Biomolecule loaded bioadhesive for the rotator cuff tendon repair by promoting endogenous progenitor cell response.

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INTRODUCTION: Rotator cuff (RC) tendon-related pathologies account for 70% of shoulder pain and dysfunction in the adult population, which can occur due to either mechanical overuse or inflammation. Though surgical intervention to repair RC tear using a suture anchor is routinely done, the failure rate of these treatments is high, and the re-tears are a common occurrence because of poor regenerative capability of tendon. While tissue engineering scaffold has been developed to bridge the tendon tissue, these approaches fail to close the tear gap on partially ruptured tendon, which occurs in a majority of the incidence, which leads to fatty infiltration and weak mechanical strength of RC [1]. Currently, many cell-based approaches have been evaluated for the RC tendon repairment with some short-term successes [2]. However, the translational potential of these strategies is limited due to high cost and long processing time for stem cell extraction, scaffold seeding and then implantation [3]. To overcome this limitation, we have developed a new treatment in which torn RC tendon tissue was reattached with a biomolecule-releasing bioadhesive and then secured with a suture anchor. The bioadhesive can actively promote tendon tissue regeneration by promoting endogenous progenitor cell responses via localized release of growth factor at the tear site. This study aims to test its biological activities and safety *in vitro* and therapeutic efficacy using an in vivo chronic rotator cuff tear rat model.

METHODS: A click-chemistry based chitosan (CS) adhesive invented in our laboratory was modified for rotator cuff tear treatment [4]. *Boyden Chamber cell migration study:* Rat tail tendon cells were freshly extracted and cultured in DMEM 10% 1% (10% Fetal bovine serum and 1% Penn strep) until passage 2. 24 hours starved cells were loaded in the top chamber and various growth factors (PDGF, VEGF, SDF 1a and TGF-1b) diluted media was loaded in the lower chamber. After 72 hours, the membrane of the chamber was stained with DAPI to analyze the migrated cells. *Animal model:* This study is approved by the IACUC. Sprague-Dawley rats (200-300g weight, n = 12) were divided into 3 chronic tear groups: (1) healthy, (2) suture only treatment, (3) suture + bioadhesive + growth factor. A chronic RC tendon tear model was made, by partially cutting the tendon (50%) and leaving it without treatment for 4 weeks [5]. After 4 weeks, the torn tendon was exposed and repaired. PDGF-releasing adhesive was injected into the tear and the torn tendon was firmly pressed together to facilitate bonding prior to suture anchor placement. The deltoid muscle and subcutaneous skin were closed with sutures and skin staples. *Histological analysis* was done at 0, 2 post- second surgery, analyses of cell/tissue responses were performed to assess the integrity of the tissue (H&E), inflammatory response (CD11b), extracellular matrix (ECM) regeneration (Toluidine Blue), and progenitor cell recruitment (CD44 and CD 73) in response to the various treatments. Images were analyzed and quantified using the ImageJ software and GraphPad Prism. *Functional activity analysis* was used to evaluate the shoulder function every 72-hour intervals post the repair surgery, using inked paw prints on white paper in an in-house walkway system to measure the rat's stride length (distance from hand strike to next hand strike of the same hand) and step length (distance from hand strike to next hand strike of the opposite hand).

RESULTS: The adhesive and protein release properties were previously evaluated. The protein release study previously showed the burst release in the first 18 hours followed by a sustained release for up to 14 days. After 72 hours of incubation, the migration study revealed a substantial (~80% of total cells) migration of tendon cells in response to PDGF. Notably, the PDGF group exhibited significantly higher cell density (> 2x) per unit area compared to other growth factors, such as VEGF, SDF-1a, and TGF-1b (**Figure 1A**). The tissue regenerative properties of PDGF-releasing bioadhesive were then evaluated *in vivo*. The adhesive + PDGF treatment groups displayed increased cellularity at the tear site, contrasting with decreased cellularity in suture-only samples. The release of PDGF is likely to promote the recruitment of CD44+/CD73+ progenitor cells, since the progenitor cell count was found to be significantly higher (>3x) in the PDGF adhesive group than the suture-only group (**Figure 1B**). PDGF-releasing adhesive treatment is also found to promote extracellular matrix production. Specifically, toluidine blue staining revealed that glycosaminoglycan (GAG) intensity in PDGF-releasing adhesive treated tendons is ~2x higher than those with suture control (**Figure 1C**). Functional gait analysis showcased the PDGF releasing adhesive group possesses significantly better overall gait performance (stride length (**Figure 1D**) and width) post-surgery than suture-only group.

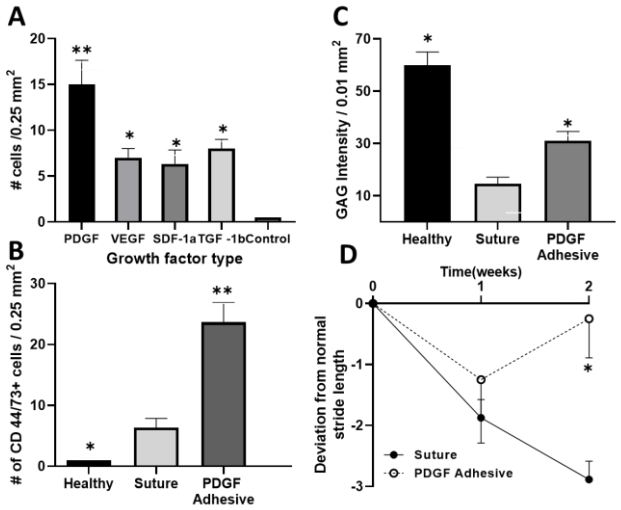

*Figure 1: (A) In vitro migration study indicating # of migrated cells after 72 hours incubation (B) Colocalized CD44+/CD73+ progenitor cell density. (C) Tendon GAG intensity. (D) Relative deviation of Stride length from healthy marked as 0. Data represented as mean ± SD. Student's t –test: ** indicates p< 0.01*

DISCUSSION: All the results support our general hypothesis and objective that growth factor releasing bioadhesive can be made to enhance endogenous progenitor cell recruitment and response and to promote tissue regeneration of torn tendon. PDGF was selected for the *in vivo* study since many others, and we have found that PDGF presents great potency for recruiting tendon cells and progenitor cells [6]. The chronic and partial transected tendon tear model was used in this study to simulate the most common rotator cuff tear condition. By analyzing the rotator cuff and surrounding tissue, we have observed that there is a significant increase of CD44+/CD73+ progenitor cells near the tear area. In fact, studies have shown that abundant CD44+/CD73+ progenitor cells can be found in the tissue surrounding RC tendon, namely the synovial and bursa lining [7]. While long-term study is still ongoing, our results have shown that the active recruitment of progenitor cells to the torn RC tendon tissue can not only increase tissue GAG production but also enhance the shoulder function recovery based on gait analyses.

SIGNIFICANCE/CLINICAL RELEVANCE: This work is aimed at developing a new treatment for chronic partial torn RC tendon in which current treatment is not effective and associated with a high failure rate. In fact, our results have shown that PDGF bioadhesive application can significantly enhance torn RC tissue and functional recovery by eliciting the regenerative activities of endogenous progenitor cells in the joint capsule. This new treatment has great translational potential since it complements current treatment with a suture anchor and can promote RC tissue regeneration with patients' own progenitor cells.

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