What is Wound Slough? A pilot study to define the proteomic and microbial composition of wound slough and its implications for wound healing.

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International Wound Infection Institute

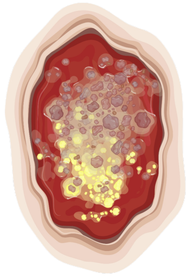
Slough: composition, analysis and effect on healing. Kalan et al., 2023

Introduction

Chronic Wounds impact **2.5–4.2%** of the general adult population in the US and UK.

Slough is a well-known feature of non-healing wounds.

- Slough is visually and texturally heterogenous in presentation and varies from wound to wound.
- Ultimately, the **tissue and microbial composition of wound slough is not well defined.**
- The implications of slough composition on a wound's ability to heal are also unknown.



Methods

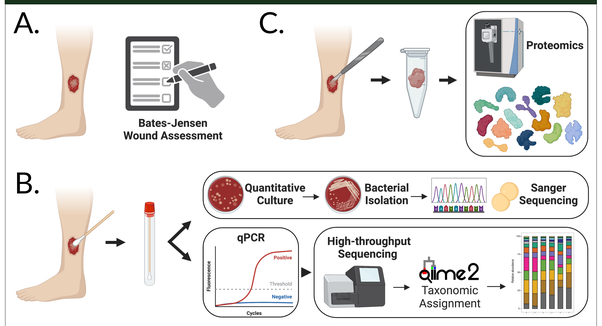


Figure 1 Sample collection: 23 subjects with chronic wounds and visible slough were enrolled. **A:** Clinical wounds status was measured via the Bates-Jensen Wound Assessment. **B:** Swabs of the wound surface were collected via the Levine technique and sent for bacterial 16S ribosomal gene sequencing. **C:** Wound slough was removed with sharp debridement and sent for proteomics via liquid chromatography mass spectrometry (LC-MS).

Subjects

	Subject 001	Subject 002	Subject 003	Subject 004	Subject 005	Subject 006	Subject 007	Subject 008	Subject 009	Subject 010
Subject Age(yr)	70	74	72	77	62	43	77	73	57	59
Biologic Sex	M	M	M	F	F	M	F	F	F	F
Race	White	White	Black	White	White	White	White	White	White	White
Wound Age(yr)	15	3	2	0.15	0.12	0.1	1.17	1.25	0.33	1
Wound Etiology	Venous Stasis Ulcer	Venous Stasis Ulcer	Surgical Infection	Trauma	Trauma	Venous Stasis Ulcer	Surgical Infection	Venous Stasis Ulcer	Pressure Ulcer	Pressure Ulcer
Wound Location	Left Medial Ankle	Left Anterior Shin	Right Lateral Ankle	Right Lateral Shin	Left Anterior Shin	Right Posterior Lower leg	Right Lateral Ankle	Left Medial Shin	Sacrum, coccyx	Sacrum, coccyx
Bates-Jensen Score	40	35	34	34	35	26	46	40	42	42
Outcome 3 mo. after Sample Collection	Deteriorated	Ongoing	Ongoing	Healed	Healed	Healed	Deteriorated	Ongoing	Ongoing	Deteriorated

Table 1: Subject and Wound Characteristics for the 10 subjects w/ completed sample processing. Wound etiology and characteristics as well as patient co-morbidities were extracted from the medical record. Whether the wound healed, was stable, or deteriorated 3-months following sample collection was also recorded.

Aim

To determine the proteomic and microbiologic components of slough as well as interrogate the associations with wound healing.

Slough Microbiome

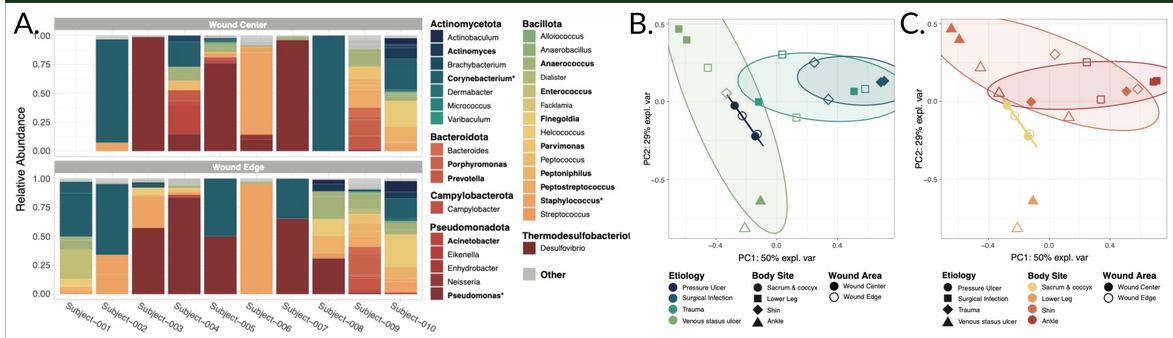
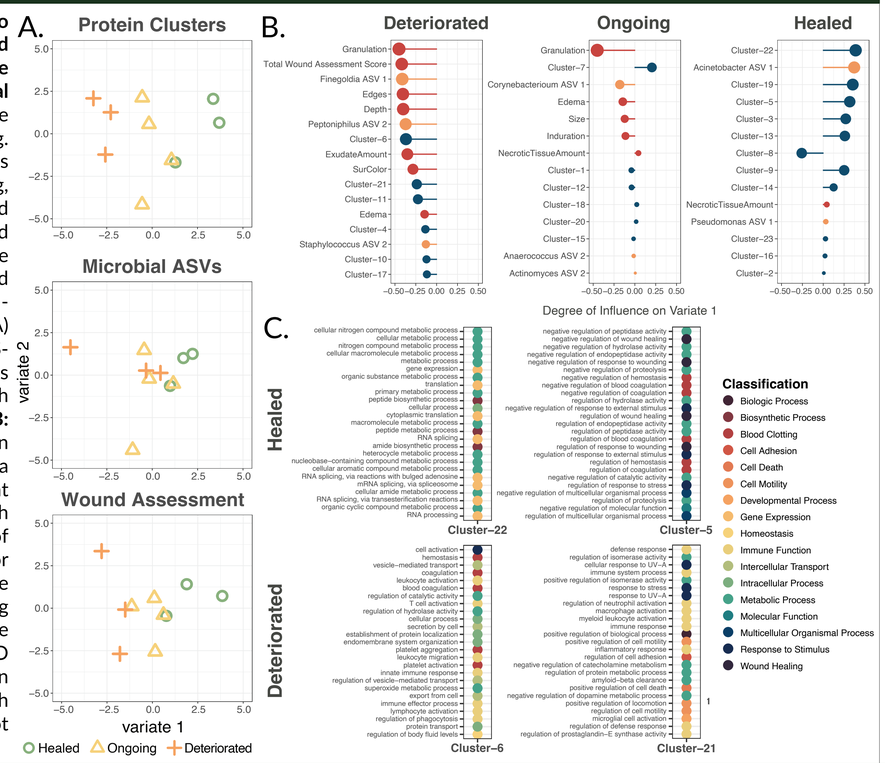


Figure 2: Slough microbiome associates with the wound's etiology and location on the body. A: Relative abundance plots of the microbial communities at the wound edge (top row) and center (bottom) in each subject. Taxa comprising ≥ 1% of sample reads are represented. Overall slough microbiome is enriched for skin associated taxa (eg. *Corynebacterium*, *Staphylococci)* and anaerobes (e.g. *Anaerococcus*, *Finegoldia*, *Peptoniphilus*, *Prevotella*, *Prophyromonas*). **B-C:** PCA plot illustrating that wound microbial communities cluster by the wound's etiology (B) and body site (C) respectively (p-values < 0.01 for both, PERMANOVA).

Implications for Wound Healing

Figure 4: Chronic wounds that go on to heal can be distinguished from those that get worse via the proteomic, microbial, and clinical features of slough. Proteins were grouped by k-means clustering. To predict the variables associated with wound healing, the protein cluster, microbial, and the Bates - Jensen Wound Assessment datasets were integrated into a supervised Partial Least Squares - Discriminant Analysis (PLS-DA) via the *mixOmics* package. **A:** PLS-DA plots indicating that features of each dataset can distinguish wound healing outcomes. **B:** Variable plots of the protein clusters (blue), microbial taxa (orange) and wound assessment criteria (red) that distinguish each outcome group along variate 1 of the PLS-DA plots. A longer vector to the right indicates a variable with greater influence pulling samples to the right along the variate 1 axis. **C:** Enriched GO processes within the top protein clusters that distinguish slough from wounds that healed or got worse from the other outcomes.



Slough Proteome

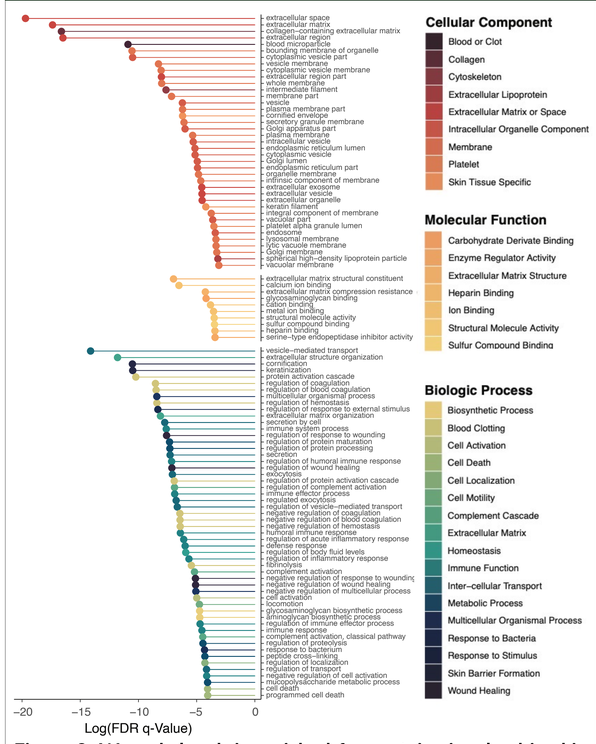


Figure 3: Wound slough is enriched for proteins involved in skin barrier formation, wound healing, blood clotting, and various immune functions. The most abundant proteins across all slough samples were input as a ranked list to the Gene Ontology enRIchment analysis (*GOrilla*). Significantly enriched GO terms are listed by their description and ordered by FDR-qValue. GO terms associated with extracellular and cellular components, molecular functions, and biologic processes are in reds, orange-yellows, and yellow-greens to blues respectively.

Conclusion

The proteomic and microbial composition of slough is associated with wound healing outcome 3 months later.

- Wound slough contains complex microbial communities as well as a milieu of skin and immune associated proteins.
- The slough microbiome associates with the wound's etiology and its location on the body.
- Slough enriched for chronic inflammatory proteins, anaerobic microbial taxa, and with higher Bates-Jensen Wound Assessment Scores were associated with wounds that clinically deteriorate 3-months later.

Future works will utilize these methods to evaluate the composition of wound slough from more subjects.