Senescent-associated Extracellular Matrix Production and Delayed Wound Healing by Senescent Dermal Fibroblasts

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Introduction

Senescent cells play an important role in development, accumulate with age and are implicated in some age-related diseases. However, their role during wound healing is still controversial. While early clearance of these cells seems to prevent wound closure in adults, chronic wounds have been shown to benefit from their removal. It is not clearly understood why these cells have such different temporal effects.

Wound healing is a complex process supported by the generation of a provisional extracellular matrix (ECM) by fibroblast cells in the stromal tissue of an organ. Senescent cells are capable of altering this extracellular environment on account of their secretory phenotype and this altered ECM can persist beyond the lifespan of the cells it originated from

Here, we use a high-throughput Wound-on-Chip (WoC) platform to understand how:
- Senescent cells alter the ECM architecture and composition around them, and
- Senescent cells affect gap closure during provisional matrix generation

Methods

- Used two-photon polymerization (2PP) 3D printing and stereo- lithography (SLA) 3D printing to fabricated micropillar devices
- Microtissues composed of normal human dermal fibroblasts from neonatal foreskine (NHDF-neo) or senescent dermal fibroblasts were seeded
- Senescent fibroblasts were induced with repeated treatments of 100 uM of hydrogen peroxide (H_2O_2) for 2 hours every 48 hours over 7 days

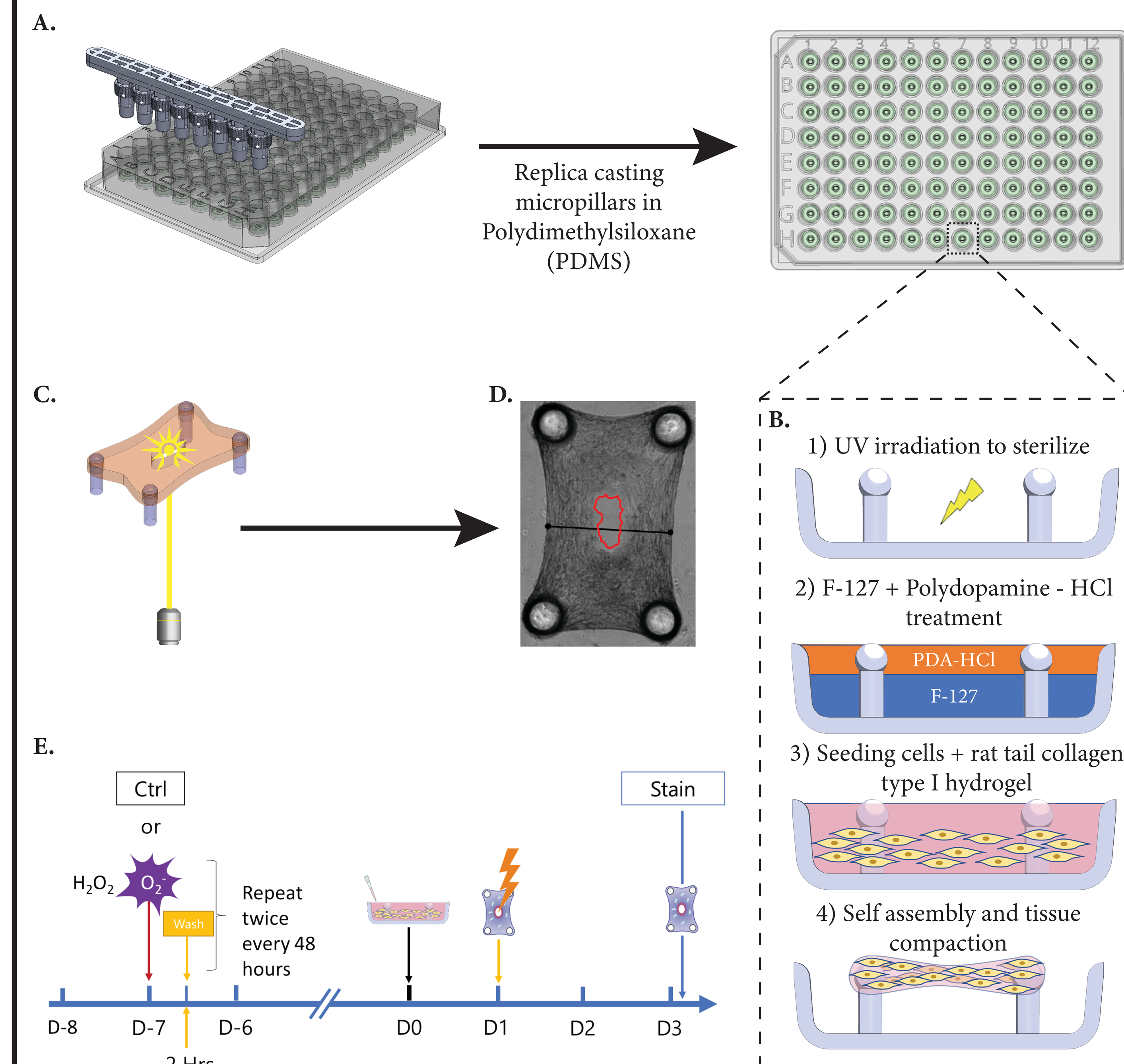


Figure 1 Fabrication and experimental setup (A) Overview of microtissues fabrication. (B) Fibroblasts in a collagen matrix compact in the microtissue around cantilevers. (C) Tissues are injured using a Q-switched Nd:YAG laser. (D) Tissues are tracked over time with brightfield microscopy and analyzed using an automated image processing pipeline. (E) Timeline of senescence induction and utilization in microtissues.

Results: Wound closure dynamics

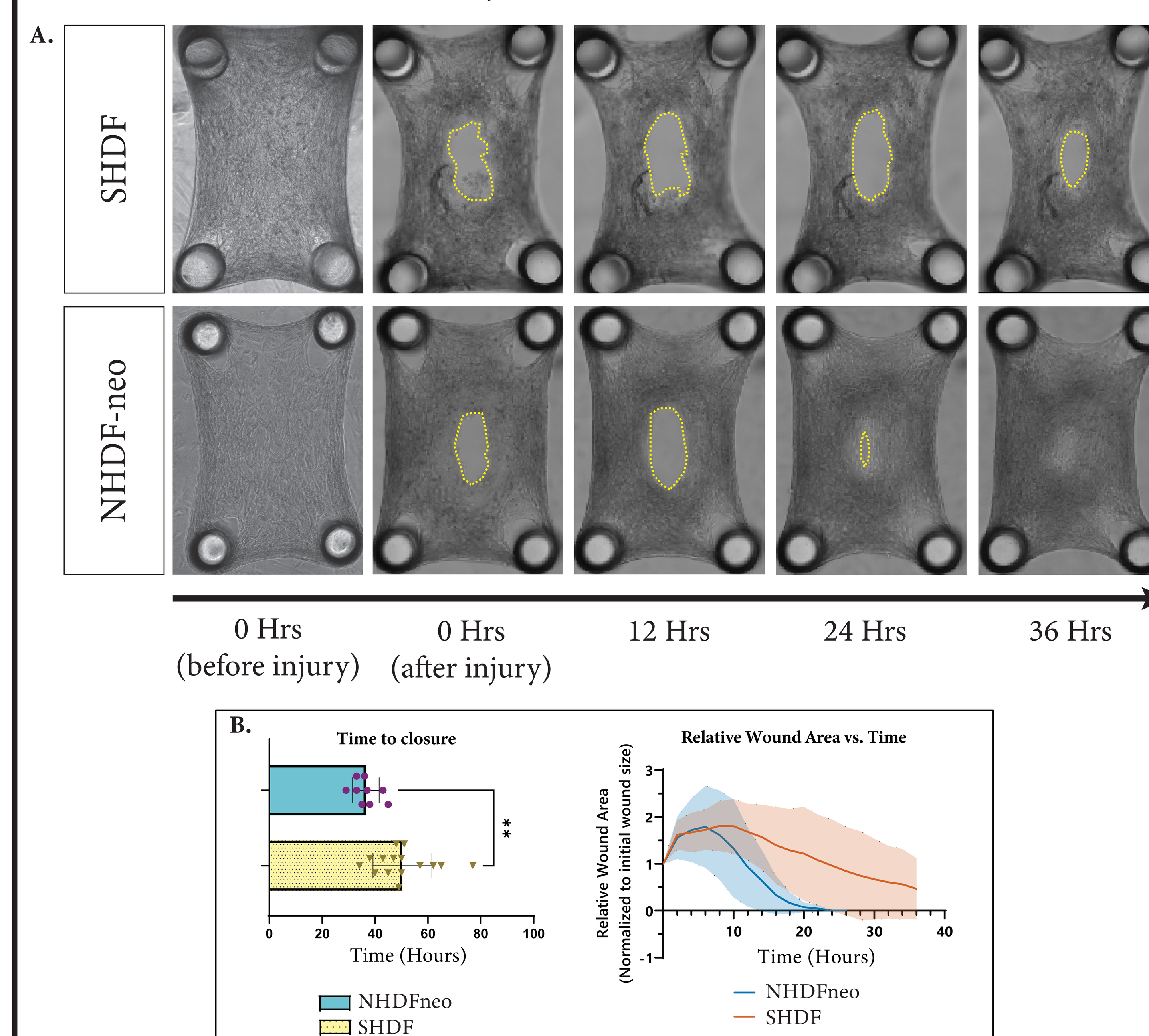


Figure 2 (A) Representative brightfield images of NHDFneo and SHDF microissues imaged immediately before and after laser ablation, 12 hours,24 hours and 36 hours after laser ablation respectively. All tissues were injured with 1 mJ of energy. Tracked injury edge marked with yellow dashed outline (B) Time to complete tissue closure for each cell type and Wound area vs time - analyzed using the automated wound tracking pipeline (N=3, n=8). Student's t-test used for comparison (** p<0.005)

Results: Senescence detection

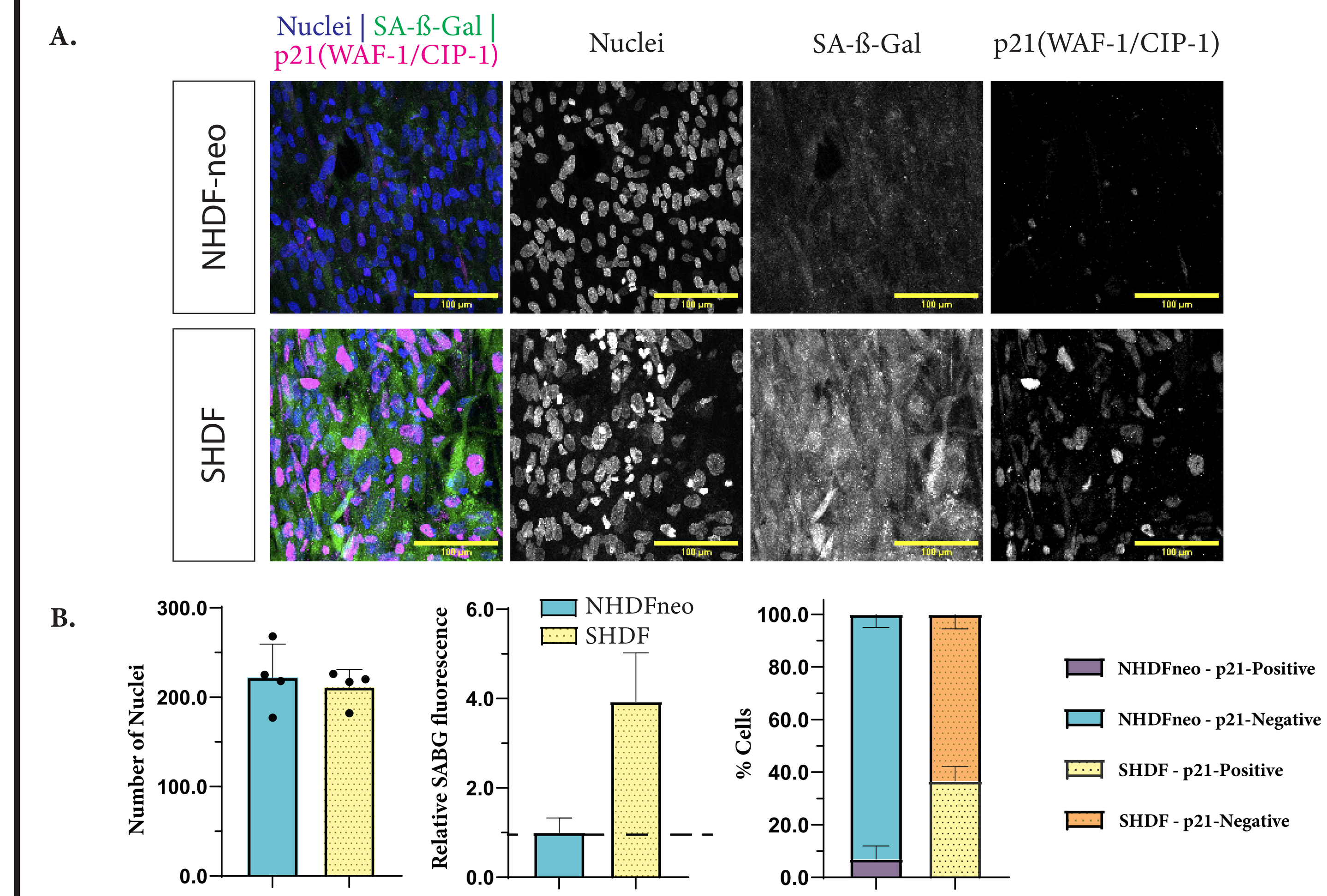
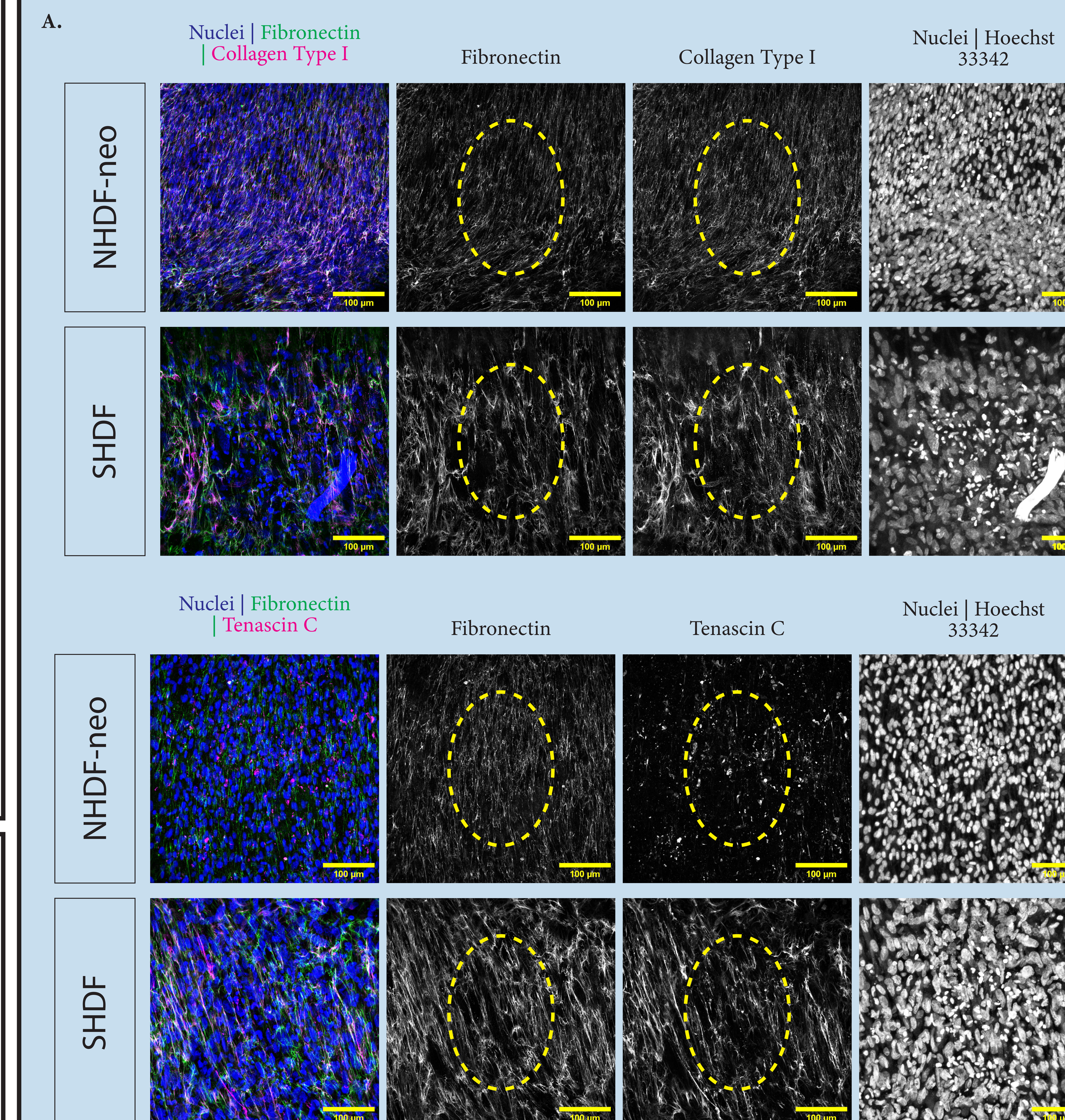


Figure 3 (A) Maximum intensity projections of confocal fluorescent images and (B) quantifications of nuclei, senescent associated beta galactosidase expression, and p21 expressing cells,

Results: Provisional extracellular matrix generation

Senescent Dermal Fibroblasts produce more provisional Fibronectin and Tenascin C than normal human dermal fibroblasts during wound closure



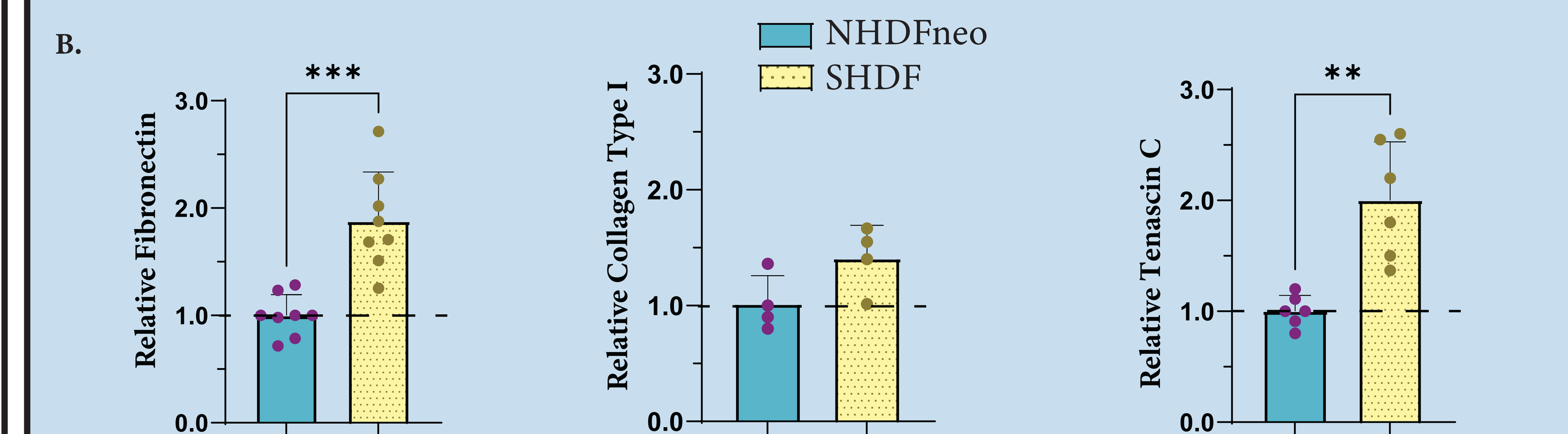


Figure 4 Maximum intensity projections of confocal fluorescent images and quantifications of fibronectin, collagen type I and tenascin C. (A) Representative images of NHDFneo and SHDF microtissues with original area of injuries marked in yellow dashed lines. (B) Quantification of relative fibronectin, collagen type I, and tenascin C production in ROIs marked in yellow dashed lines from sum slice projections of z-stacks (N=3, n=4).

Results: Extracellular matrix protein quantification

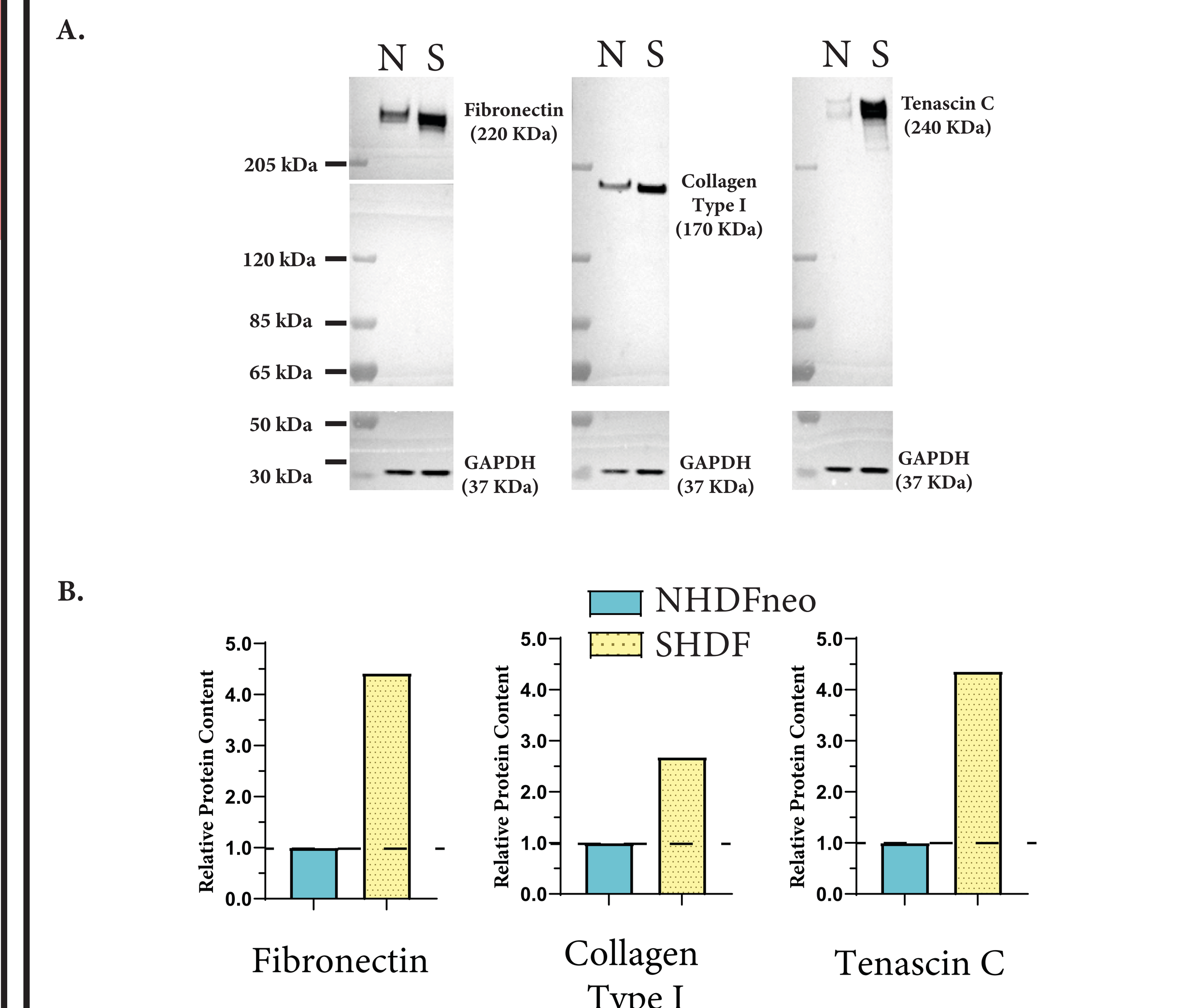


Figure 4 (A) Representative western blots and (B) quantification of relative fibronectin, collagen type I, and tenascin C production in NHDFneo and SHDF cells when cultured on tissue culture plastic in 2D. Protein quantity is normalized to relative to band intensities in the the NHDFneo lanes.

Discusssion

SHDF cells exhibited:
- slower time to closure and closure dynamics than NHDFneo cells
- thicker and longer fibers of fibronectin and collagen type I than NHDFneo
- fibrillar tenascin C organization that seems to interact/overlap with fibronectin expression
- higher expression of tenascin C and fibronectin proteins in 2D culture

Our data support the hypothesis that ECM synthesis, composition and organization is a critical factor driving wound closure. Additionally, over-production of some ECMs may even inhibit normal wound closure.

The feedback loop between ECM and cells has been reported in literature extensively. This study and platform open the door to exploring how senescence-altered extracellular matrix composition and architecture may affect cell and tissue behavior.

Acknowledgements

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References

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