The balance between cell contractility and adhesion modulates provisional matrix assembly during wound closure

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Abstract

Upon injury of fibrous tissues, resident fibroblasts migrate into the wound bed, depositing a fibrous provisional matrix and pull the margins of the wound closer to accelerate wound closure. While cellular contractility and cell adhesion are two processes regulating wound healing, it remains unclear how these processes coordinate provisional matrix assembly during stromal closure. To address this question, in this study we manipulated cell contractility and cell-matrix adhesion of Normal Human Dermal Fibroblasts Neonatal (NHDFneos) and Normal Human Lung Fibroblasts (NHLFs) and assessed wound closure in a 3D microtissue model of stromal wound healing.

Briefly, NHDFneos and NHLFs were embedded in a type I collagen gel and seeded into μTaq devices composed of microwells with microPillars to form 3D microtissues. Twenty-four hours after the tissues formed, a pulsed nanosecond Nd:Yag Laser was used to create full-thickness wounds in the center of the tissue. Both NHDFneo and NHLF microtissues were treated with contractility or adhesion modulators before injury and wound closure dynamics were assessed for 24 hours post-injury using time-lapse microscopy. Traction force microscopy was performed on the individual cell types seeded on collagen type I coated polyacrylamide gels (Young's modulus = 5 kPa) under different treatment conditions using a Nikon-Eclipse-Ti microscope. Immunofluorescent staining against phosphorylated-paxillin was performed to visualize focal adhesions. The size of focal adhesions was looked at qualitatively as a metric for adhesion. In these experiments, staining against cellular fibronectin was performed to visualize provisional matrix assembly of fibers in microtissues 24 hours post-injury.

At baseline, NHDFneo healed the wounds in the microtissues within 24 hours post injury, while NHLF microtissues failed to close the wounds. On 2D substrates, NHDFneos exhibited lower contractility and smaller adhesions compared to NHLFs. Treatment with manganese increased focal adhesion size and contractility of NHDFneos as well as concurrent inability to heal full-thickness wounds in microtissues. Manganese-treated NHDFneo microtissues revealed clustering of cellular fibronectin with little to no fibrillogenesis throughout the tissue post-injury, while untreated tissues assembled fibronectin fibers throughout the remodeled tissue post-injury. Conversely, treatment of NHLFs with a low dose FC11, a FAK inhibitor, lowered contractility and the size of focal adhesions to comparable levels as untreated NHDFneos. NHLF microtissues treated with FC11 healed within 24 hours post injury while untreated microtissues failed to heal (N = 3, n = 20 tissues per treatment group). Interestingly, NHLFs treated with blebbistatin and Y-27632, a myosin II and ROCK inhibitor respectively, exhibited lower contractility, but continued to fail healing microtissues. Together, our data support the hypothesis that the balance between contractility and adhesion levels modulates wound closure in our microtissue system, which may inform new mechanotherapeutic strategies for treating impaired wound healing.

Platform

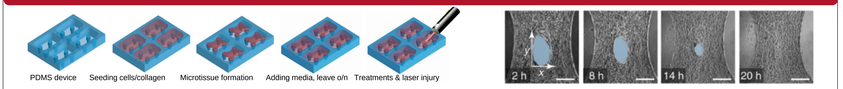

Figure 1: Graphical representation of wound healing platform (Left) *PDMS device molded into individual wells to form 4 micropillars. Cells are then seeded in a collagen suspension and allowed to form during a short incubation period before media is added and left overnight to fully compact. After ~24 hrs. media is changed, and treatments are added. Then, tissues are injured with a Nd:Yag laser.* ***(Right)*** *Time-lapse imaging of tissues is set up shortly after injury for ~24 hrs. to allow quantification of healing dynamics.* ***Figure adapted from*** *Sakar, M.S., et al., Cellular forces and matrix assembly coordinate fibrous tissue repair. Nature Communications, 2016. 7(1): p. 11036.*

Motivation

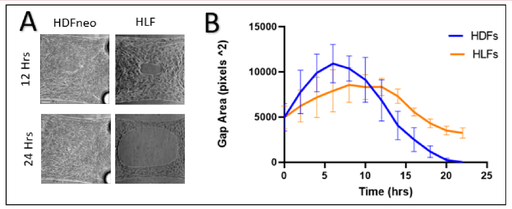

Figure 2: Cell type differences in stromal wound closure. ***(A)*** *Time-lapse images capturing HDFneo and HLF tissues 12 hrs. and 24 hrs. post injury. Some HLF tissues ripping the wound back open by 24 hrs. Scale bar = 100 um.* ***(B)*** *Quantification of HDFneo vs. HLF tissue healing over 24 hrs. post injury revealing a difference in healing capabilities.*

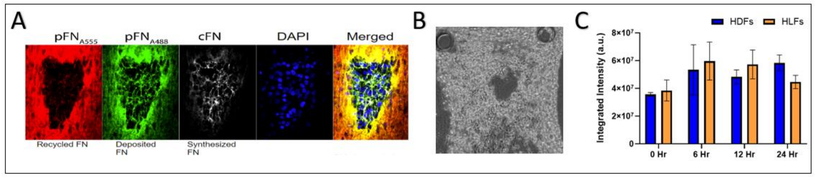

Figure 3: Fibronectin production is essential for the healing of wounds. (A) *Recycled FN is fluorescently tagged FN added in culture during tissue formation and is reused by cells to close the wound. Deposited FN is fluorescently tagged fibronectin added in culture after injury for cells to use to close the wound. Cellular FN, stained with immunofluorescence, is produced by the cells during wound closure. Merged image showing cellular and deposited FN making up the provisional matrix within the healed wound.* ***(B)*** *MEF cells with fibronectin knockout fail to heal in the microtissue system.* ***(C)*** *Integrated intensity quantifications of fibronectin production for HDF and HLF tissues at different time points after injury reveal no significant differences between the two cell types.*

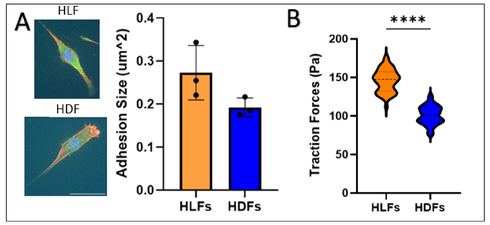

Figure 4: Cell type differences in adhesion and contractility. (A) *Quantification of adhesion size in HLF vs. HDFneo cells adherent to 5kPa polyacrylamide substrates (red: phalloidin, green: p-paxillin). Scale bar = 50 um.* ***(B)*** *Quantification of traction forces generated by HLF vs. HDFneo single cell traction force levels. Forces were determined to be statistically significant via unpaired t-test. Compared to HDFs, HLFs have larger adhesions and exhibit increasing contractility levels.*

Hypothesis

We hypothesize that the balance between cell-ECM contractility and adhesion modulates provisional matrix assembly to close wounds in engineered fibrous microtissues.

Contractility and adhesion manipulations affect healing dynamics

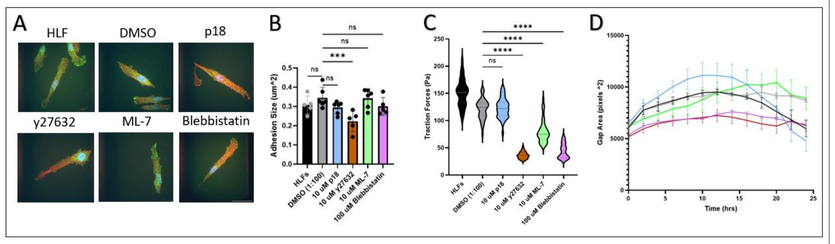

Figure 5: Treatment of HLFs with contractility inhibitors did not aid in healing, despite a shift in adhesion size and traction forces. ***(A)*** *IF staining of p-paxillin (green) and phalloidin (red) for cells of different groups. Scale bar = 50 um.* ***(B)*** *Adhesion size quantifications measured for single cells of different groups. Sizes were determined to be statistically significant via one-way ANOVA.* ***(C)*** *Traction forces measured for single cells of different groups. Forces were determined to be statistically significant via one-way ANOVA.* ***(D)*** *Gap area of tissues quantified manually every 2 hrs. post-injury for 22 hrs. N = 3, n = ~15 tissues per group.*

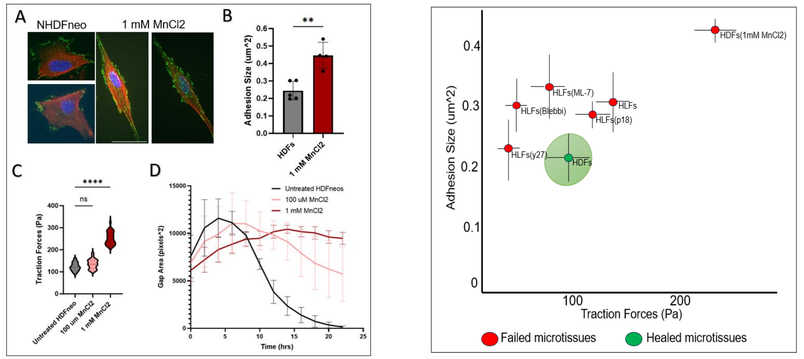

Figure 6 (Left): Treatment with MnCl2 in HDFneo cells and tissues reveals an increase in adhesion size and traction forces along with failure of wound closure. (A) *IF staining of p-paxillin (green) and phalloidin (red) for cells of different groups. Scale bar = 50 um.* ***(B)*** *Adhesion size quantifications measured for single cells of different groups. Sizes were determined to be statistically significant via unpaired t-test.* ***(C)*** *Traction forces measured for single cells of different groups. Forces were determined to be statistically significant via one-way ANOVA.* ***(D)*** *Gap area of tissues quantified manually every 2 hrs. post-injury for 22 hrs. N = 3, n = ~15 tissues per group.* ***Figure 6 (Right): Proposed phase diagram of healing vs. failing tissues.*** *Manipulations placed on the diagram, based on single cell adhesion size and traction force, along with the tissues ability to heal 24 hrs. post injury.*

Coarse grain model to simulate altering contractility or adhesion levels

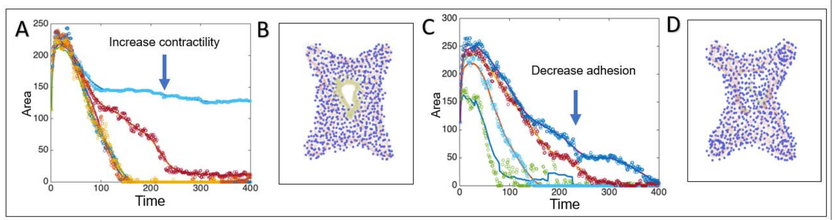

Figure 7: Coarse grain model simulations predicts modulation of healing when contractility and adhesion are altered in fibroblast tissues. (A-B): *Simulations showing increased contractility healing outcomes, revealing that increasing contractility levels, causes a halt in healing in microtissues.* ***(C-D):*** *Simulations showing decreased adhesion healing outcomes, revealing that decreasing adhesion can cause tissues to become weak in the area of the original injury even after healing.*

FAK inhibitor rescues healing in lung tissues

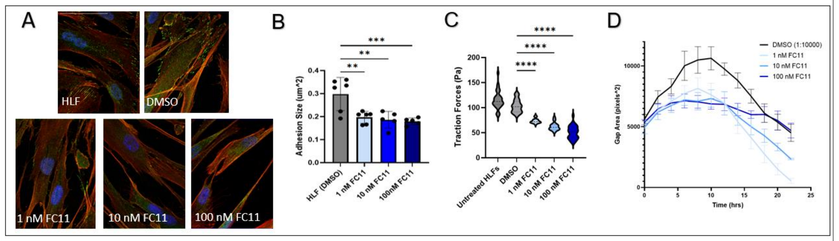

Figure 8: FAK inhibitor, FC11, rescues closure in HLF microtissues. ***(A)*** *Immunofluorescent staining for p-paxillin (green) and phalloidin (red) for cells of different groups. Scale bar = 50 um.* ***(B)*** *Adhesion size quantifications measured for single cells of different groups. Sizes were determined to be significant via one-way ANOVA.* ***(C)*** *Traction forces measured for single cells of different groups. Forces were determined to be significant via one-way ANOVA.* ***(D)*** *Gap area of tissues quantified manually every 2 hrs. post-injury for 22 hrs. N = 4, n = ~22 tissues per group.*

Optimal range needed for healing and failure

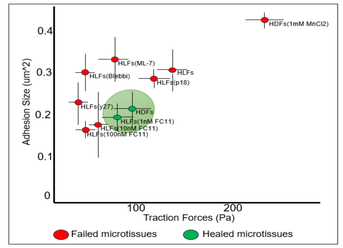

Figure 9: Phase diagram of healing and failing microtissues based on single cell contractility and adhesion. *An optimal range is proposed based on two manipulations that heal (shaded green). Manipulations that fall outside the range (red), fail to heal.*

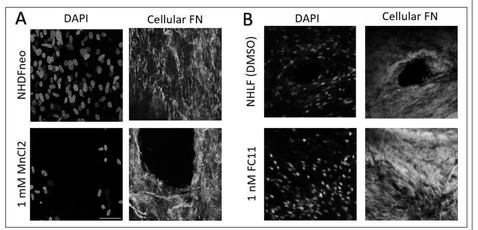

Figure 10: Provisional matrix assembly is affected in failing HLF and HDF microtissues. ***(A)*** *DAPI (left) and cellular fibronectin (right) stains for untreated and MnCl2 treated HDF tissues. Scale bar = 50 um.* ***(B)*** *Dapi (left) and cellular fibronectin (right) stains for untreated and FC11 treated HLF tissues.*

Impact

Discussion

- Together, our data support the hypothesis that the balance between contractility and adhesion levels modulate wound closure in our microtissue system.
- Our proposed concept could inform new mechanotherapeutic strategies for treating impaired wound healing.

Conclusions and future work

- In this work, we were able to use levels of contractility and adhesion size in two fibroblast cell types to push tissues to either fail or heal in our model system.
- An optimal range for healing vs. failing tissues was proposed based on collected data.
- Differences in provisional matrix assembly were observed when adhesion and contractility levels operated outside of this range.
- Future work will investigate the relationship between cell adhesion and contractility in modulating provisional matrix assembly during aberrant wound healing.

Acknowledgements

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