bonaventure were the first to use the three-dimensional alginate system to culture chick embryo growth plate chondrocytes in serum free medium containing insulin, transferrin and selenium (ITS) following expansion of the cells as a monolayer in serum-containing media [27,35]. Our group further refined this system by eliminating the expansion step, increasing the plating density and removing all additives from the media [37,38]. Under these conditions, the chick embryo chondrocytes retained their phenotype and continued to produce a cartilage matrix. Adult human articular chondrocytes harvested from the joints of patients undergoing total knee arthroplasty (TKA) for severe OA were cultured on alginate beads in serum free medium; however, the medium contained ITS and bovine serum albumin [39–42]. These cultures were used to measure the effect of insulin-like growth factor on proteoglycan synthesis. In these experiments, chondrocytes from all regions of the surface of the joint were pooled before plating.

The ultimate goal of our study was to refine the methodology and expand the utility of HOAC cell cultures in order to develop a reproducible platform for analyses of HOAC metabolism and measurements of clinically-relevant endpoints of OA, and ultimately, to test the efficacy of potential disease modifying OA drugs (DMOADs). In this report we describe the development of a serum, hormone and albumin free, three-dimensional alginate culture system for primary, human, osteoarthritic chondrocytes (HOACs) isolated from patients undergoing total knee arthroplasty. The endpoints of these experiments were cell yield and survival, the production and degradation of ECM molecules, and synthesis of the inflammatory cytokine interleukin-1 beta (IL-1β) in HOAC cultures established from the femoral condyles and tibial plateaus, as well as from the sides of greatest and least pathology. Our hypothesis was that HOACs would retain the OA phenotype for five days in culture.

Table 1
Comparison of cell yield and viability in different regions of the joint.

Sample Identification	Total Cell x10^6	Total Live Cell x10^6	% Viability
Lateral Femoral Condyle	2.55	1.75	69
Medial Femoral Condyle*	2.75	1.55	83
Lateral Tibial Plateau	1.38	0.775	57
Patella	0.65	0.325	50

Cartilage was isolated from the femoral condyles, tibial plateau and patella of Patient 1, a 73-year old female undergoing TKA. Tissues were dissociated in enzymes overnight. The trypan blue dye exclusion test was used to determine cell viability. % Viability = (total number of live cells ÷ total number of cells) × 100. * = side of greatest pathology. More living cells were obtained from the femoral condyles than the tibial plateau. The patella yielded the least amount of living cells.

2. Methods

2.1. Tissue procurement

Human knee joints were obtained from OA patients undergoing TKA following consent as outlined in the regulatory protocol approved by Cooper University Hospital's Institutional Review Board. All patients gave their informed consent to the study. This study was carried out in accordance with the Declaration of Helsinki and approved by the Cooper University Hospital's Institutional Review Board (protocol number 14-058EX).

Written informed consent was obtained from the patients listed in Tables 1 and 2 for publication of their individual details and accompanying images in this manuscript. The consent form is held by Cooper University Hospital in the patients' clinical notes and is available for review.

Standard total knee replacement cutting guides were used to remove articular cartilage and 3–4 mm of bone from the tibial plateaus and femoral condyles. Tissue was placed in a container with sterile saline. The extent of tissue damage was stage IV as radiographically evaluated according to Ficat classification [43,44].

2.2. Histological assessment of cartilage degradation

A small portion of the tissue was isolated from Patient 2 in the surgery-suite with a circular saw prior to chondrocyte isolation, and fixed in 2.5% paraformaldehyde for a minimum of 18 h at 4 °C. Tissue was decalcified with 10% ethylene diamine tetraacetic acid (EDTA) (Thermo Fisher Scientific, Philadelphia, PA) while rocking at 4 °C. The EDTA solution was changed every 48–72 h for 6–8 weeks. Tissues were dehydrated through a series of ethanols and zylenes, embedded in paraffin, sectioned at 4 µm and stained with hematoxylin and eosin (H & E, Poly-Scientific Research, Bay Shore, NY) or Saffranin O (ThermoFischer Scientific) [45,46]. The extent of tissue damage was graded by the Osteoarthritis Research Society International standard (OARSI) [47].

2.3. Preparation of primary cultures of HOACs

Cartilage was harvested from the femoral condyles, tibial plateau and patella, minced and digested overnight with 200 U/ml collagenase (Worthington Biochemical Corporation, Lakewood, NJ), 0.3% trypsin, 50 U/ml penicillin/streptomycin, and 15 U/ml amphotercin B in

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