Modelling cellular crosstalk driving extracellular matrix accumulation in fibrotic skin diseases

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Background

While most cutaneous wounds heal with minimal scarring, some develop excessive fibrotic tissue known as keloids, which extend beyond the original wound area. The mechanisms driving keloid formation remain incompletely understood. Using single-cell RNA sequencing, we identified Schwann cells, fibroblasts, and M2-polarized macrophages as key contributors to keloid pathology (Figure 1)[1]. We propose that crosstalk between Schwann cells and M2 macrophages promotes extracellular matrix accumulation through increased matrix protein expression and reduced MMP9 activity, ultimately leading to keloid formation (Figure 2).

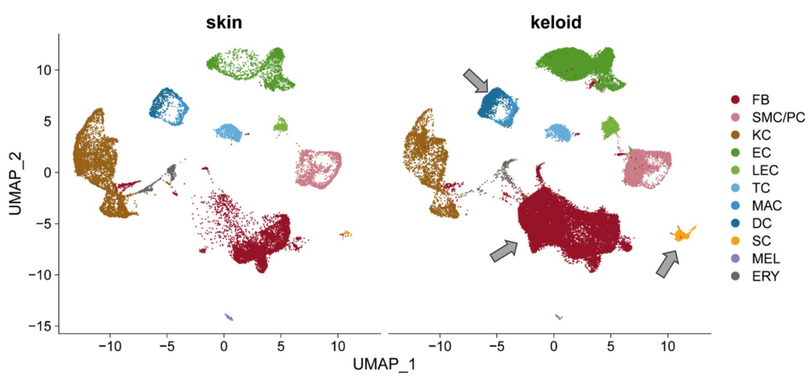

Figure 1: Single cell RNA sequencing analysis of healthy skin compared to keloids. Grey arrows indicate the relevant populations of fibroblasts, Schwann cells and macrophages [1].

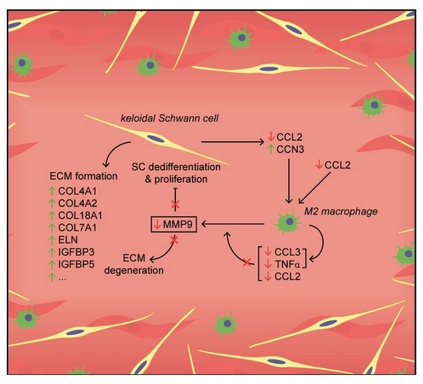

Figure 2: Overview of Schwann cell localization and proposed mode of action within keloids [1].

Objective

To further investigate the proposed mechanism of changes in the extracellular matrix (ECM) production and composition, we are developing an *in vitro* model of keloid formation. The model is applied to on the one hand test the formation of matrix using primary skin and keloid tissue and on the other hand to test the interactions of various cell types involved in pathological scar formation, in particular Schwann cells, fibroblasts, and macrophages. Using this innovative model, we aim for a better understanding of the fibrotic processes.

Results

In vitro cultured scar and keloid cells recapitulate in vivo processes

To investigate ECM formation, we have cultured dermal cells from dissociated skin and keloids for 4 weeks in our self assembled matrix models with subsequent microscopy analysis (Figure 3). Differences in matrix structure and composition were observable between the conditions recapitulating the increased matrix secretion and alignment observable *in vivo* (Figure 4).

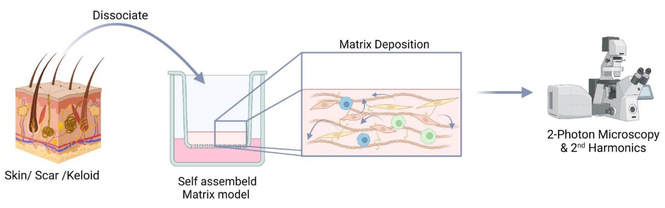

Figure 3: Overview of the self assembled matrix model. Figure created using BioRender.

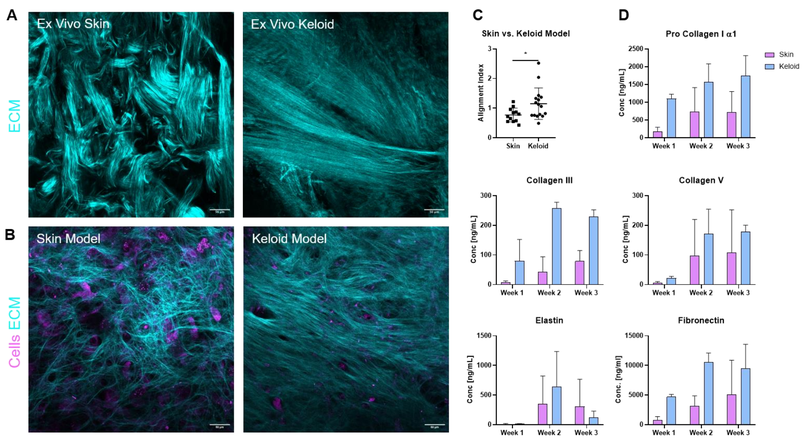

Figure 4: Self assembled matrix model of skin and keloid compared to ex vivo. (A) Images of collagen fibres (cyan) of ex vivo skin and Keloid taken using a 2-photon microscope with 2nd harmonics generation. (B) Images of collagen fibres (cyan) and cells (magenta) of skin and keloid model taken using a 2-photon microscope with 2nd harmonics generation. (C) Fiber alignment analysis of microscopy images. (D) ELISA results of pro-Col1α1, Col3, Col5, Elastin and Fibronectin concentration in the supernatant of the models. Mean +SD depicted.

Keloid fibroblast supernatant induces Schwann cell proliferation

To investigate the effect of healthy and keloid fibroblasts on Schwann cells we have treated them with the respective supernatant. Keloid fibroblasts induce expression of genes related to collagen modification and cell proliferation in the Schwann cells (Figure 5).

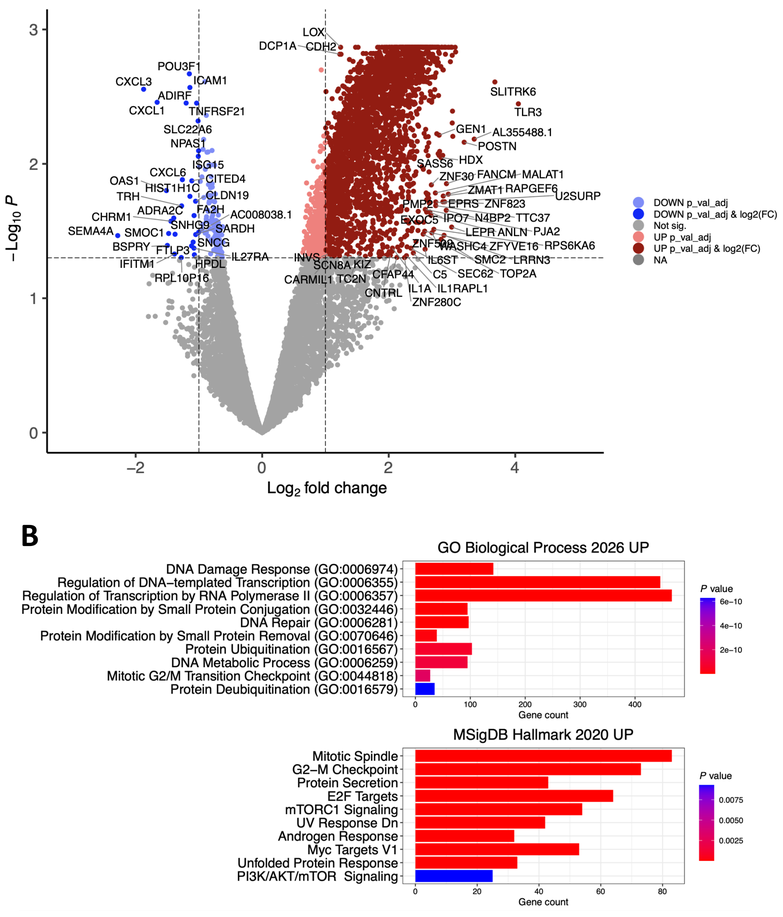

Figure 5: Up and downregulated genes in Schwann cells upon keloid and healthy fibroblast supernatant treatment. (A) Volcano plot depicts selected up- and downregulated genes in keloid vs. healthy fibroblast supernatant treated Schwann cells. (B) Bar graphs showing GO-terms of significantly upregulated genes in keloid fibroblast supernatant treated Schwann cells.

Co-culturing M2 macrophages with Schwann cells induces a pro-fibrotic phenotype in Schwann cells

We performed RNA-sequencing of Schwann cells in co-culture with monocytes and macrophages polarized according to an established protocol to investigate changes in gene expression due to their crosstalk (Figure 6) [2]. M1 macrophages induced a pro-inflammatory response, and M2 macrophages caused the upregulation of ECM related genes.

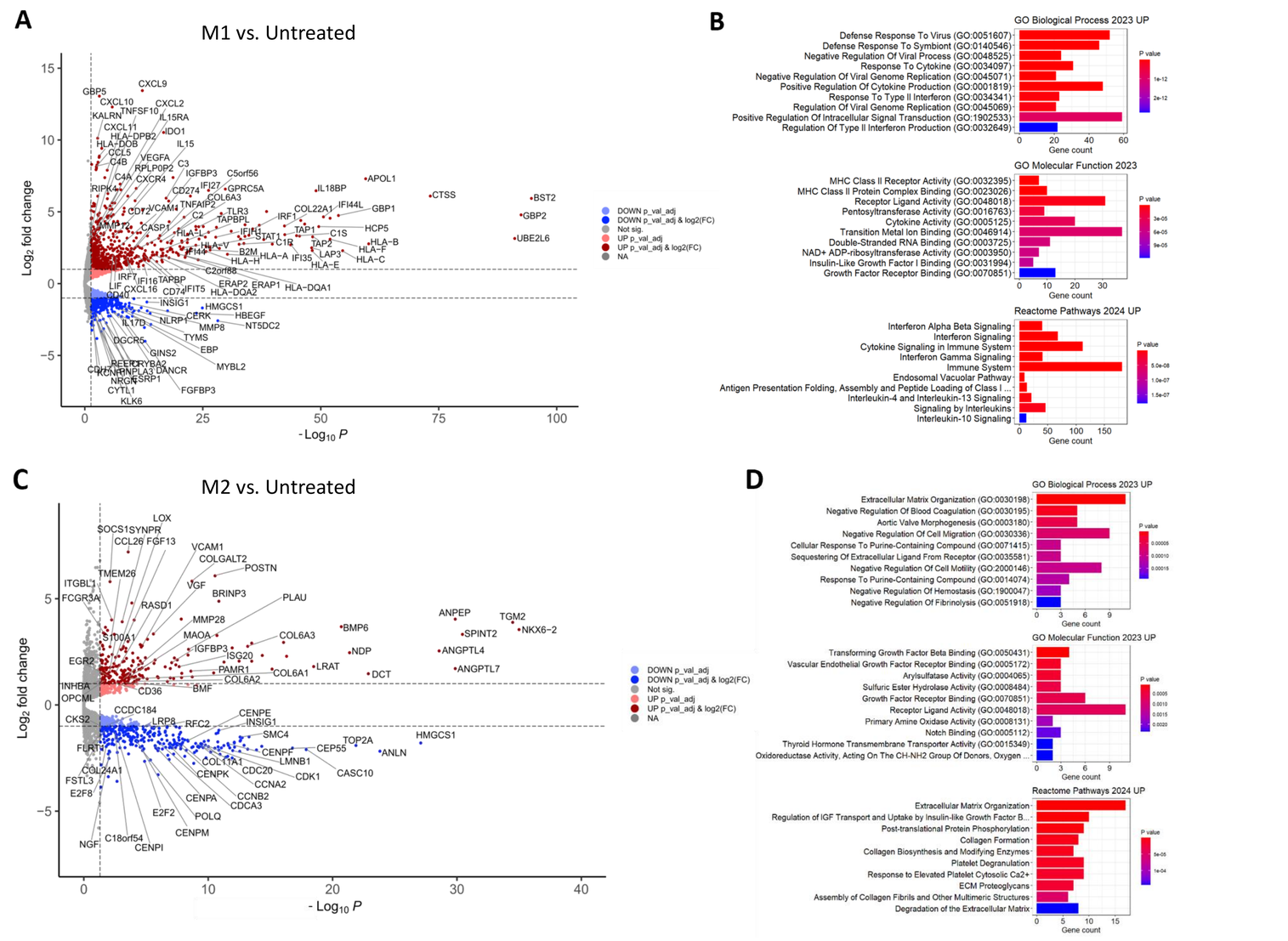

Figure 6: Up and downregulated genes in Schwann cells upon co-culture with M1 and M2 macrophages compared with the untreated condition. (A&C) Volcano plot depicts selected significantly up- and downregulated genes. (B&D) Bar graph shows GO-terms of significantly upregulated genes in co culture with M1 and M2 macrophages.

Monocytes and macrophages influence fibroblast ECM secretion and organization

To investigate how monocytes and polarized macrophages influence the ECM of healthy and keloid fibroblasts we have performed co-culture experiments (Figure 7). Pro-inflammatory macrophages suppressed ECM deposition, whereas pro-fibrotic macrophages promoted the formation of a more highly aligned matrix. In contrast, monocytes enhanced ECM secretion into the supernatant.

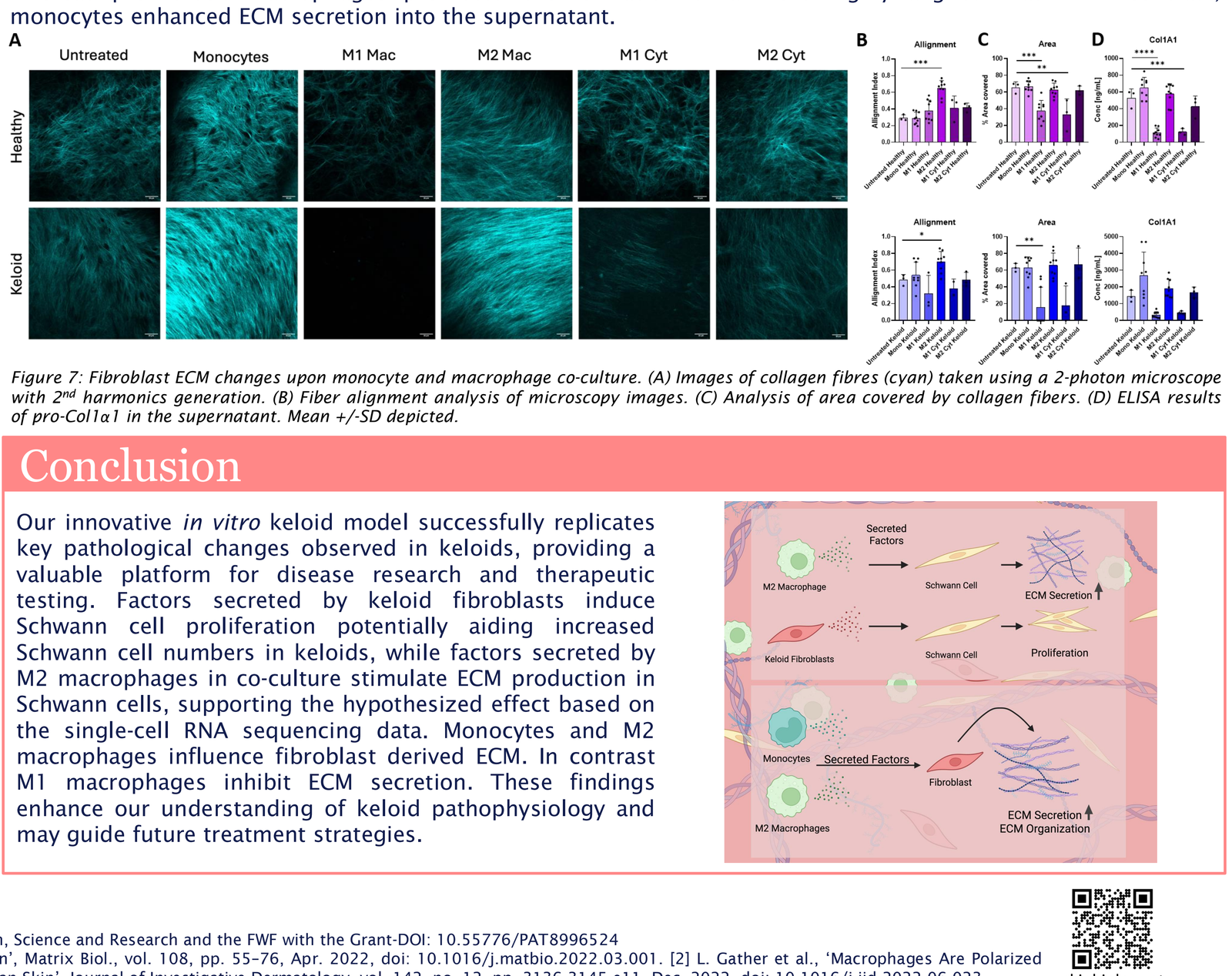

Figure 7: Fibroblast ECM changes upon monocyte and macrophage co-culture. (A) Images of collagen fibres (cyan) taken using a 2-photon microscope with 2nd harmonics generation. (B) Fiber alignment analysis of microscopy images. (C) Analysis of area covered by collagen fibers. (D) ELISA results of pro-Col1α1 in the supernatant. Mean +/-SD depicted.

Conclusion

Our innovative *in vitro* keloid model successfully replicates key pathological changes observed in keloids, providing a valuable platform for disease research and therapeutic testing. Factors secreted by keloid fibroblasts induce Schwann cell proliferation potentially aiding increased Schwann cell numbers in keloids, while factors secreted by M2 macrophages in co-culture stimulate ECM production in Schwann cells, supporting the hypothesized effect based on the single-cell RNA sequencing data. Monocytes and M2 macrophages influence fibroblast derived ECM. In contrast M1 macrophages inhibit ECM secretion. These findings enhance our understanding of keloid pathophysiology and may guide future treatment strategies.

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References: [1] M. Direder et al., 'Schwann cells contribute to keloid formation', Matrix Biol., vol. 108, pp. 55–76, Apr. 2022, doi: 10.1016/j.matbio.2022.03.001. [2] L. Gather et al., 'Macrophages Are Polarized toward an Inflammatory Phenotype by their Aged Microenvironment in the Human Skin', Journal of Investigative Dermatology, vol. 142, no. 12, pp. 3136-3145.e11, Dec. 2022, doi: 10.1016/j.jid.2022.06.023.

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