Diabetic Complications Consortium

Application Title: Characterization of Cellular and Molecular Heterogeneity in Human Diabetic Foot Ulcers

Principal Investigator: Horsley

1. Project Accomplishments:

Chronic, non-healing wounds are a significant concern to diabetic mellitus patients. To uncover potential mechanisms impairing diabetic wound healing, we compare the cellular complexity and differential gene expression between diabetic foot ulcers (DFUs) and non-diabetic foot ulcers (NDFUs) in humans. Diabetic and non-diabetic adults undergoing skin wound debridement were consented (HIC# 1609018360) to donate discarded tissue for this study. Wound healing requires the coordination of several different types of cells to regenerate after skin injury. Consequently, we have utilized the emerging single-cell transcriptomic sequencing to investigate the milieu heterogeneity of these wounds at the single-cell level. We have compared scRNAseq profiles from 5 DFUs and 3 NDFUs from individuals of both sexes and an average age of 57.5+/- 7 years. A total of 30,973 cells from all 8 samples were integrated using Scanorama batch correction. The transcriptomes of these cells were visualized on 2D graph using Uniform Manifold Approximation and Projection (UMAP) technique. Five major cell types: epidermal keratinocytes, fibroblasts, endothelium, pericytes, and immune cells were identified using cluster analysis and marker genes identification. Gene ontology analysis revealed groups of genes associated with keratinization, keratinocyte differentiation and epidermis development that were down-regulated in DFUs with respect to NDFUs, whereas genes associated with immune and inflammatory response, proteolysis, cell signaling, and collagen catabolic process were up-regulated in DFUs. Moreover, we identified two subpopulations of fibroblasts distinctively expressing different sets of immune response genes between DFU and NDFU. This expression pattern could underlie differences in healing rates and morbidity between diabetic and non-diabetic foot ulcers and continue to be investigated.

2. Specific Aims:

Aim 1: Establish protocols to collect, store and characterize cells within human diabetic foot ulcers and share data with community and consortium. We will use the expertise of our multidisciplinary team in human diabetic wounds (Hsia), skin cell isolation and characterization (Horsley), and data analysis (Gerstein) to collect and store tissues within diabetic foot ulcers. We will also host our data online for the public and consortium for collaboration and scientific advancement.

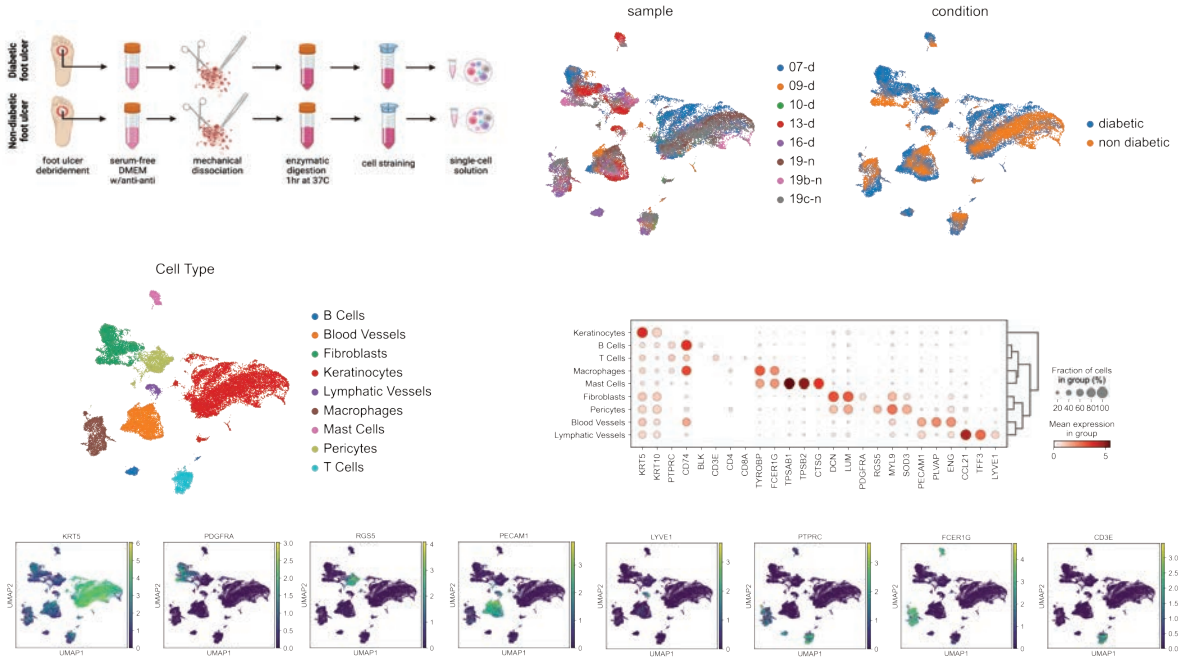


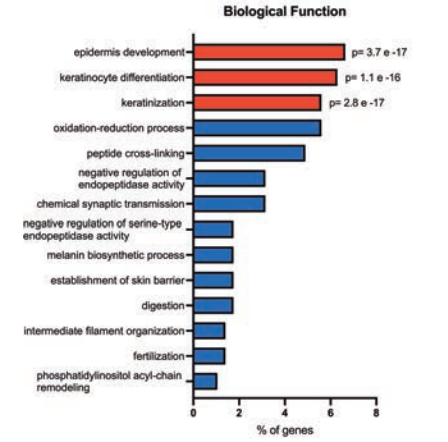
Figure 1. Isolation protocol and single cell RNA sequencing plots demonstrating feasibility and breadth of cell types.

We were able to successfully isolate cells from diabetic foot ulcers and control foot ulcers. We were ablet to identify a variety of cell types including keratinocytes, immune cells, and fibroblasts.

Aim 2: To utilize single cell resolution technologies to identify cellular heterogeneity with diabetic skin.

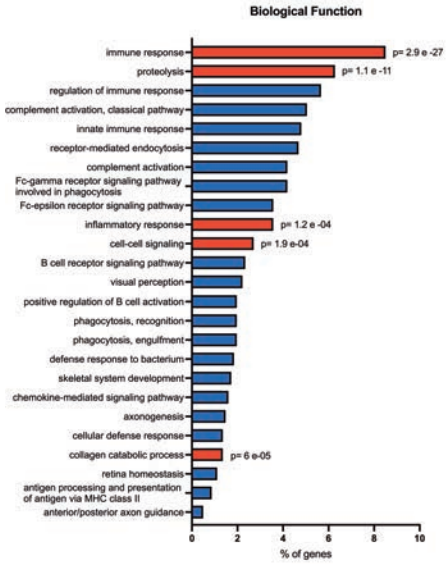
First, we analyzed whether the bulk diabetic and control samples displayed global gene expression changes (Figure 2). We found that genes downregulated in diabetic samples were associated with reduced keratinization. In contrast, the diabetic cells upregulated gene expression associated with inflammation, cell signaling, and collagen catabolism.

To determine if specific cell types contributed to these bulk gene expression changes, we first analyzed keratinocytes given the downregulation of genes involved in epidermal differentiation. Indeed, keratinocytes displayed downregulation of many genes involved in keratinocyte development, differentiation, and keratinization (Figure 3). Keratinocytes in diabetic foot wounds also upregulated genes in involved in immune response and changes in proteolysis and collagen catabolism (Figure 3). These data suggest that keratinocytes in diabetic foot wound are unable to fully promote reepithelization and contribute to changes in inflammation and ECM repair.



Genes Downregulated in DFUs

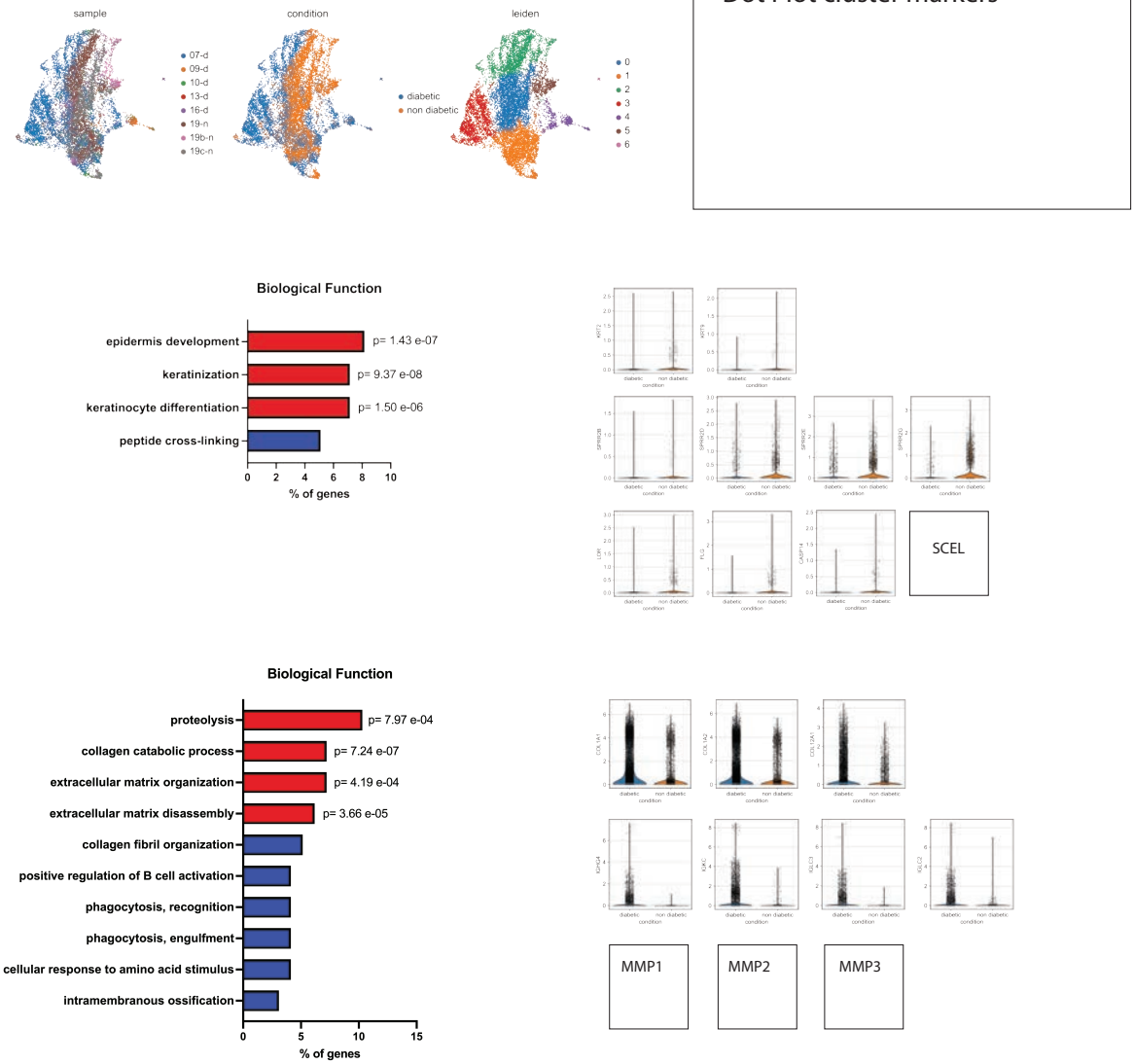
Epidermis Development
SPRR2E, SPRR3, SPRR2G, CDSN, KRT2, KLK5, LCE1E, KLK7, KRT9, LCE3D, ACER1, SCEL, CASP14, KRT17, DCT, TGM5, SPRR2B, SPRR1B, SPRR2D
Keratinocyte differentiation
FLG, SPRR2E, SPRR3, SPRR2G, CDSN, CRCT1, LCE1E, LOR, LCE3E, LCE3D, ACER1, SCEL, LCE2A, DSG4, SPRR2B, SPRR1B, TGM3, SPRR2D
Keratinization
SPRR2E, SPRR3, SPRR2G, KRT2, LCE1E, LOR, LCE3E, HRNR, LCE3D, CASP14, LCE2A, KRT17, SPRR2B, SPRR1B, TGM3, SPRR2D



Genes Upregulated in DFUs

Immune response
ADAMDEC1, CXCL9, IL26, CCL4L2, IGHV3-23, IGHV4-34, IGHV1-46, FASLG, IGHV4-39, CXCL13, JCHAIN, IGLV2-8, VPREB3, FCGR3A, FCGR3B, TNFSF11, CCR6, HLA-DOA, HLA-DOB, CR2, CHIT1, NCR3, AIM2, IGHD, IGKV4-1, TLR10, PKHD1L1, HLA-DQB1, CCL14, CCL13, IGHV2-5, CXCR5, IGLV3-1, IGHV1-69, KIR2DL3, CRHR1, IGLV2-11, VTN, IGLV1-40, HLA-DMB, IGLV3-27, IGLV1-47, IGKC, IGLV6-57, CNR2, IGKV1-39, IGLV2-14, IGLV3-21, IGLV1-44, IGKV3-15, IGHA1, CCL18, IGHA2, HLA-DQA2, HLA-DQA1, HLA-DRB5, IGHV3-53, IL10RB, IGHV3-11, GZMA, IGKV1-5, TNFRSF10C, KIR3DL1, PRG4, GZMH, CXCL11, CXCL12, IGKV3-20, IL17A
Proteolysis
ADAMDEC1, IGHV3-23, IGHV4-34, IGHV4-39, IGLV2-8, IGHG3, IGHG4, IGHG1, IGHG2, IGLC3, IGLC2, PRSS57, MMP12, MMP11, MMP13, IGKV4-1, PAPPA2, IGHV2-5, IGLV3-1, IGHV1-69, PRSS45, C2, IGLV2-11, ADAM28, IGLV1-40, PRSS50, IGLV3-27, IGLV1-47, IGKC, IGLV6-57, IGKV1-39, IGLV2-14, IGLV3-21, NRIP2, ADAMTS18, IGLV1-44, IGKV3-15, AMZ1, IGHV3-53, IGLC7, IGHV3-11, IGKV1-5, ECEL1, IGLC6, PGA5, PGA4, GZMK, GZMM, PIP, CTRC, IGKV3-20
Inflammatory response
CCL14, CCL13, CXCL9, IL26, CCL4L2, CXCR6, CXCL13, CRHBP, CNR2, ADORA1, FFAR3, CCL1, S1PR3, CCL18, PTGDR, IL10RB, CD180, TNFRSF10C, CYBB, CXCL11, NCR3, AIM2, CXCL12, TLR10, IL17F, SIGLEC1, CDO1, FOLR2, IL17A
Cell-cell signaling
FCRL2, CCL13, CXCL9, VIPR2, IL26, SIRPG, GATA4, FASLG, ADRB1, ECE2, BARX1, CXCL13, WISP2, CXCL11, FGF14, ADORA1, INHA, CCL18, WNT2, FGF12, SIGLEC6, IL17A
Collagen catabolic process
MMP12, COL1A1, MMP11, MMP13, COL1A2, COL25A1, COL11A1, COL4A4, COL12A1, COL8A1, COL19A1

Figure 2. Bulk changes in gene expression in healthy and diabetic foot ulcer samples.



ECM genes:

KLK12	Kallikreins (keratinization)
CTSK	Cathepsin (bone remodeling and resorption)
CAPN12	Calpain
COMP	cartilage oligometric matrix protein (ECM protein)
POSTN	Periostin ECM protein - wound healing)
LUM	Lumican (collagen fibrill org, epithelial cell migration and tissue repair)
MFAP2	Microfibril Assoc Prot 2 (degradation of ECM, elastic fibre formation)
FN1	Fibronectin 1 (wound healing)
TIMP2	MMP inhibitors
DPT	Dermatopontin (link btw dermal fibroblasts & ECM. Inh cell prolif, accelerates collagen fibril formation)
NTRK2	Neurotrophic tyrosine receptor kinase (cell diff)

Figure 3. Comparison of keratinocyte heterogeneity and gene expression in healthy and diabetic foot wounds.

To further explore changes in cell populations between health and diabetic foot wounds, we analyzed fibroblasts. Interestingly, we found that diabetic fibroblasts clustered in

distinct clusters compared to non-diabetic food wounds (Figure 4). Non-diabetic fibroblasts in foot wounds clustered into 3 main clusters, while diabetic fibroblasts clustered into 2 additional clusters. Foot wound fibroblasts in diabetic patients upregulated genes involved in signal transduction, cell adhesion, and extracellular matrix organization.

Interestingly, Cluster 3, which is enriched in diabetic patients and cluster 4 expressed IL-6, indicating that both distinct populations had an inflammatory signature (Figure 4). To examine the differences between these fibroblast populations, we compared the genes differentially expressed each population. While 10 genes were shared between clusters 3 and 4, 67 genes were unique to the diabetic enriched cluster 3 (Figure 4). These unique genes were involved in translation and inflammation, suggesting that diabetic fibroblasts are more inflammatory than their counterparts in non-diabetic foot wounds (Figure 4).

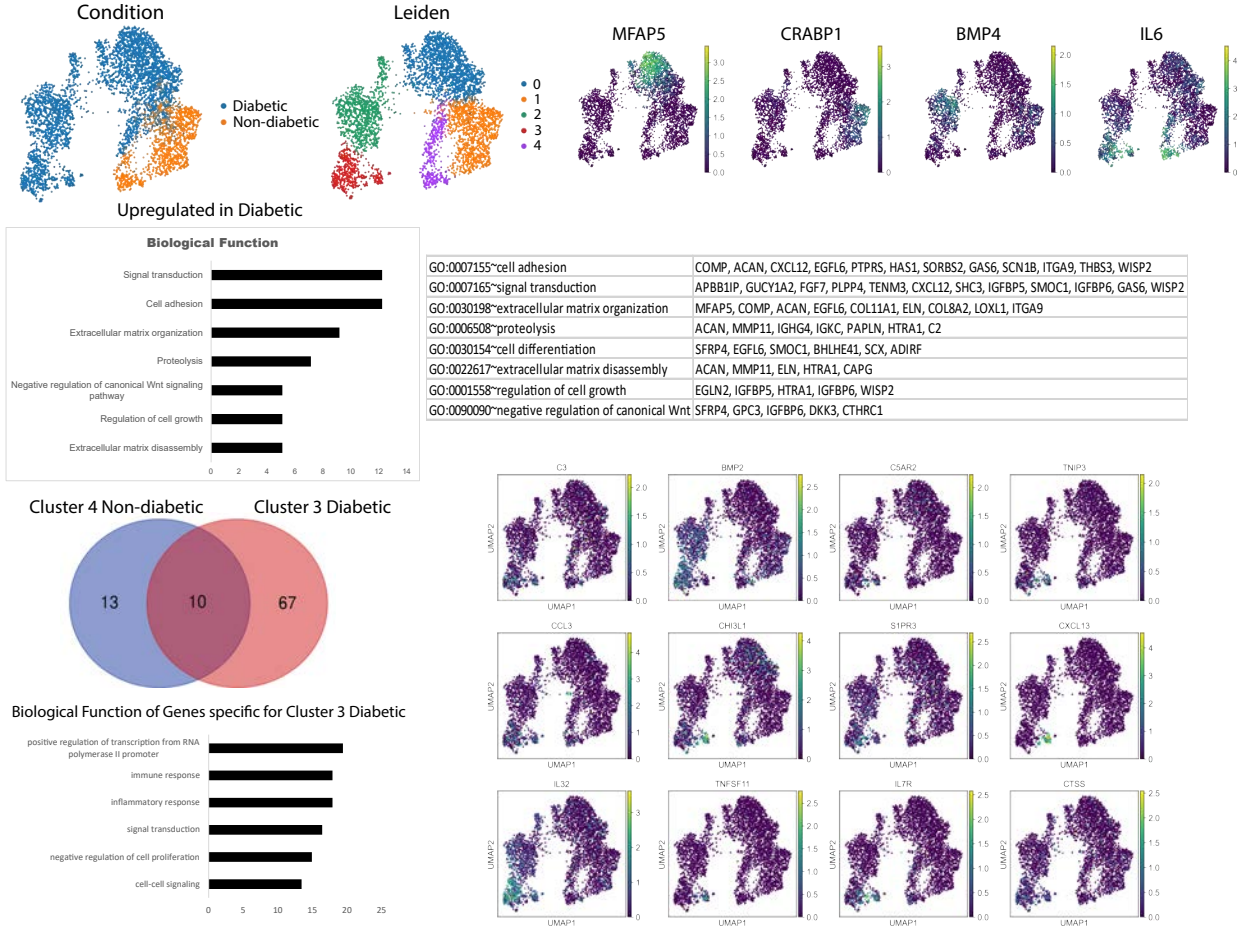


Figure 4. Comparison of fibroblast heterogeneity and gene expression in healthy and diabetic foot wounds.

To investigate whether changes in inflammatory cells were detects in healthy vs. diabetic foot wounds, we analyzed macrophages in these patient samples. The diabetic

macrophages upregulated genes involved in cell adhesion, extracellular matrix, and metabolism (Figure 5).

Taken together, these data indicate that changes in cellular heterogeneity underlies defects in diabetic foot wounds. Our data may provide novel insights into mechanisms why which diabetic foot wounds display healing defects as well as providing markers for exploring the functional role of these cells and molecular changes in wound repair.

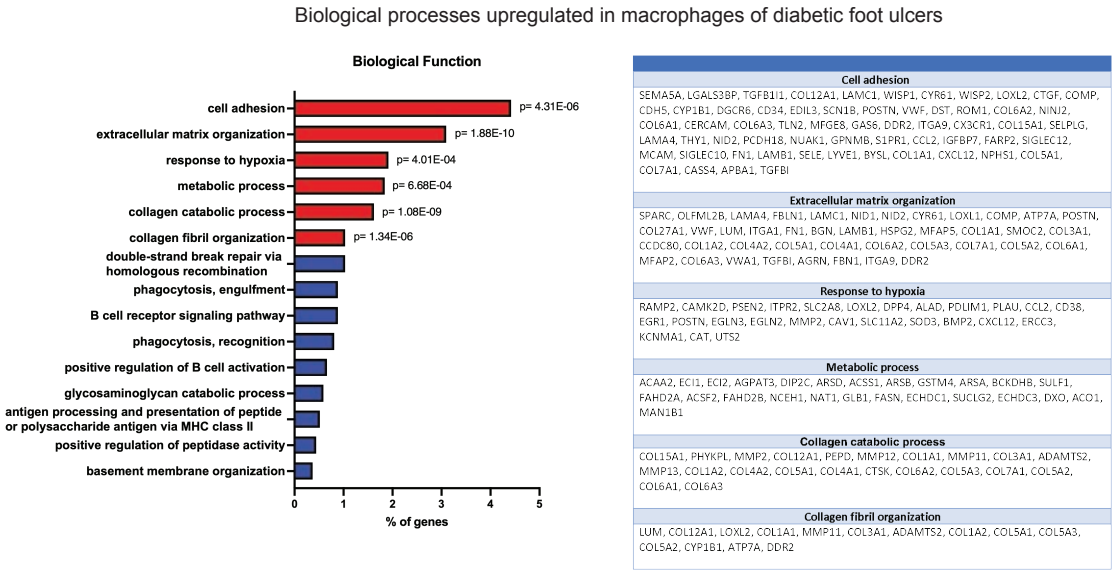
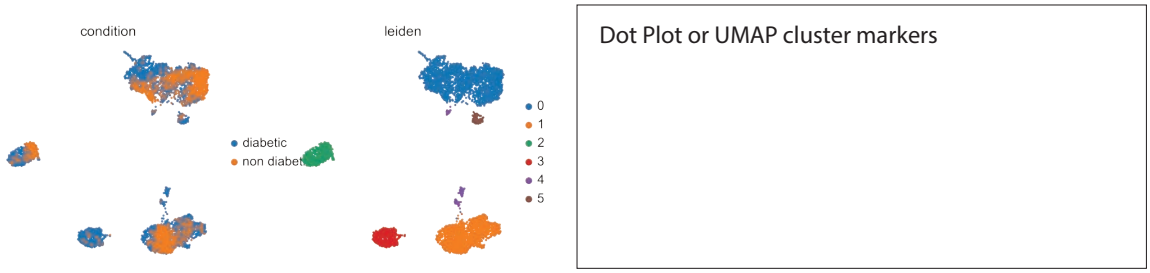


Figure 5. Comparison of genes upregulated in macrophages in diabetic foot wounds compared to healthy controls.



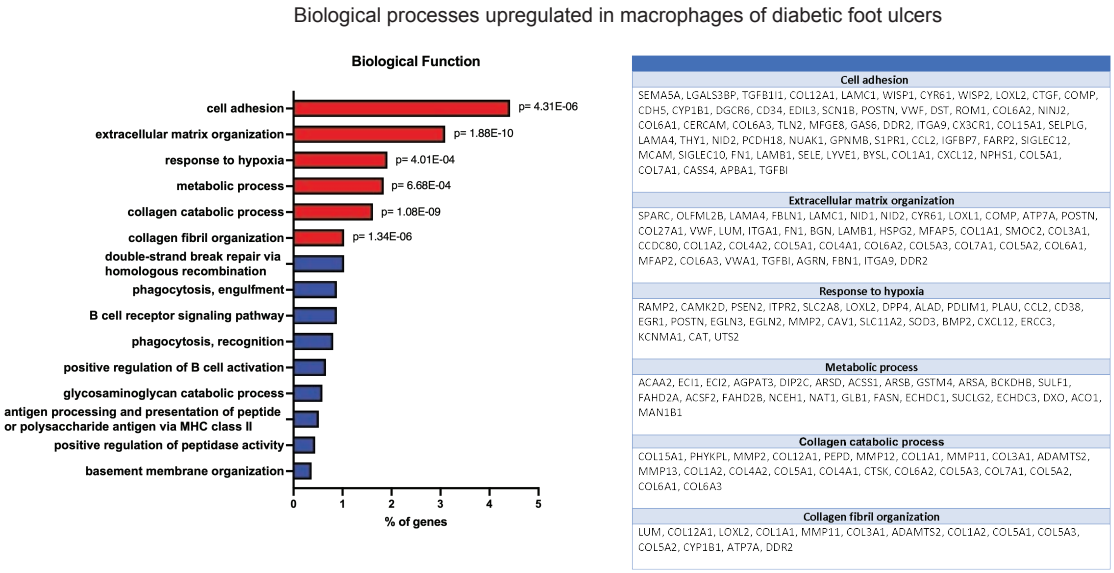


Figure 4. Comparison of macrophages in healthy and diabetic foot wounds.

Aim 3: To visualize cell types in the cellular context with diabetic skin wounds.

We were unable to complete this aim given time constraints with the COVID-19 pandemic.

3. Publications:

Data to be submitted in 2021.