**Analysis of immune cell phenotype and heterogeneity in young versus aged wounds**

## 1. Names and affiliations and disciplines of the research team

Dr Kimberly Mace, Lydia Becker Institute of Immunology and Inflammation, School of Biological Sciences, Faculty of Biology, Medicine and Health; Discipline: wound healing and regulation of inflammation in response to injury

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## 2. Outline of proposed research

*Background and rationale*

Complications associated with impaired wound healing in the elderly can result in increased morbidity and reduced quality of life as well as increased mortality rate (Thomason HA et al., 2009). In addition, the cost to national health services across the globe is significantly increasing due to these complications (Jaul E et al., 2017). Understanding the underlying mechanism resulting in impaired wound healing is critical to developing therapies and technologies to enhance tissue repair and regeneration.

Inflammation during wound healing is necessary to kill pathogens and clear the wound bed of debris and dead cells. Following an acute inflammatory phase, myeloid cells, comprised of neutrophils and macrophages, must switch phenotype from pro-inflammatory to pro-healing in order to promote the next phase of wound healing, the proliferative/regenerative phase. When this fails to occur, a chronic inflammatory environment develops, resulting in delayed and impaired healing.

We and others have shown that the acute inflammatory response in aged wounds is perturbed (Swift ME et al., 2001). However, the cause of the aberrant behaviour of myeloid cells in response to injury is not understood. Orchestration of the injury response is complex and the heterogeneity of cell types and their functions remain uncharacterised during this process. Therefore we perform single cell sequencing on dissociated wound cells from the inflammatory phase of wound healing (Day 3) in young versus aged mice in order to gain insight into the aberrant cell behaviours observed during impaired wound healing. The results from these analyses, in conjunction with tissue level histological and single molecule RNA *in situ* studies, as well as high dimensional flow cytometry data **already obtained** from myeloid cells in young and aged wound tissue**,** will allow us to complete our manuscript describing the underlying mechanisms contributing to impaired immune responses during wound healing in the elderly. This will be the first single cell analysis of aged wounds to our knowledge and will be submitted to a high impact journal.

*Plan of work*

Single cell gene expression analyses are necessary to characterise the phenotype of wound resident and immune cells at high resolution. We have already completed one round of single cell sequencing and high dimensional flow analyses of myeloid cells in wound tissues of young and aged mice. However, it is

essential repeat these experiments and analyses in order to publish our findings. Therefore, we will isolate wound tissue from 3 young versus 3 aged mice, dissociate the tissue into single cells as previously described (Torbica T et al., 2019) and perform a second round of flow cytometry and single cell sequencing. We already have funding to repeat the high dimensional flow analyses and are **therefore requesting funds to cover single cell sequencing costs only**.

Mace lab members will perform the wounding experiments (Home office licence in place to cover in vivo work: PPL P83A89CE6) and tissue dissociation to obtain single cell suspension. Cells will be passed to the Genomics facility directly for immediate sequencing. The Genomics facility provides basic data processing such as sequencing read filtering, trimming, and normalisation as well as mapping. Ronshaugen lab members will perform more advanced single cell data analyses using a workflow to identify cell types via hierarchical clustering and t-distributed stochastic neighbour embedding (t-SNE) in order to visualise cell heterogeneity and identify differences between young and aged myeloid cells.

Typically, thousands of cells can be individually sequenced from a heterogeneous population. At Day 3 of wound healing, approximately 50% of the cells in the tissue are myeloid cells. These cell populations can be analysed based on lineage-specific transcripts, but other cell populations, such as keratinocytes and fibroblasts will also be able to be analysed, making this a robust and unbiased approach to understanding differences in wound healing and developing new hypotheses regarding how ageing impacts cell behaviour during tissue repair and regeneration.

*Timeline*
June 2020 – wounding and sequencing will take place
July 2020 – data analyses will be perfomed

*References*
Jaul, E., & Barron, J. (2017). Age-Related Diseases and Clinical and Public Health Implications for the 85 Years Old and Over Population. *Frontiers in Public Health, 5*, 335.

Swift ME, Burns AL, Gray KL, DiPietro LA. (2001 ). Age-related alterations in the inflammatory response to dermal injury. *J Invest Dermatol*. 117(5):1027-35.

Thomason, H., & Hardman, M. (2009). Delayed wound healing in elderly people. *Reviews in Clinical Gerontology, 19*(3), 171-184. doi:10.1017/S095925980999027X

Torbica T, Wicks K, Umehara T, Gungordu L, Alrdahe S, Wemyss K, Grainger JR, Mace KA (2019). Chronic inflammation in response to injury: retention of myeloid cell in injured tissue is driven by myeloid cell-intrinsic factors. *J Invest Dermatol* 139:1583-92.

## 3. Summary budget of project costs

£6,000 - Genomics facility charges for sequencing of 2 RNA single cell samples (3 young versus 3 aged wound-dissociated cell samples, pooled into 2 groups) on 10X Chromium single cell sequencing platform – to be performed by University of Manchester Genomic Technologies Core Facility.