Establishment of a protocol for multicolor flow cytometry of wounded porcine skin

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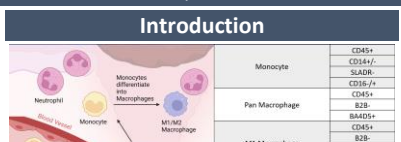
Abstract

Due to their similarity to human skin, pigs are increasingly used to study wound healing. Both pig and human skin have firm attachment, sparse hair coat, thick epidermis and dermis, no *panniculus carnosus*, and heal by re-epithelialization with minimal contraction.

Flow cytometry is a robust method to analyze the inflammatory milieu at the wound site. Although efforts have been made to optimize porcine skin digestion for flow cytometric analysis, it is currently not a common practice and there are no widely used protocols.

Our lab optimized a method to isolate single cells from porcine skin following excision injury using an enzymatic digestion that yield consistently robust single cell isolation with high viability. We also optimized reliable antibody panels, for the identification of granulocytes (CD45+B2B+), monocytes subsets (CD45+B2B-SLADR-CD14+CD16+/-), as well as M1 inflammatory (CD45+B2B-SLADR+CD80+) and M2 reparative (CD45+B2B-SLADR+CD163+) macrophages. Development of a reliable method to isolate single cells from porcine skin opens the door for studies to assess the cellular changes during porcine wound healing.

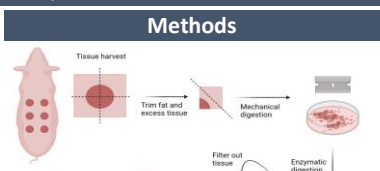
Introduction



Monocyte	CD45+
	CD14+/-
	SLADR-
	CD16-/+
Pan Macrophage	CD45+
	B2B-
	BA4D5+
M1 Macrophage	CD45+
	B2B-
	BA4D5+
	CD80+
M2 Macrophage	CD45+
	B2B-
	BA4D5+
	CD163+
Neutrophil	CD45+
	B2B+

Figure 1: Cell types of interest and their marker expression. Monocytes subsets are CD45+2B2-SLADR-CD14+CD16+/-), pan macrophages are CD45+B2B-BA4D5+, M1 inflammatory Macrophages are CD45+2B2-SLADR+CD80+, M2 reparative macrophages are CD45+2B2-SLADR+CD163+, and Neutrophils are CD45+2B2+.

Methods



Results

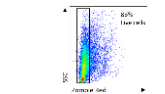


Figure 2: Enzymatic digestion of porcine skin allows isolation of live cells. Flow cytometric analysis demonstrates an average of 80+% viable cells following mechanical and enzymatic digestion.

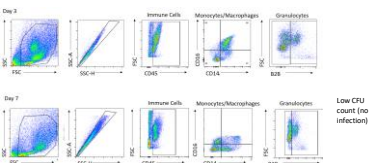


Figure 3: Changes in the inflammatory milieu from day 3 to day 7 post excision wounding. Flow cytometric analysis demonstrates observable changes in the inflammatory milieu in wounds at day 3 to day 7 post excision injury, particularly in monocyte/macrophage populations.

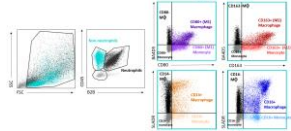
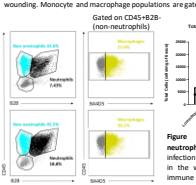


Figure 4: Separation of monocyte and macrophage subsets. Cells isolated from day 10 post excision wounding. Monocyte and macrophage populations are gated on non-neutrophil population.



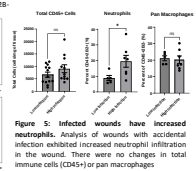


Figure 5: Infected wounds have increased neutrophils. Analysis of wounds with accidental infection exhibited increased neutrophil infiltration in the wound. There were no changes in total immune cells (CD45+) or pan macrophages

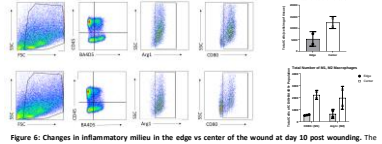


Figure 6: Changes in inflammatory milieu in the edge vs center of the wound at day 10 post wounding. The edge of the wound (top flow panel) has a trending increase in immune cell infiltration (CD45+) and CD80+ (M1) and Arg1+ (M2) macrophages at day 10 post wounding.

Conclusions and Future Directions

- Optimization of live cell isolation from porcine skin via enzymatic digestion
- Identification of reliable antibody panels for the characterization of granulocytes and monocyte/macrophage subset populations during the healing processing of porcine wounds

Acknowledgements

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