



Brief communication

Molecular resurfacing of cartilage with proteoglycan 4

K. Chawla^{a,*}, H.O. Ham^{a,d}, T. Nguyen^{a,1}, P.B. Messersmith^{a,b,c,d,**}^a Department of Biomedical Engineering, Northwestern University, Evanston, IL, USA^b Department of Materials Science and Engineering, Northwestern University, Evanston, IL, USA^c Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL, USA^d Chemistry of Life Processes Institute, Northwestern University, Evanston, IL, USA

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ABSTRACT

Early loss of proteoglycan 4 (PRG4), a lubricating glycoprotein implicated in boundary lubrication, from the cartilage surface has been associated with degeneration of cartilage and early onset of osteoarthritis. Viscosupplementation with hyaluronic acid and other macromolecules has been proposed as a treatment of osteoarthritis. However, the efficacy of viscosupplementation is variable and may be influenced by the short residence time of lubricant in the knee joint after injection. Recent studies have demonstrated the use of aldehyde (CHO) modified extracellular matrix proteins for targeted adherence to a biological tissue surface. It is hypothesized that CHO could be exploited to enhance the binding of lubricating proteoglycans to the surface of PRG4-depleted cartilage. The objective of this study was to determine the feasibility of molecular resurfacing of cartilage with CHO-modified PRG4. PRG4 was chemically functionalized with aldehyde (PRG4-CHO) and aldehyde plus Oregon Green (OG) fluorophore (PRG4-OG-CHO) to allow for differentiation of endogenous and exogenous PRG4. Cartilage disks depleted of native PRG4 were then treated with solutions of PRG4, PRG4-CHO, or PRG4-OG-CHO and then assayed for the presence of PRG4 by immunohistochemistry, ELISA, and fluorescence imaging. Repletion of cartilage surfaces was significantly enhanced with the inclusion of CHO compared with repletion with unmodified PRG4. These findings suggest a generalized approach which may be used for molecular resurfacing of tissue surfaces with PRG4 and other lubricating biomolecules, perhaps leading in the future to a convenient method for overcoming loss of lubrication during the early stages of osteoarthritis.

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1. Introduction

Articular cartilage is a stratified connective tissue located at the ends of long bones, and normally provides a load-bearing, low friction, wear-resistant surface. The compressive modulus of cartilage increases with depth from the articular surface and directly correlates with GAG content [1]. In the superficial zone, which corresponds to the uppermost 10% of the total cartilage thickness, the cells have a flattened morphology, are organized in clusters parallel to the articular surface [2], and exist at a high cell density compared with deeper zones [3]. Upon damage due to disease, injury or as a consequence of aging, articular cartilage possesses limited capacity for self-repair [4,5]. Osteoarthritis (OA), the most common

degenerative joint disease, begins with superficial fibrillation [6] and can result in extensive degeneration and eventual loss of cartilage.

The zonal organization of cartilage has implications for the biomechanical properties of the tissue. Boundary lubricants encoded by the gene *PRG4* [7] include: the protein products superficial zone protein (SZP) [8,9], secreted by chondrocytes of the superficial zone [9]; synoviocytes [8,9] and meniscal cells [10]; lubricin [11], abundant in synovial fluid [12]; megakaryocyte stimulating factor [13]; and proteoglycan 4 (PRG4). These highly homologous molecules are thought to contribute to the low friction properties of articular cartilage and can be referred to collectively as PRG4 [14]. The role of PRG4 as a boundary lubricant is supported by its presence at the surface of articular cartilage [9], its abundance in synovial fluid, its mutated form resulting in camptodactyly-arthropathy-coxa vararthritis syndrome [15], and its reduction of the friction coefficient when applied between natural and artificial materials [16–19]. In addition, expression and localization of PRG4 was found to be down-regulated in several animal models of OA [20–23] suggesting a relationship between loss of PRG4 and pathogenesis of OA.

Current clinical strategies for treating OA include intra-articular hyaluronic acid (HA) viscosupplementation. The efficacy

* Correspondence to: K. Chawla, Department of Chemistry, University of California, Irvine, CA 92697, USA. Tel.: +1 949 8249172; fax: +1 949 8242210.

** Corresponding author at: Northwestern University, Department of Biomedical Engineering, 2145 Sheridan Rd., Evanston, IL 60208, USA. Tel.: +1 847 467 5273; fax: +1 847 491 4928.

E-mail addresses: kanika@uci.edu (K. Chawla), philm@northwestern.edu (P.B. Messersmith).

¹ Present address: Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093, USA.

of viscosupplementation is variable [24,25] and may be influenced by the short residence time of HA in the knee joint after injection [24]. Further, treatment of rat knee joints with injections of recombinant lubricin [26], which bound to the cartilage surface, has indicated chondroprotective effects during the progression of OA [27], suggesting the benefits of lubricating molecules in the knee joint as a possible therapy for OA. While promising, one drawback of this potential treatment is the lack of semi-permanent adhesion to the cartilage surface, as in current viscosupplementation treatments. Approaches that enhance the presence of lubricating biomolecules at the tissue surface may offer advantages by increasing the biolubricant concentration at the boundary layer.

Recent studies have established chemical modification of extracellular matrix (ECM) proteins with aldehyde (CHO) groups as a viable approach for adhering biomolecules to a tissue surface. Chondroitin sulphate, one of the main ECM components in cartilage, was multifunctionalized with methacrylate and CHO groups in order to generate a bioadhesive which could be used for cartilage integration [28,29]. It was hypothesized that functionalization of lubricating biomolecules with CHO would enhance binding to the surface of PRG4-depleted cartilage. Thus, the objectives of this study were to prepare CHO-modified PRG4 (PRG4-CHO), and to determine whether the presence of CHO enhances immobilization of PRG4 to articular cartilage tissue surfaces.

2. Materials and methods

2.1. Cartilage harvest

Cartilage disks (6 mm diameter; ~0.3 mm thick, representing ~15% of the total cartilage thickness) were harvested from the patellofemoral groove and femoral condyles of bovine calve stifle joints (Research 87, Boylston, MA) using aseptic technique. Disks were cut to include the intact articular surface.

2.2. Cartilage culture and PRG4 purification

Cartilage disks were incubated in medium (low-glucose Dulbecco's modified Eagle's medium supplemented with 0.1 mM

non-essential amino acids, 0.4 mM L-proline, 2 mM L-glutamine, 100 U ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin, and 0.25 µg ml⁻¹ amphotericin B) supplemented with 0.01% bovine serum albumin, 10 ng ml⁻¹ human transforming growth factor-β1 and 25 µg ml⁻¹ ascorbic acid [30]. Disks were cultured for 12–19 days with culture medium collected every 3 days. PRG4 was purified as previously described [9,30]. Briefly, pooled culture medium was fractionated by anion-exchange chromatography. The sample was applied on DEAE-Sepharose, previously equilibrated with 0.15 M NaCl, 0.005 M EDTA, and 0.05 M sodium acetate, pH 6.0. The 0.3–0.6 M eluate was collected, concentrated with a Centricon Plus 100 kDa MW cutoff filter, and then quantified for protein content by bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL). The presence of PRG4 protein was confirmed by Western blot analysis using monoclonal antibody 4D6. Samples (0.5 µg of total PRG4 per lane) were separated on a 4–15% gradient polyacrylamide gel by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and transferred overnight to a polyvinyl difluoride membrane. The membrane was probed with mAb 4D6 using the ECL Plus detection system (GE BioSciences, Piscataway, NJ). Production of monoclonal antibody 4D6 was directed against purified PRG4 at the Monoclonal Antibody Facility at Northwestern University (Chicago, IL). Mouse hybridoma cell lines secreting PRG4-specific antibody were generated and then purified (data not shown).

2.3. Chemical modification of PRG4

PRG4 was chemically modified with CHO only (PRG4-CHO) or both CHO and Oregon Green (PRG4-OG-CHO) as shown in Fig. 1. OG functionalization was employed to distinguish between applied (exogenous) and native (endogenous) PRG4. PRG4-CHO was prepared by modifying PRG4 with succinimidyl 4-formylbenzamide (Solulink, San Diego, CA, hereafter referred to as CHO) at a PRG4:CHO molar ratio of 1:1000 in 100 mM PO₄ buffer, pH 8.5, overnight at 4 °C with shaking. To prepare PRG4-OG-CHO, purified PRG4 in solution was combined with OG 488 carboxylic acid, succinimidyl ester (OG-SE, Molecular Probes, Carlsbad, CA) at a PRG4:OG molar ratio of 1:100 and agitated for 3 h at room temperature. Excess and unreacted OG-SE was removed by illustra NAP-5 column purification (GE Healthcare, Piscataway, NJ). PRG4-OG was

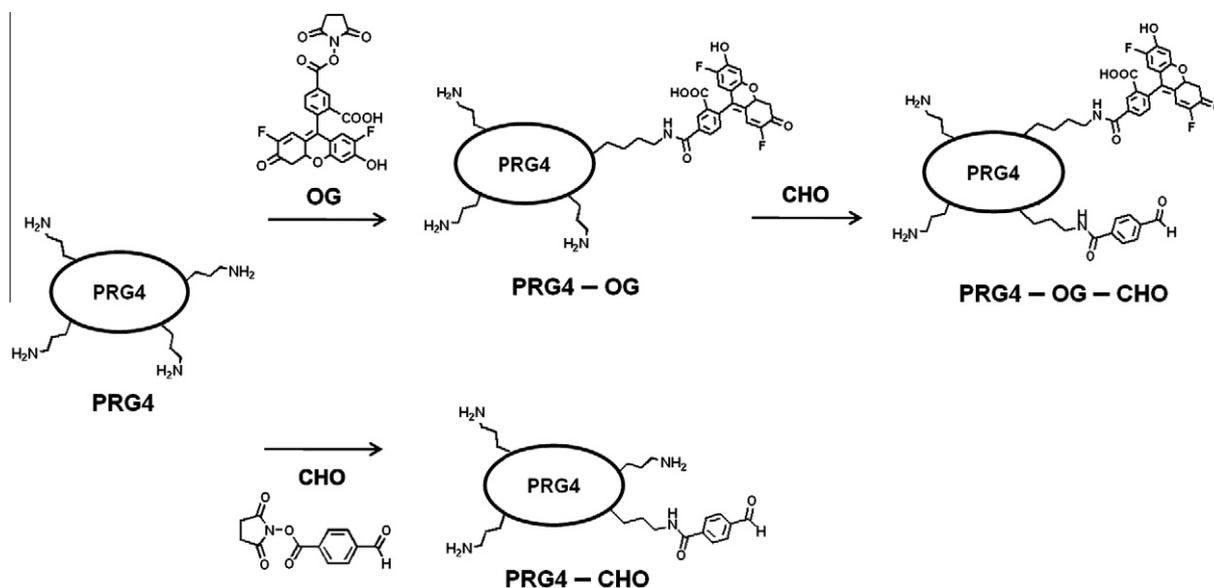


Fig. 1. Chemical modification of PRG4 with OG and CHO. Purified PRG4 was reacted with succinimidyl ester of OG 488 carboxylic acid or succinimidyl-4-formylbenzamide (S-4FB), generating PRG4-OG or PRG4-CHO, respectively. A portion of PRG4-OG was further reacted with S-4FB to generate PRG4 conjugated with both OG and CHO (PRG4-OG-CHO).

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