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Toward understanding the role of cartilage particulates in synovial inflammation

A.M. Silverstein †, R.M. Stefani †, E. Sobczak †, E.L. Tong †, M.G. Attur §, R.P. Shah ‖, J.C. Bulinski ¶, G.A. Ateshian † ‡, C.T. Hung † *

† Department of Biomedical Engineering, Columbia University, New York, NY, USA
‡ Department of Mechanical Engineering, Columbia University, New York, NY, USA
§ Division of Rheumatology, Department of Medicine, New York University School of Medicine and NYU Langone Medical Center, New York, NY, USA
‖ Department of Orthopedic Surgery, Columbia University, New York, NY, USA
¶ Department of Biological Sciences, Columbia University, New York, NY, USA

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SUMMARY

Objective: Arthroscopy with lavage and synovectomy can remove tissue debris from the joint space and the synovial lining to provide pain relief to patients with osteoarthritis (OA). Here, we developed an *in vitro* model to study the interaction of cartilage wear particles with fibroblast-like synoviocytes (FLS) to better understand the interplay of cartilage particulates with cytokines on cells of the synovium.
Method: In this study sub-10 μm cartilage particles or 1 μm latex particles were co-cultured with FLS ±10 ng/mL interleukin-1α (IL-1α) or tumor necrosis factor-α (TNF-α). Samples were analyzed for DNA, glycosaminoglycan (GAG), and collagen, and media samples were analyzed for media GAG, nitric oxide (NO) and prostaglandin-E2 (PGE2). The nature of the physical interaction between the particles and FLS was determined by microscopy.
Results: Both latex and cartilage particles could be phagocytosed by FLS. Cartilage particles were internalized and attached to the surface of both dense monolayers and individual cells. Co-culture of FLS with cartilage particulates resulted in a significant increase in cell sheet DNA and collagen content as well as NO and PGE2 synthesis compared to control and latex treated groups.
Conclusion: The proliferative response of FLS to cartilage wear particles resulted in an overall increase in extracellular matrix (ECM) content, analogous to the thickening of the synovial lining observed in OA patients. Understanding how cartilage particles interface with the synovium may provide insight into how this interaction contributes to OA progression and may guide the role of lavage and synovectomy for degenerative disease.

Introduction

Tissue wear occurs as the result of articulation between load bearing surfaces[1]. Increased cartilage wear, leading to formation of loose particles in the joint space, has been linked to aging, overuse and obesity[2], often leading to osteoarthritis (OA). Several studies have investigated the use of synovial fluid aspirates to diagnose OA by characterizing cartilage wear particles in the synovial joint[3–5]. Clinically, it has been observed that cartilage and bone debris are bound to the surface or embedded deep within the synovial membrane of patients suffering from capsular synovial hyperplasia and metaplasia[6]. It has been hypothesized that the fibrotic shortening of the synovial capsule and synovitis result in OA-associated pain and contribute to further degradation of cartilage[6,7]. To provide pain relief to patients with OA, surgeons often use arthroscopy with lavage and synovectomy to remove tissue debris from the joint space and the synovium, however its success as a preventative measure has been widely debated[8,9]. Characterizing the interaction between cartilage particulates and the synovium is important for understanding why lavage is successful in some but not all patients;

*Address correspondence and reprint requests to: C.T. Hung, Columbia University, Department of Biomedical Engineering, 1210 Amsterdam Avenue, 351 Engineering Terrace, New York 10027, NY, USA. Fax: 1-212-854-8725.
E-mail addresses: ams2396@columbia.edu (A.M. Silverstein), rms2216@columbia.edu (R.M. Stefani), es3351@columbia.edu (E. Sobczak), elt2128@columbia.edu (E.L. Tong), mukundan.attur@nyumc.org (M.G. Attur), rs3464@cumc.columbia.edu (R.P. Shah), jcb4@biology.columbia.edu (J.C. Bulinski), ateshian@columbia.edu (G.A. Ateshian), cth6@columbia.edu (C.T. Hung).

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it may also explain how increased concentrations of cartilage debris may contribute to the progression of osteoarthritic changes via the synovium. Despite mounting *in vitro* and *in vivo* evidence of the deleterious effects of cartilage particulates on joint health, the mechanisms that mediate their negative interactions with synovial cells remain poorly understood.

In vivo studies have investigated the biological effects of cartilage wear particles on synovial joint health and disease progression in animal models[10–12]. For the latter, animals developed synovitis shortly after joint injection with particulates, which persisted and was accompanied by the infiltration of mononuclear cells and capsular, fibrotic thickening of the synovium[10]. Notably, the injected particles were still present and embedded within the synovium months later[10,11].

Monolayer cell and particulate co-cultures have previously been used to study the effects of biologic and synthetic particles on cells. While the most common method of determining particle-induced effects is to apply a specific number of particles per cell[13–15], measuring particulate mass per number of cells is also commonly used[16,17]. Adding wear particles of any material to cells can change cellular viability, metabolic activity, function and expression of OA specific markers. Specifically, treatment of human fibroblast-like synoviocytes (FLS) or chondrocytes with cartilage particles results in increased proteinase activity[16] and tumor necrosis factor-α (TNF-α) gene expression[17]. Lacking the availability of techniques to measure precisely the number or size of particles being used for treatment, previous studies examined biologic wear particles by mass, with an undefined size distribution. As such, it has been difficult to determine if the mechanism involved in driving the response of FLS to cartilage particulates is mediated by phagocytosis, cell surface contact or a combination of both.

Studies typically use synthetic particles that are 0.2–7 μm in size[13–15,18,19], as particles <15 μm in diameter, are readily phagocytosed by chondrocytes, macrophages, FLS and mesenchymal stem cells (MSCs)[20,21]. Compared to these non-biologic (metal or plastic) wear particles, biologic (cartilage) wear particles are more complex to study because they are composed of extracellular matrix (ECM) proteins that can be phagocytosed or degraded by, or bound to cells. This study aims to better understand the interplay of cartilage particulates with cytokines on cells of the synovium to foster the development of effective strategies to mitigate the inflammatory effect of cartilage particulates in OA.

Methods

Experimental design

Three studies are discussed in this paper (outlined in Fig. 1). Study 1 optimized the treatment dosage of sub-10 μm cartilage or 1 μm latex particles. Particle concentrations up to 1000 particles per cell were cultured for 5 days with confluent FLS monolayers to determine a dose that would elicit a cellular response without compromising cell viability ($n = 3–4$/group). The lowest particle dose that resulted in significantly altered metabolic activity, as determined by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay, while preserving cell viability, was selected for subsequent experiments. A dosage of 250 particles per cell was selected for future experimentation for both latex and cartilage particles.

In study 2, cells were co-cultured for 5 days with ±250 cartilage particles (CART) per cell in the presence or absence of 10 ng/mL of interleukin-1α (IL-1α, Life Technologies, Carlsbad, CA) or TNF-α (Life Technologies) ($n = 6–8$/group). These concentrations are previously established for studies of cytokine effects on joint tissues[22–24]. FLS were also cultured ±250 latex particles per cell to establish a baseline non-biologic particle response. In study 3, designated donor cultures were maintained in media in the presence (FLS + CART) or absence (FLS only) of 250 cartilage particles per cell for 5 days. In parallel, cell-free cultures with (CART only) or without particles (media only) were maintained for 5 days under the same conditions. Conditioned media was removed from the four donor culture groups (media only, CART only, FLS only, FLS + CART), and particles were removed via filtration with a 0.22 μm filter in the particle groups. This conditioned media was split with fresh growth media and added to the recipient FLS monolayers for 5 days ($n = 5–8$/group).

Isolation and culture of FLS and cartilage explants

Synovial tissue was harvested from three freshly slaughtered juvenile bovine knee joints (2–4 weeks old) and digested in collagenase type II (Worthington Biochemical Corporation, Lakewood, NJ) for 2 h with stirring at 37°C. Digested cells (FLS) were filtered through a 70 μm porous nylon mesh. Viable cells were counted and plated at a density of 1.76×10^3 cells/cm^2. To obtain a pure cell population, FLS were expanded for two passages in α-Minimal Essential Medium (α-MEM, Life Technologies) containing 10% fetal bovine serum (FBS), 1% antibiotic-antimycotic and bFGF-2 (Life Technologies)[25,26]. Articular cartilage explants were cored using a disposable biopsy punch (10 mm diameter, Acuderm® Inc., Fort Lauderdale, FL) and cultured in cartilage explant media[27] supplemented with 1% antibiotic-antimycotic, 1% non-essential amino acids (Sigma–Aldrich, St. Louis, MO), 1 mg/mL bovine serum albumin (BSA), 50 μg/mL ascorbic acid (Sigma–Aldrich), 0.01 μg/mL hydrocortisone (Sigma–Aldrich) and 0.002 μg/mL insulin (BD Biosciences, San Jose, CA) until use.

Creation and characterization of in vitro synovium model

Confluent FLS were trypsinized, counted, re-suspended and plated at a density of 50×10^4 cells/cm^2 in α-MEM (10% FBS, 1% antibiotic–antimycotic) supplemented with 50 μg/mL ascorbic acid to allow the formation of a dense cell monolayer. After 5 days, confluent monolayers were fixed in 4% paraformaldehyde (PFA, Sigma–Aldrich) overnight at 4°C before being washed in phosphate buffered saline (PBS, Life Technologies). Cell monolayers were stained to determine the presence of Type I collagen (rabbit polyclonal anti-collagen I, Abcam, Cambridge, MA), lubricin (rabbit polyclonal anti-lubricin, Abcam) and DNA (4′,6-Diamidino-2-Phenylindole, Dihydrochloride, DAPI, Life Technologies).

Particulate preparation and characterization of particles

Cartilage particles were generated aseptically from the superficial and middle zones by manually abrading cartilage submerged in phosphate buffered saline (PBS) with waterproof 120 grit sandpaper (McMaster-Carr, Elmhurst, IL). Potential residual sandpaper particulates were removed gravimetrically and via sub-sequent filtration, as was confirmed through microscopic inspection of the particulate solution. The resulting cartilage particle solution was sequentially filtered with 70 μm, 40 μm, and 10 μm porous nylon mesh filters to achieve a sub-10 μm particulate size. An aliquot of either cartilage particles or 1 μm latex particulates (Sigma–Aldrich) was further diluted in PBS, counted and sized using a Multisizer 4 Coulter Counter (Beckman Coulter, Brea, CA) to determine the concentration of both the cartilage particulate and latex particle solutions[28]. An average of four aliquots was used to determine the particle concentration of the stock solutions and the average