Tethering Lubricin/PRG4 to Improve Regenerative Healing of Fibrocartilaginous Tissues

COLUMBIA
COLLEGE OF DENTAL MEDICINE

[+]Julian I. Bensadoun, [+]Jeffrey T. Felix, Solaiman Tarafder, Chang H. Lee*
+Co-first authors; *Faculty mentor

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INTRODUCTION

- We have devised a regenerative approach for injured fibrocartilaginous tissues such as knee meniscus and TMJ discs (**Fig. 1**).
- Lubricin/PRG4, an abundant protein in synovial fluids (SF) and on articular surfaces, may disrupt healing by preventing cell/tissue adhesion.
- This study is designed to 1) investigate the mechanism of lubricin/PRG4 retention on the injured surface of intra-synovial tissues and 2) to explore strategies to facilitate healing of fibrocartilaginous TMJ disc and knee meniscus tissues by tethering the infiltrated lubricin/PRG4 on injured surface.

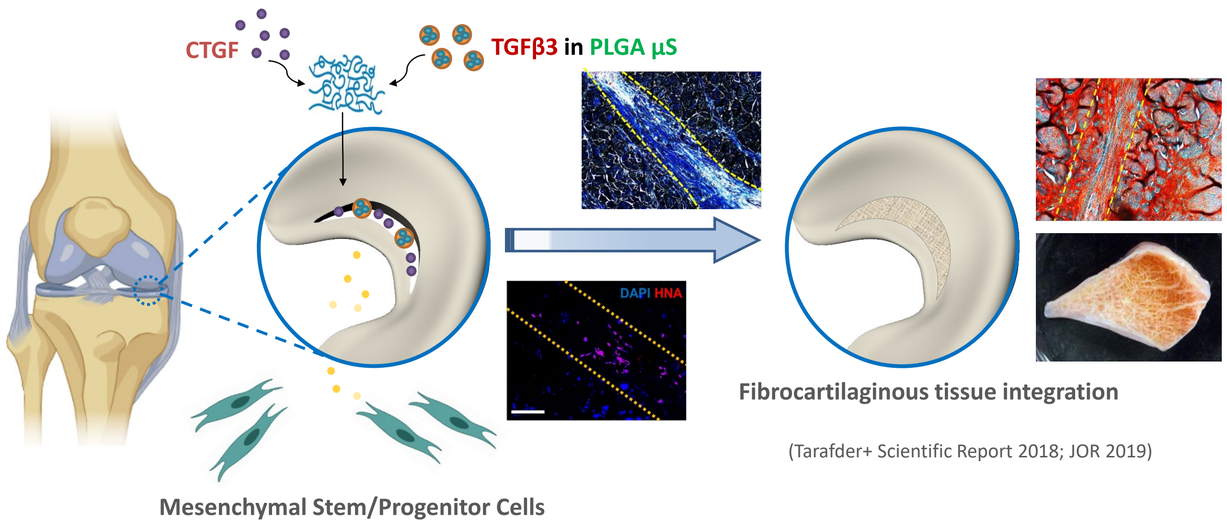


Fig. 1. Regenerative strategy to heal injured fibrocartilaginous tissues by stem cell recruitment and controlled delivery via bioadhesives.

RESULTS

Injured menisci are infiltrated by lubricin/PRG4 associated with HA and COL-II

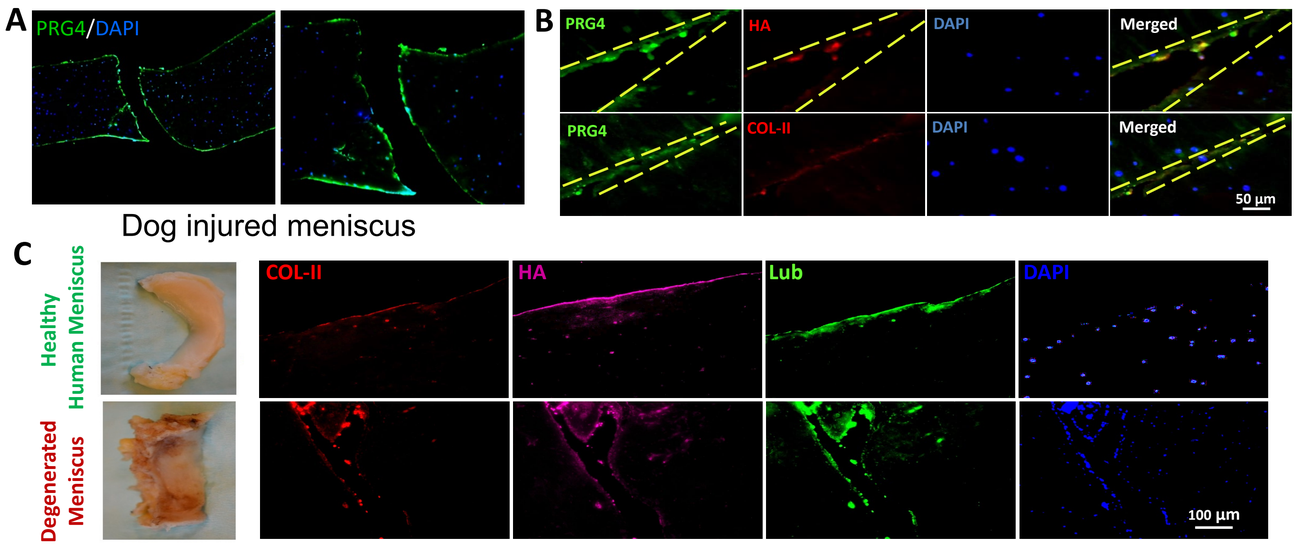


Fig. 2. PRG4 is retained on injured canine meniscus tissue (**A**). Lubricin/PRG4 is associated with Hyaluronic acid (HA) and Collagen II (COL-II) deposition (**B**). In human samples, healthy meniscus showed consistent lubricin/PRG4 expression only on surface but infiltrated into the injured surface in the degenerated tissues (**C**).

HA and COL-II pre-coating promotes Lubricin/PRG4 deposition on meniscus surface

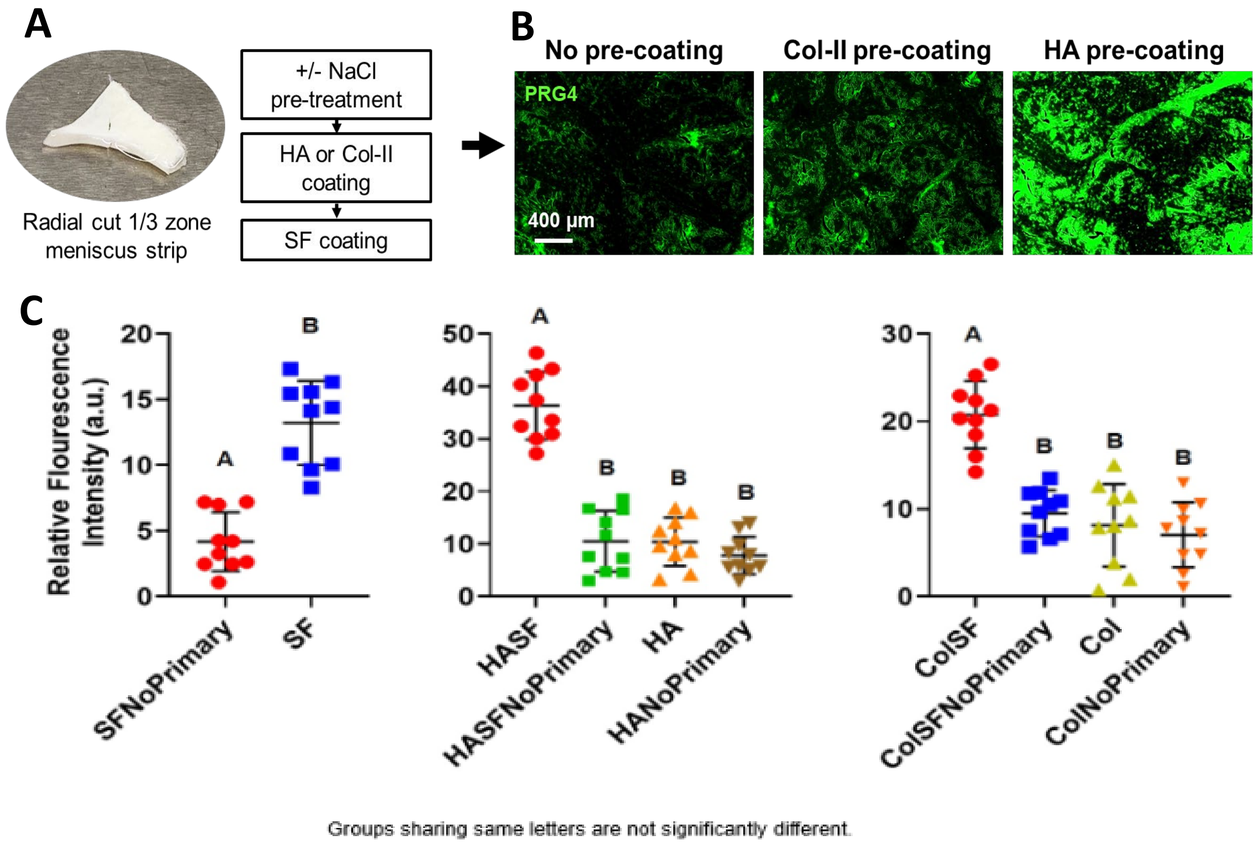
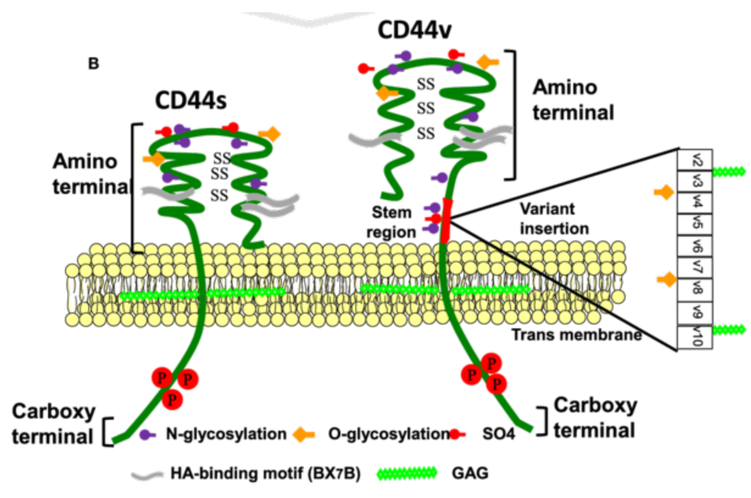


Fig. 3. Canine meniscus sections were treated with NaCl to deplete the surface of PRG4 and were coated with SF with and without a COL-II or HA pre-coating (**A**). Pre-coating with HA significantly improved PRG4 retention (**B**). Quantitatively, HA pre-coating showed the highest fluorescence intensity ($p<0.0001$; $n = 10$), and Col-II pre-coating yielded an increased fluorescent intensity compared to control ($p<0.0001$; $n=10$) **(C)**.

CD44 to bind through lubricin/PRG4



Characteristics of CD44

- Transmembrane glycoprotein (MW of 85–200 kDa).
- Widely distributed in the central nervous system, the lung, and the epidermis.
- Acts as a cell surface adhesion receptor with strong affinity to PRG4.
- Interacts with various ligands including HA, osteopontin (OPN), collagens, and matrix metalloproteinases (MMPs).

In vitro binding test of CD44 with lubricin/PRG4

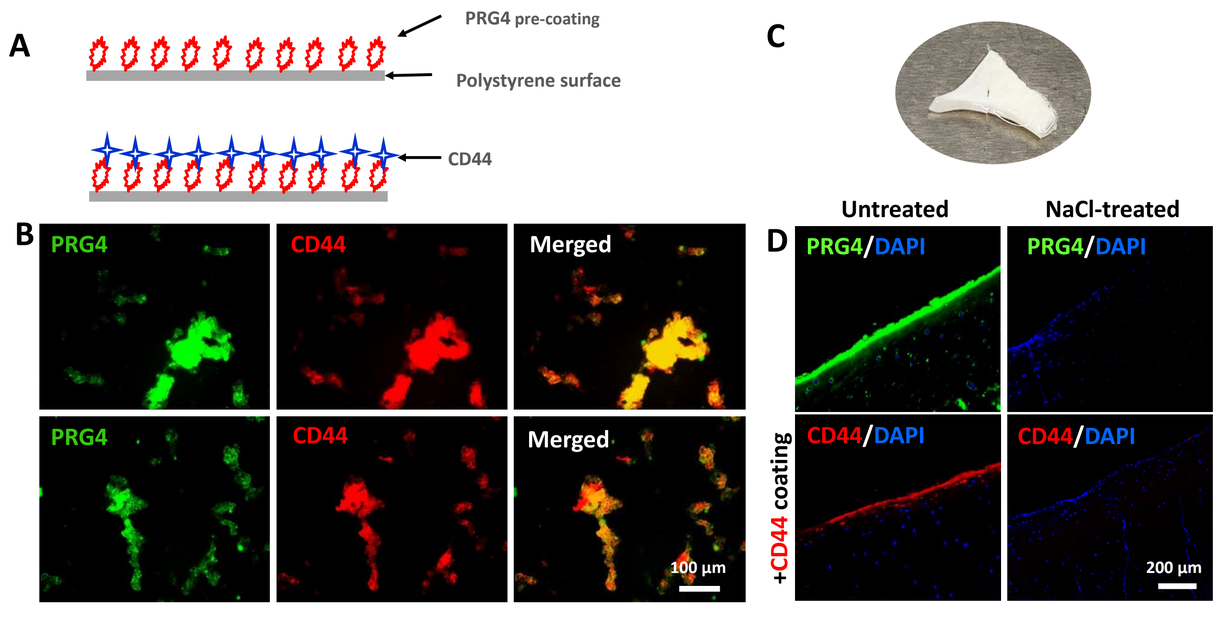


Fig. 4. PRG4 coating on a CD44-precoated polystyrene culture plate showed co-localization of PRG4 and CD44 (**A, B**). Similarly, CD44 binding was prominent on the PRG4-abundant injured meniscus surface compared to a PRG4-depleted injured meniscus surface (**C, D**).

CD44-bound bioadhesives improve healing of lubricin/PRG4-infiltrated meniscus injuries

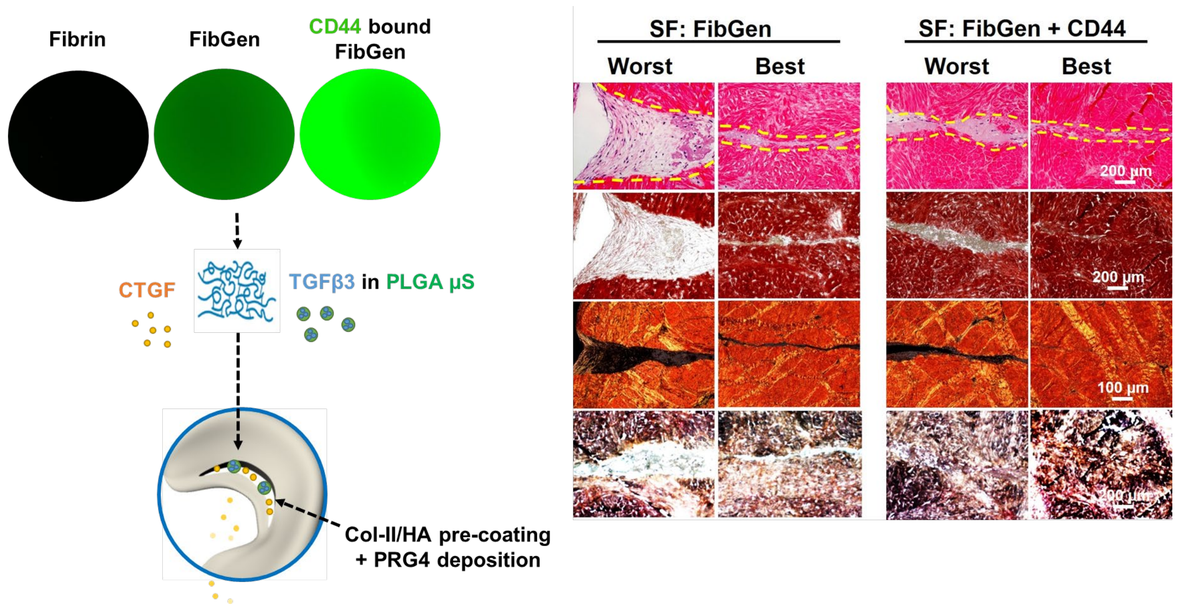


Fig. 5. CD44-bound fibrin crosslinked with genipin (FibGen) was prepared, confirmed by fluorescence dye, and applied to our explant healing model (**A**). After 6 weeks, CD44-bound FibGen showed improved healing of injured meniscus tissue (**B**).

DISCUSSION

- Our data suggest that hyaluronic acid (HA) plays an important role in allowing the binding of lubricin/PRG4 to torn meniscus surfaces.
- CD44 is a surface receptor expressed in various cells including synovial MSCs that has a strong binding affinity both to lubricin/PRG4 and HA.
- To tether the surface pre-coated lubricin, we developed CD44-bound bioadhesives.
- Our CD44-bound FibGen tightly binds Fibrin/Fibrinogen, and has improved adherence to torn meniscus tissue via the interaction with HA and through tethering of lubricin/PRG4.
- CD44 bound FibGen may serve as an efficient bioadhesive to support healing of clinically relevant meniscus tears by endogenous stem cell recruitment.

ACKNOWLEDGEMENTS

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