Modulating early matrix remodeling in cartilage repair inhibits contraction but exacerbates fibrosis

Hanna Solomon[1,2], Maddie Hasson[1,2], Lorenzo Fernandes[1,2], Jay M. Patel[1,2]
[1] Emory University Department of Orthopaedics, Atlanta GA. [2] Atlanta VA Medical Center, Decatur GA.
Disclosures: *Jay M. Patel (3B - NovoPedics Inc; 4 – NovoPedics Inc; 4 – Forsagen LLC)*

Introduction: Focal cartilage injuries are a common musculoskeletal malady, which is a cause of concern due to its lack of ability to regenerate on its own. The most common form of repair is marrow stimulation (e.g., microfracture; MFx), which involves puncturing the subchondral bone to recruit marrow elements into the defect to create a provisional clot for tissue regeneration. However, marrow stimulation has poor long-term outcomes, typically resulting in inadequate defect fill and inferior fibrous tissue formation, both of which cause wear to the repair and surrounding cartilage, thereby accelerating OA. The augmentation of the MFx clot has been long attempted with bioactive factors, scaffolds, and cell implantation. These efforts have focused on improving chondrogenesis; yet we may need enhancements that maintain volumetric fill and battle fibrosis. Specifically, the role of early remodeling of the provisional marrow clot is unexplored, though it likely has implications in eventual tissue repair. Our objective was to understand the role of early matrix remodeling (via fibrinolysis) on volume maintenance and fibrotic potential of simulated MFx environments.

Methods: The Early MFx Environment: In a rabbit model, full thickness trochlear defects were created in 3 animals, followed by microfracture with awl. Tissues were retrieved at 1 week for histology (Gomori Trichrome, COL1, α-smooth muscle actin [α-SMA]). Simulated MFx Environment: Marrow-derived cells (MDCs) were isolated from juvenile bovine femoral condyles and encapsulated within fibrin gels. Fibrin Microgel Studies (10uL): To understand how aprotinin (fibrinolysis inhibitor) affects early remodeling, matrix deposition, and mechano-sensation of MDCs in fibrin gels, fibrinogen (final concentration: 10mg/mL) was mixed with thrombin (5U/mL), calcium chloride (20mM), and juvenile bovine marrow-derived cells (200k/mL) in an 8-well chamber slide and allowed to gel for 60 minutes. Gels were cultured for 3 days in aprotinin (100 KIU/mL) and/or TGF-β3 (10ng/ml), fixed/stained (Phalloidin, α-SMA, YAP/TAZ, fibronectin), and imaged with confocal microscopy (Nikon A1R) to visualize cell response. A subset of gels were fabricated with fibrinogen-AlexaFluor488 to visualize fibrin remodeling. Macro-Scale Fibrin Gel Studies (100uL): To investigate macro-scale gel contraction and cell behavior, fibrinogen (final concentration: 50mg/mL) was combined with thrombin (10U/mL), calcium chloride (20mM), and MDCs (2M/mL), and gelled. Constructs were cultured for 7 days in chemically-defined media (control, aprotinin, TGF-β3, aprotinin+TGF-β3) and imaged at the terminal point to measure contraction. Terminal gene expression of urokinase-type plasminogen activator (uPa), α-SMA, and plasminogen-activator inhibitor (PAI-1) was performed.

Results: The early MFx environment from our rabbit trochlear model (**Fig 1A**) showed significant signs of contraction (**Fig 1B**) and fibrosis (α-SMA and COL1; **Fig 1C**) as early as 1 week post-operatively. Furthermore, in simulated MFx gels, we showed that the common chondrogenic agent TGF-β3 increases fibrinolysis even further via higher expression of uPa (**Fig 1D**), leading to rapid gel contraction. Interestingly, TGF-β3 also caused α-SMA stress fiber formation (**Fig 1E**), with increased co-localization with F-actin (**Fig 1F**), indicating early fibrotic activity. With regards to cell microenvironment, TGF-β3 led to considerable remodeling of the fibrin network, a response that was mitigated by inhibiting fibrinolysis with aprotinin (**Fig 2A**). Aprotinin also led to decreased production of fibronectin, both in the absence and presence of TGF-β3 (**Fig 2B; Fig 2C**). Interestingly, aprotinin and TGF-β3 both increased YAP/TAZ nuclear localization in MDCs, with an additive effect when both were applied (**Fig 2D**). Finally, gels cultured with aprotinin alone maintained their area compared to the control, whereas gels treated with TGF-β3 contracted significantly (**Fig. 3A**); aprotinin partially reduced this TGF-β3-mediated contraction. Aprotinin and TGF-β3 both increased pro-fibrotic expression (α-SMA, PAI-1) (**Fig 3B; Fig 3C**), a result that was additive in combination.

Discussion: In this study, microfracture exhibited contraction and fibrosis as early as one week, and the addition of TGF-β3, a known chondrogenic agent, led to increased fibrinolysis, contraction, and early fibrosis. Aprotinin demonstrated less multi-scale fibrin remodeling and less fibronectin deposition, whereas TGF-β3 increased fibrin remodeling and fibronectin deposition. Interestingly, both aprotinin and TGF-β3 synergistically increased YAP/TAZ activity, which was unexpected since we theorized it would follow the same trend as fibronectin deposition. While antifibrinolytics may prevent marrow clot contraction, they seem to enhance the fibrosis of MDCs.

Significance/Clinical Relevance: Antifibrinolytics may prevent marrow clot contraction and maintain defect fill, but they enhance fibrosis of marrow-derived cells in the early repair environment. Thus, alternative methods that combat both contraction and fibrosis may be required.

References: [1] Erggelet+, J Clin Orthop, 2016. [2] Smith+, Bioconjug Chem, 2007.

Acknowledgement: Emory Orthopaedics; Regenerative Engineering Medicine (REM).

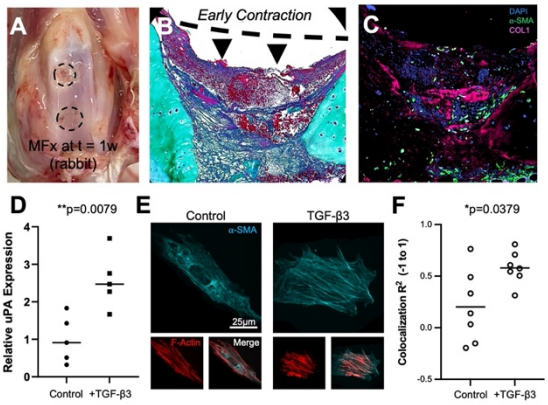

Figure 1. (A) Macro-scale image of MFx at one week in a rabbit model. (B) Gomori Trichrome and (C) α-SMA/COL1 staining of defect. (D) uPA expression of control and TGF-β3 group in 3D gels cultured for 7 days. (E) MDCs cultured for 3 days (+/- TGF-β3) in fibrin gels, stained for α-SMA. (F) α-SMA colocalization with F-Actin in control and TGF-β3 group.

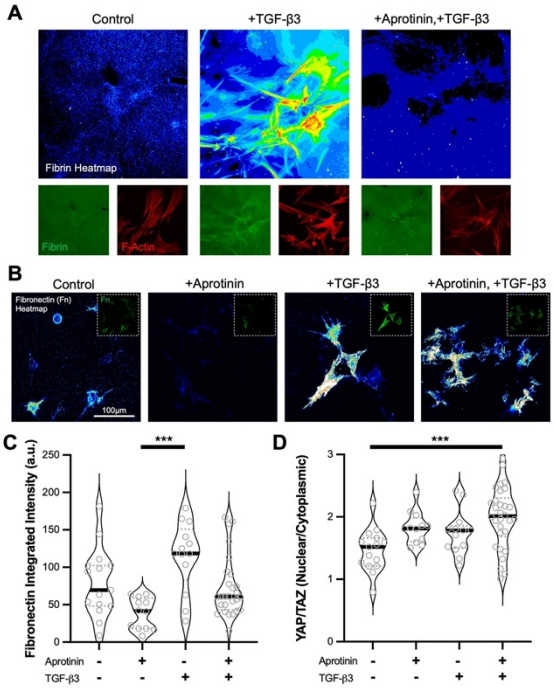

Fig 2. (A) MDCs cultured for 3 days in labeled fibrin gels (+ aprotinin and/or TGF-β3). (B) MDCs cultured for 3 days (with aprotinin and/or TGF-β3) and stained for fibronectin. (C) Fibronectin deposition per cell. (D) YAP/TAZ nuclear localization of MDCs. *** represents p<0.001.

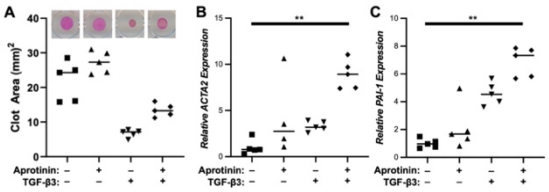

Fig 3. (A) Areas of fibrin gels cultured out for 7 days and measured at the terminal point. (B) α-SMA (ACTA2) and (C) PAI-1 expression of MDCs in fibrin gels at 7 days. ** represents p<0.01.