Chemically Functionalized Proteoglycan 4 (PRG4) for Cartilage Resurfacing

Kanika Chawla[1], Hyun Ok Ham[2], Trung Nguyen[3], and Phillip B. Messersmith[2]
[2]Department of Biomedical Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL
[1]current address: Department of Chemistry, University of California-Irvine, Irvine, CA; [3]current address: Sirigen, Inc., San Diego, CA

Statement of Purpose: Early loss of proteoglycan 4 (PRG4), a lubricating glycoprotein implicated in boundary lubrication[1], from the cartilage surface has been associated with degeneration of cartilage[2]. Treatment of rat knee joints with recombinant lubricin[3] injections has indicated chondroprotective effects[4], 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. Recent studies have indicated the versatility of chemically modifying extracellular matrix proteins with aldehyde groups (CHO) for targeted adherence to a biological tissue surface[5]. We hypothesized that chemically functionalized PRG4 (PRG4-CHO) would enhance deposition of PRG4 onto the cartilage surface. The objectives of the study were to determine: (1) feasibility of resurfacing cartilage with PRG4-CHO, and (2) whether CHO enhances surface modification of cartilage with PRG4.

Methods: Articular cartilage disks were harvested from bovine calf knees (n=5). PRG4 was purified as previously described[1,5]. Purified PRG4 was functionalized with Oregon Green (OG) 488 carboxylic acid, succinimidyl ester (PRG4-OG) to distinguish between exogenous and endogenous PRG4. PRG4-OG was then functionalized with succinimidyl 4-formylbenzoate to yield PRG4-OG-CHO. Tissue samples were incubated in 1% SDS followed by incubation in PBS or a PRG4 solution: PRG4, PRG4-CHO, PRG4-OG, PRG4-OG-CHO. The amount of PRG4 adhered to articular cartilage was determined by guanidine extraction and indirect ELISA[6]. Modified surfaces were visualized by immunohistochemistry (IHC) Sections were reacted with mAb 4D6 for PRG4, or mouse IgG, and detected with rhodamine-conjugated secondary antibody.

Results: CHO functionalization of PRG4 enhanced the concentration of PRG4 adherent to the cartilage surface (**Fig. 1**). Samples repleted with non-functionalized PRG4 had similar amounts of PRG4 at the surface (0.5±0.1 μg/cm^2, p=0.9) compared to undepleted control samples. The amount of PRG4-CHO and PRG4-OG-CHO on cartilage surfaces was 2.0-fold higher than non-functionalized PRG4. PRG4-CHO and PRG4-OG-CHO adhered to the cartilage surface in similar amounts (1.1±0.3 μg/cm^2, p=0.9). Samples depleted of PRG4 by SDS treatment had 5.0-fold less PRG4 than samples repleted with PRG4-CHO, or PRG4-OG-CHO (p<0.001). IHC revealed PRG4 was present at the surface of control (**Fig. 2C**) samples but absent from surfaces treated with SDS (**Fig. 2F**) treated samples. Samples which were further repleted with PRG4 (**Fig. 2I**), PRG4-CHO (**Fig. 2L**), or PRG4-OG-CHO (**Fig. 2O**) showed presence of PRG4 at the surface. Functionalization with OG (**Fig. 2N**) indicated PRG4 at the surface to indeed be exogenous PRG4, distinct from endogenous PRG4 (**Fig. 2O**).

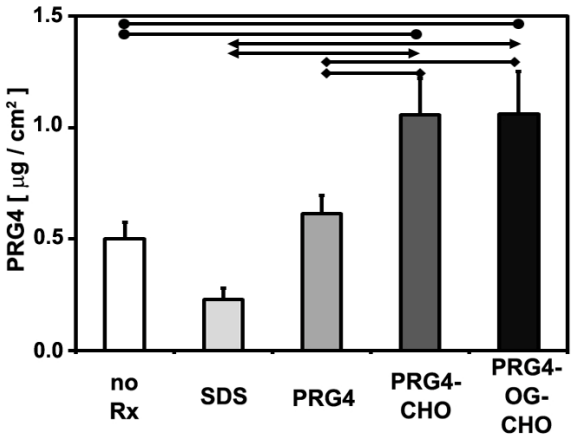

Figure 1. PRG4 content of cartilage disks which were dissociated (□) and then repleted with PRG4 (▨), PRG4-CHO (■), and PRG4-OG-CHO (■). Mean±SEM, n=4-6, (▲) p<0.001, (●) p<0.01, (♦) p<0.05.

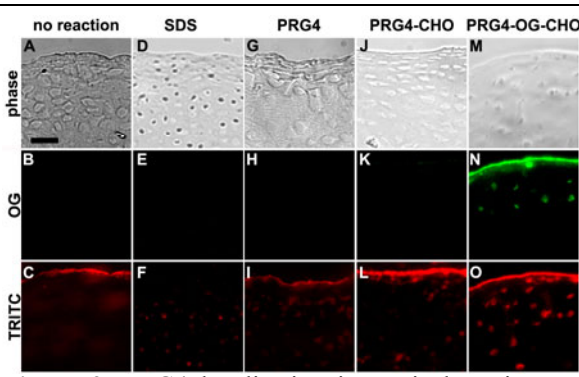

Figure 2. PRG4 localization in vertical sections of control (**A-C**), SDS-treated (**D-F**), PRG4 (**G-I**), PRG4-CHO (**J-L**), or PRG4-OG-CHO (**M-O**) repleted cartilage samples. Bar=50 μm.

Conclusions: These findings are the first to demonstrate that PRG4 can be chemically functionalized and significantly enhance the adherence of PRG4 to cartilage surfaces. The increase in PRG4 concentration at the cartilage surface, due to chemical modification, suggests a possible therapeutic benefit of locally immobilizing lubricating molecules at the cartilage surface. In the future, our studies will explore the lubrication potential of modified cartilage surfaces as well as the translation of these studies to the *in vivo* environment to determine duration of efficacy.

References: 1. Schmidt TA. Arth Rheum. 2007;56: 882-891. 2. Young AA. Arth Res. Ther. 2006;8:R41. 3. Jones AR. J. Orthop Res. 2007;25:283-292. 4. Flannery CR. Arth Rheum. 2009;60:840-847. 5. Schumacher BL., ABB, 2004; 311: 144-152. 6. Nugent-Derfus GE. J Orthop Res. 2007; 25: 1269-1276.