Title (bolded)

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Note: Please list all authors and affiliations, and underline the presenting author. The below format (Intro/Methods/Results…) are recommended but not mandatory. Abstracts in a paragraph form (without subsections) will also be accepted. Figures are recommended but optional. Please use Arial font, Size 10 for everything except the title (Size 12). Margins should be at least 0.5" on each side. Your abstract MUST fit within one 8.5" x 11" page, and abstracts can only be submitted as a PDF. Delete this note before submission.

Introduction: Describe the motivation behind your work, background details, gaps in the field, and relevance to Veterans. A hypothesis/study objective is also highly recommended.

Methods: Briefly describe methods used to conduct your research.

Results: Provide research observations and findings, with references to figures (**Figure 1A**) and/or tables (**Table 1**).

Discussion:

Significance/Clinical Relevance (optional**):** This may be combined with the discussion.

References (optional**)**: Citation information can be shorted, e.g., [1] Kowalski + JTERM, 2022.

Acknowledgement (optional**):** Funding, support, personnel not included as authors.

Figures (optional): Provide up to two figures (can have subpanels), making sure that text within figures is legible. Provide enough information for readers to interpret figures (subpanel descriptions, sample sizes, p-values, etc). If figures/tables are included, it is recommended that they appear at the bottom or sides of the abstract, with square text wrapping.

The influence of initial clot properties and cell contractility on the microfracture environment

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Introduction: Cartilage injuries are one of the most common musculoskeletal injuries. Due to the tissue's inherent lack of regenerative capacity, it is unable to self-heal. One of the most common cartilage repair techniques is microfracture, which involves puncturing the subchondral bone to recruit marrow elements. While this treatment provides short-term relief, inferior fibrous tissue often forms, which has limited functional properties and is highly susceptible to wear. Certainly, the deposition of fibrous tissue after microfracture has been established, yet the early players in the microfracture clot remain unexplored. Thus, the purpose of this study was to evaluate the influence of initial clot properties and cellular contractility on contraction of and regeneration within the microfracture clot. Establishing a clearer picture of cell behavior within the microfracture clot may lead to an enhanced understanding of its fibrosis propensity and provide routes towards new cartilage formation.

Methods: Cell Culture: To simulate the microfracture environment, we isolated marrow-derived cells from juvenile bovine femoral condyles. Bone marrow chunks were rinsed vigorously in heparin media (0.2% w/v), and the resulting solution was processed through a Ficoll gradient to isolate mononuclear cells. These cells were expanded, and P1-P2 cells were utilized for the described studies. 3D Macro-Scale Fibrin Gel Studies: Fibrinogen (final concentration: 50mg/mL) was combined with thrombin (1-20U/mL), calcium chloride (20mM), and PBS, and gelled at 37°C for 60 minutes. Gels were mechanically tested in unconfined compression between two platens to 20% strain to obtain elastic modulus. Fibrin gels were seeded with marrow-derived cells and cultured for 4 weeks in control media or media containing Fasudil (Rho inhibitor). Clots were imaged three times per week to measure temporal clot area. Both thrombin concentration during gelation (2, 20 U/mL) and Fasudil concentration during culture (0, 10, 50μM) were tested to elucidate the roles of clot structure and cell contractility, respectively. 2D Micro-Scale Fibrin Studies: Fibrinogen (final concentration: 5mg/mL) labeled with CruzFluor 405 (1% w/w) was mixed with thrombin (1-5U/mL) and calcium chloride (20mM) in an 8-chamber slide and allowed to gel for 60 minutes. Marrow-derived cells were then seeded on top of gels, cultured for 24 hours (control or 50uM Fasudil), and fixed/stained (Phalloidin 555). Samples were imaged with confocal microscopy (Nikon A1R) to visualize the contracting fibrin network.

Results: Mechanical testing revealed a nonlinear trend in the elastic moduli of fibrin clots with varied thrombin concentrations, generally increasing from 5-20 U/mL of thrombin but peaking at 2 and 20 U/mL of thrombin (**Figure 1A**). After culturing fibrin gels (gelled with 2 and 20 U/mL of thrombin) over a four-week period, the low-thrombin gel experienced significant contraction, whereas the high-thrombin gel maintained a larger area (**Figure 1B)** At the micro-scale, the low-thrombin gels exhibited thicker fibrin fibers, whereas high-thrombin gels yielded thinner fibers (**Figure 1C**). With regards to cellular contractility, 3D fibrin gels, when in the presence of Fasudil, maintained a significantly larger area than the gels cultured in control media, indicating a dose-dependent decrease in contraction after four weeks (**Figure 2A**). Tracking clot area over time revealed that clots cultured in 50 μM Fasudil experienced little to no contraction over the four weeks, whereas the control group contracts significantly (**Figure 2B**). Micro-scale images of cells on 2D gels showed that 50μM Fasudil showed less actin stress fibers and less fibrin densification than cells cultured in control media (**Figure 2C**).

Discussion: The results of this study show that the microscale properties of the microfracture clot, specifically the fiber thickness and porosity of the fibrin network, may have a significant impact on macroscale clot contraction over time. When treated with Fasudil, a Rho inhibitor, clots resist contraction on both a micro- and macro-scale level. Higher thrombin concentrations and treatment with Fasudil both modify the extracellular microenvironment and cellular machinery in a way that prevents macroscale contraction, which may be potential avenues for preventing early microfracture fibrosis.

References:
[1] Wolberg+, Blood Rev, 2007. [2] Shi+, Lab Invest., 2017. [3] Moriyama+, Drug News Perspect., 2004 [4] Iseki+, Am J Sports Med., 2019.

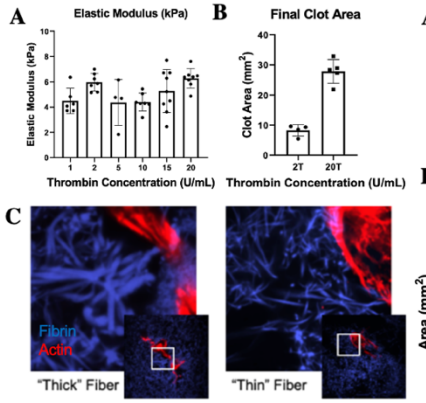

Fig 1. Initial properties, contraction, and structure of gels with varied thrombin concentrations. (A) Elastic moduli of 3D fibrin gels with 1-20 U/mL thrombin (n=8). (B) Final area of 3D fibrin gels containing 2 and 20 U/mL of thrombin after 4 weeks (n=5). (C) Confocal microscopy images of marrow-derived cells cultured on 2D fibrin gels with low (left) and high (right) thrombin concentrations. Fibrin shown in blue, actin shown in red.

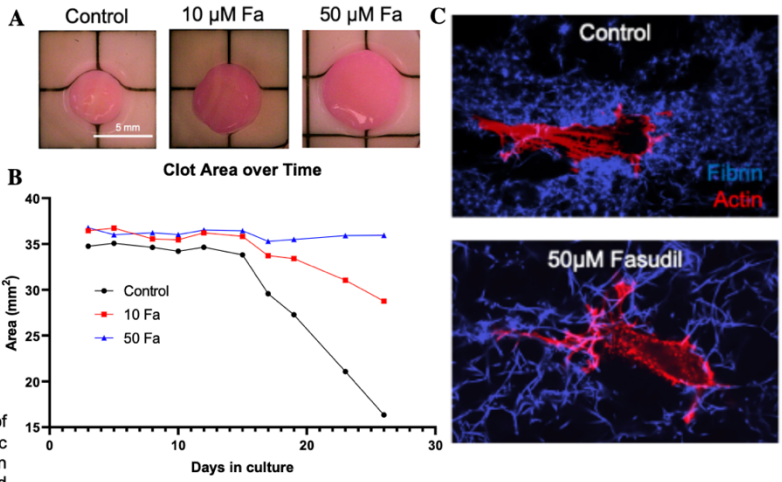

Fig 2. Macroscale contraction and microscale cell properties under the presence of Fasudil. (A) Representative images of clots cultured in 0, 10, and 50μM Fasudil media after 4 weeks. (B) Clot area tracked throughout the 4-week culture period (n=3). (C) Confocal microscopy images of marrow-derived cells cultured on 2D fibrin gels in control and 50 μM Fasudil media. Fibrin shown in blue, actin shown in red.