**Diabetic Complications Consortium**

**Application Title:** Defining Host-Microbiome Interactions in Diabetic Wound Healing
**Principal Investigator:** Lindsay Kalan, PhD

## 1. Project Accomplishments:

Chronic non-healing wounds, such as diabetic foot ulcers, arise when normal tissue repair processes are interrupted. These types of wounds are associated with severe morbidity and diminished quality of life. Further, the microbiome of chronic wounds is highly complex and diverse, comprising bacteria and fungi from both unaffected skin and the environment. Current treatment strategies include surgical debridement of wound tissue, aiming to both reduce microbial burden and stimulate cellular regeneration by inducing an acute wound. However, the mechanisms underlying this response are poorly characterized. Understanding how the microbiome modulates host responses associated with tissue repair may lead to novel diagnostic markers to predict healing outcomes. In this pilot study we proposed to employ dual transcriptome profiling (RNA-seq) of the host and microbiome to simultaneously characterize host and microbial gene expression in response to debridement. Our goal is to identify molecular signatures associated with cellular responses to promote healing or microbial signatures associated with persistence and biofilm formation that impede healing.

This project has two aims: 1) Define host-microbiome responses to sharp debridement and 2) Identify microbial signatures associated with impaired wound healing in diabetic foot ulcers. The study design includes recruitment from the Madison Veterans Hospital Diabetic Foot Ulcer Clinic. The original study design included if 1) they are >18 years old, 2) have a Wagner grade 1 diabetic foot ulcer (uninfected, but colonization allowed) for >1 month, 3) have palpable pedal pulses. Patients were excluded if 1) they are not receiving sharp debridement as part of routine clinical care, 2) use of a skin substitute is planned (which precludes future sharp debridement), or 3) they have received systemic antimicrobials in the past 2 weeks. We aimed to enroll 40 patients to achieve our target of 30 study participants.

We have achieved our enrollment goal and have obtained specimens from 48 patients resulting in a total of 144 unique samples for processing to satisfy the goals of our aims. A single set of samples has three total specimens including the pre-debridement sample, post-debridement sample, and healthy skin sample from the contralateral foot. The baseline samples from the first 10 patients was used for optimizing extraction of RNA from these types of samples. Our initial extraction processes found highly degraded and low quality RNA that would not be suitable for high-throughput sequencing. We were able to work through these challenges to optimize the extraction process to yield both RNA and DNA suitable for sequencing on the remaining 38 patient samples.

## 2. Specific Aims:

### Results

**Specific Aim 1**. Define host-microbiome responses to sharp debridement
We created RNAseq libraries and had them sequenced on the NovoSeq at the UW Biotechnology Center. This sequence run was successful, resulting in yields of >100 million reads per sample on average (**Fig. 1**) for both the pre-debridement and post-debridement samples.

To process raw data reads from multiple lanes were merged into per-sample fastq files and adaptor and low quality reads were removed with cutadapt. STAR aligner was used to map

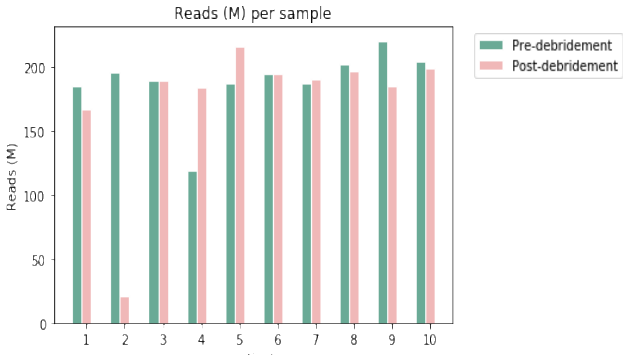


**Figure 1**: Total number of reads generated from RNAseq libraries of pre- and post-debridement samples organized by patient.

reads to GENCODE's hg38 transcriptome. Transcripts mapped to the hg38 were then quantified using RSEM. ENSEMBL ID's were translated to GO terms and functional descriptions with R package *biomart*. Visualization of related GO terms were made with NaviGO to identify similar gene ontologies.

Our initial analysis of the data is consistent with skin and wound healing processes. For example, in the top 100 expressed genes we detected several genes encoding keratins, genes involved in the inflammatory response, and long noncoding RNAs known to be involved in the regulation of tissue repair. The top 15 expressed genes are listed in **Table 1**.

**Table 1: Top 15 Expressed Genes from Diabetic Foot Ulcer Samples Collected at Visit 0**

| Ensemble Gene ID | Gene Description | Gene Name |
|---|---|---|
| ENSG00000251562.8 | metastasis associated lung adenocarcinoma transcript 1 | MALAT1 |
| ENSG00000274012.1 | RNA component of signal recognition particle 7SL2 | RN7SL2 |
| ENSG00000210082.2 | mitochondrially encoded 16S rRNA | MT-RNR2 |
| ENSG00000281181.1 | novel transcript, similar to RNA 1 YAM1 | FP236383.5 |
| ENSG00000283293.1 | RNA component of 7SK nuclear ribonucleoprotein | RN7SK |
| ENSG00000205420.11 | keratin 6A | KRT6A |
| ENSG00000186847.6 | keratin 14 | KRT14 |
| ENSG00000186832.9 | keratin 16 | KRT16 |
| ENSG00000245532.9 | nuclear paraspeckle assembly transcript 1 | NEAT1 |
| ENSG00000128422.17 | keratin 17 | KRT17 |
| ENSG00000169429.11 | C-X-C motif chemokine ligand 8 | CXCL8 |
| ENSG00000206585.1 | RNA, variant U1 small nuclear 7 | RNVU1-7 |
| ENSG00000096696.14 | desmoplakin | DSP |
| ENSG00000282885.2 | novel transcript | AL627171.4 |
| ENSG00000185479.6 | keratin 6B | KRT6B |
| ENSG00000135046.14 | annexin A1 | ANXA1 |

Our microbiome profiling was successful and consistent with our previous work in regards to overall taxonomic composition for both bacteria and fungi. We are in the process of completing data analysis to identify transcriptional profiles associated with healing outcomes and specific microbiome metrics.

**Specific Aim 2**. Identify microbial signatures associated with impaired wound healing in diabetic foot ulcers

## Results

We have successfully detected microbial reads from the total RNAseq data suggesting our sequence depth was sufficient. We found that percent of non-human reads per sample was highly dependent on the type of specimen collected. For example, in the pre-debridement samples a larger proportion of reads were non-human whereas swabs collected from the deep tissue post-debridement contained fewer non-human microbial reads. We optimized our classification pipelines and adjusted from the original proposal. We originally proposed to create a custom genomic database of species identified from our 16S profiling in Aim 1. We would then use this

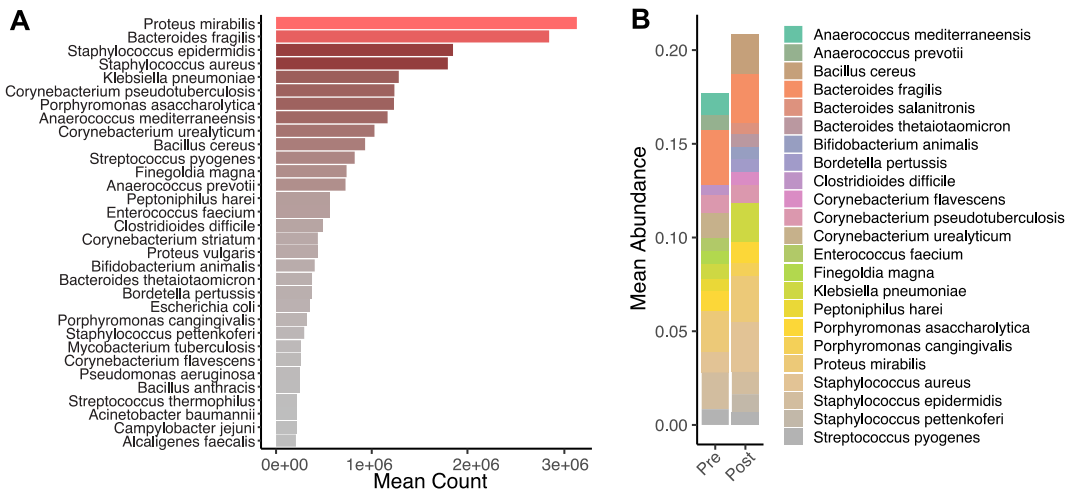


Figure 2: Microbial transcripts detected from diabetic foot ulcer samples. A) Mean transcript count by taxonomic classification across all samples (n=40). B) Mean abundance of bacterial species in pre- or post-debridement samples. Taxonomic classification and relative abundance values were computed using the Bracken package (Lu et al. 2017 PeerJ).

database to classify the non-human reads and identify metabolically active microbial taxa and corresponding upregulated genes. We have developed a pipeline to classify the non-human reads directly against public databases by adapting a custom metagenomic pipeline in our laboratory. This has sped up the computation time and power required for processing the large number of reads from multiple species. This has allowed us to identify the most metabolically active species present in the samples. **Figure 2** shows the taxa ordered by the most abundant transcripts detected and top taxa identified in pre and post debridement samples. We find organisms historically associated with DFU are metabolically active, including a large proportion of anaerobes and facultative anaerobes such as *Proteus mirabilis*, *Bacteroides fragilis*, and *Porphyromonas asaccharolytica*. Analysis is still ongoing in preparation of a manuscript.

## 3. **Publications:**

Two manuscripts are in preparation.