UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2023/24

NB: This whole report will be posted on the Society's website therefore authors should NOT include sensitive material or data that they do not want disclosed at this time.

Name of student:

Viktoria Levkanicova

Name of supervisor(s):

Tanya Shaw

Project Title: (no more than 220 characters)

What is special about the skin of the face?

Project aims: (no more than 700 words)

Our skin has distinct characteristics at different sites on our bodies, for example more or less hair and variable thickness, but this obvious variability is largely ignored in wound healing and skin disease research, and is rarely considered in the treatment of skin conditions. In particular, the skin of the face is considered special compared to other sites on the body, with evidence for superior wound healing properties in health, including faster repair and reduced scarring, and vulnerability to certain skin diseases, such as keloid scars, linear morphea, rosacea, acne, and others. These regional variations are not understood. We recently discovered that adult facial skin is remarkably different from other sites both at rest and during wound repair, with characteristics reflecting the unique development of the tissue from neural crest (1).

Our work to date has focused on the anatomical variations of dermal fibroblasts, however, we have intriguing preliminary evidence that the relative proportions of specialised cell populations in the skin of the face varies from other body sites. At the time of application, the aim of this project was to compare the immune cell composition of skin at different anatomical sites using a combination of bioinformatic and lab-based methods. The work was adjusted to include analysis of other cell types (e.g. Schwann cells, distinct fibroblast subpopulations) in healthy skin and keloid scars, using immunostaining and cell culture methods.

Project Outcomes and Experience Gained by the Student (no more than 700 words)

Bioinformatic analysis reveals distinct cell proportions in the skin of the face vs other body sites

We first used a bioinformatic approach to analyse a publicly available microarray dataset (GSE139300) comparing 150 samples of human skin from cheek or body sites, and our own bulk RNA sequencing analysis comparing mouse skin sites in homeostasis and at Day 3 after wounding (1). Analysis of key subsets of immune cells was the first goal. Most noteworthy was higher global immune cell numbers in the face, with a repressive immune signature, and evidence for less neutrophil infiltration in facial wounds.

Next, due to the wide interest in fibroblast heterogeneity, and the potential for these cells to be immune-modulatory, and key determinants of scarring, we interrogated the available data for evidence of their differing proportions in skin/wounds of the facial dermis. Most striking was the reduced proportion of mesenchymal fibroblast markers (a subset enriched in fibrotic diseases).

Finally, during the time-frame of this project, our interest in Schwann cells and their contribution to wound repair and scarring emerged. These are support/myelinating cells of the peripheral nervous system that are reported to contribute to wound repair, and are enriched in keloid scars, implicating them in the scar pathology. We started with bioinformatic approaches for their key markers, and were excited to find, based on MPZ expression, that the numbers in the beds of facial wounds was double that of body sites.

Outcomes: The work in this project has increased our excitement about the alterations in proportions of cells in skin from different sites. Plans to formally "deconvolute" the valuable human dataset that is available were circumvented by lack of availability of experts in this area. Specifically, pipelines for microarray analysis (old technology) are not as well established as we initially thought. Therefore, we quickly connected with the KCL Hub for Applied Bioinformatics (HAB) for their support, but we are awaiting our turn to work with them and are optimistic that these findings will be presented alongside the poster at the Summer Meeting.

Student impact: The student undertook online training in R studio available through the KCL HAB. She has become fluent in data access and handling, as well as important statistical methodologies. With this work she was able to successfully mine data to support existing hypotheses and generate new exciting directions.

Keloid scars have a high abundance of Schwann cells, not associated with nerves

It struck us that keloid scars occur most frequently on the face and neck (reported at least 40%), in the area where the skin is neural crest-derived. Moreover, one of the recent single cell RNA sequencing publications characterising the cellular composition of keloids reported an increased abundance of Schwann-like cells (which derive from the neural crest in embryonic development). This led us to question whether the Schwann-like cells in keloids appear equally in lesions affecting the face vs other body sites. This project analysed a large collection of keloid samples immunostained for S100B. We observed highly variable abundances, distributions and morphologies between patient samples. Next, when stratifying the samples based on anatomical site, it does seem that the keloids from the face have altered S100B patterns, but given the high variability, we now need to increase the sample size to draw conclusions from this analysis. We cautiously think that the behaviour of Schwann or Schwann-like cells in facial keloids may be distinct.

Keloid cell cultures exposed to Schwann cell culture conditions demonstrate plasticity

Having observed Schwann-like cells in keloids, we questioned whether these may be present in our primary patient-derived cell cultures. To test this, we cultured keloid cells in Schwann cell differentiation medium and were excited to see them adopt dramatic morphological changes (arborisation) and to begin expressing SOX10 and S100B (Schwann cell markers). We interpret this as evidence of remarkable keloid cell plasticity, and not as originally hypothesised, that there may be some Schwann cells present in our patient isolations.

Outcomes: Novel visualisation of diversity of Schwann cells in keloids. Evidence of remarkable plasticity of keloid-derived cultures.

Student impact: The student learned many techniques, from cell culture to immunostaining, microscopy and quantitative image analysis in FIJI.

Please state which Society Winter or Summer Meeting the student is intending to present his/her poster at:

Summer Meeting 2025

Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)

Title: Schwann cell contributions to keloid scarring

Schwann cells are the myelinating cells of the peripheral nervous system, known for the crucial role they play in nerve regeneration upon injury. Recently, new studies have suggested their active participation in skin wound healing, particularly in the stages of final wound closure via the activation of myofibroblasts and tissue remodeling which leads to scar formation. Furthermore, Schwann cells have also been implicated as major players in the pathogenesis of keloids, a type of pathological fibroproliferative scar, where they have

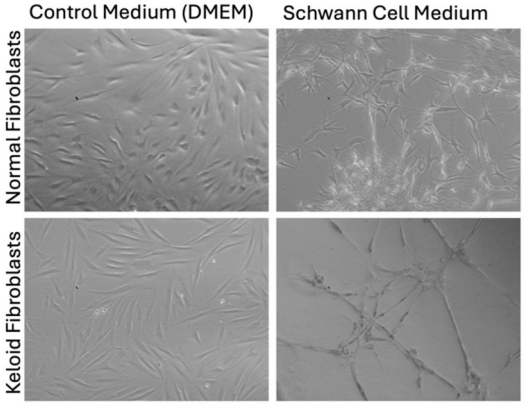
been found to influence inflammation and fibrosis through their engagement with fibroblasts and the extracellular matrix. In this study, we first used a bioinformatic approach to analyse a publicly available microarray dataset (GSE139300) comparing 150 samples of human skin from cheek or body sites, and our own bulk RNA sequencing analysis comparing mouse skin sites in homeostasis and at Day 3 after wounding. Intriguingly, using MPZ expression as a surrogate for Schwann cell number, the numbers in the beds of facial wounds was double that of body sites. This will be validated using formal deconvolution methods and immunostaining. Ultimately, we used S100B immunohistochemistry to examine the Schwann cell distribution and abundance in healthy human skin and keloid scars. Results showed highly variable abundances, distributions and morphologies between patient samples, where future work will question whether these differences correlate with anatomical site or clinical features of the scars. Finally, we questioned whether Schwann cells may be present in our primary patient-derived cell cultures. To test this, we cultured keloid cells in Schwann cell differentiation medium and were excited to see them adopt dramatic morphological changes (arborisation) and to begin expressing SOX10 and S100B (Schwann cell markers). The totality of this response leads us to interpret these observations as evidence of remarkable keloid cell plasticity.

Brief Resume of your Project's outcomes: **(no more than 200-250 words)**.
The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.

TITLE: What is special about the skin of the face?

Our skin has distinct characteristics at different sites on our bodies, but this obvious variability is largely ignored in wound healing and skin disease research. Facial skin is considered to have superior wound healing properties yet vulnerability to certain skin diseases, such as keloid scars. We recently reported that adult facial skin is remarkably different from other sites at rest and during wound repair, with characteristics reflecting the unique development of the tissue from neural crest.

This project began with analysing the anatomical variations of skin in terms of cell composition. We uncovered higher global immune cell numbers in the face, a reduced proportion of mesenchymal fibroblast markers (cells enriched in fibrosis), and an enrichment of Schwann cells. This was interesting given the reported increase in Schwann-like cell abundance in keloids and recognising that keloid scars occur most frequently on the face/neck. To expand on this, we performed S100B (Schwann cell marker) immunostaining on a large collection of keloids. Highly variable abundances, distributions and morphologies were observed; future work will question whether these differences correlate with anatomical site or clinical features of the scars. Finally, we questioned whether Schwann cells may be present in our primary patient-derived cell cultures by culturing keloid cells in Schwann cell differentiation medium. Keloid fibroblasts adopted dramatic morphological changes (arborisation; Figure) and to begin expressing SOX10 and S100B (Schwann cell markers). The totality of this response leads us to interpret these observations as evidence of remarkable keloid cell plasticity that we propose will have disease implications.



Other comments: (no more than 300 words)

None.

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Signature of student..... *.....Date……18/12/2024*

Signature of supervisor…… *..... Date…18/12/2024….*

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