

**SUPPLEMENTAL DATA FOR “TEMPORAL STABILITY IN CHRONIC WOUND
MICROBIOTA IS ASSOCIATED WITH POOR HEALING”**

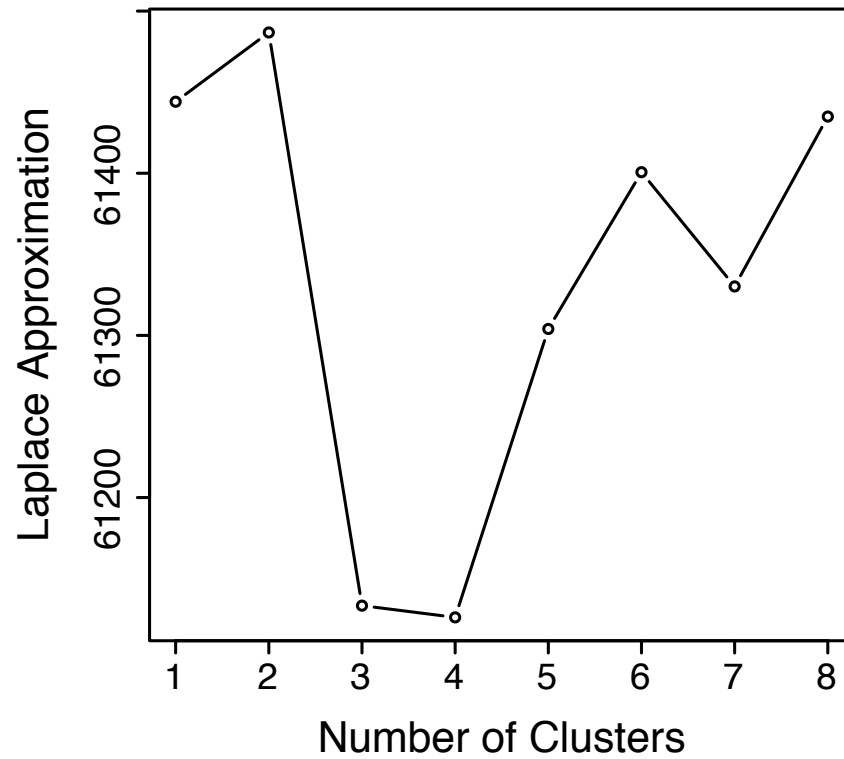


Figure S1. Laplace approximation predicts 4 clusters as optimal. The Laplace approximation of model evidence is a measure of the model fit. Lower values indicate better fits.

Table S1. Patient and ulcer characteristics for the total sample and by complication status.

‡ continuous level characteristics were analyzed with an independent samples t-test; Dichotomous characteristics were analyzed with Fisher's Exact Test; Other categorical characteristics were analyzed with Pearson's Chi-Square. An asterisk (*) indicates $p < 0.05$.

Characteristic	Total Sample (N=100)	Did not develop infection- related complication (n=69)	Developed infection- related complication (n=31)	‡p-value
Age (years), mean (SD)	54.1 (11.29)	55.4 (12.53)	52.3 (7.65)	0.136
Male sex, n (%)	78 (78.0)	56 (81.2)	22 (71.0)	0.3
White race, n (%)	91 (91.0)	62 (89.9)	20 (93.5)	0.498
Type 2 diabetes, n (%)	87 (87.0)	60 (87.0)	27 (87.1)	1
Duration of diabetes (years), mean (SD)	15.2 (11.31)	16.2 (11.55)	12.7 (10.55)	0.147
Baseline HbA1C (%), mean (SD)	8.2 (1.87)	8.32 (1.96)	8.0 (1.67)	0.461
Baseline WBC (mm ³), mean (SD)	7950.5 (1905.97)	7751.5 (1916.58)	8387.1 (1837.52)	0.121
C Reactive Protein (mg/L), mean (SD)	2.2 (4.78)	1.7 (3.04)	3.2 (7.25)	0.284
Ulcer duration (weeks), mean (SD)	31.1 (40.22)	33.5 (42.46)	25.90 (34.81)	0.352
Toe/brachial pressure index, mean (SD)	0.9 (0.25)	0.9 (0.27)	0.9 (0.22)	0.803
Ulcer surface area (cm ²), mean (SD)	2.4 (3.32)	2.0 (3.01)	3.3 (3.84)	0.101
Ulcer depth (cm), mean (SD)	0.3 (0.33)	0.2 (0.27)	0.4 (0.39)	0.002*
Ulcer location, n (%)				
Forefoot	73 (73.0)	50 (72.5)	23 (74.2)	
Midfoot	20 (20.0)	13 (18.8)	7 (22.6)	0.585
Heel	7 (7.0)	6 (8.7)	1 (3.2)	
Transcutaneous oxygen level (mmHg), mean (SD)	46.9 (15.11)	49.1 (13.80)	42.2 (17.00)	0.057

Table S2. Summary of microbial communities in DFU samples. For each bacterial taxon, the number of samples in which it was present was tabulated, and the mean relative abundance was calculated. These values were calculated for all samples, baseline only, and non-baseline samples. Taxa were filtered to those whose mean abundance was greater than 0.01.

Bacterial Taxon	Combined (n = 348)		Baseline (n = 89)		Rest (n = 259)	
	Samples	Proportion	Samples	Proportion	Samples	Proportion
Staphylococcus	345	0.2277	86	0.2188049	259	0.2317
aureus	308	0.1331	79	0.1559	229	0.1257
pettenkoferi	287	0.0532	60	0.0290	227	0.0617
unclassified	301	0.0402	69	0.0308	232	0.0435
Streptococcus	318	0.1250	81	0.1918	236	0.1025
anginosus	51	0.0052	9	0.0008	42	0.0067
unclassified	317	0.1198	81	0.1910	235	0.0958
Corynebacterium	345	0.1121	86	0.0761	258	0.1249
Anaerococcus	300	0.0700	78	0.0745	222	0.0688
Planococcaceae	221	0.0401	58	0.0457	162	0.0383
Alcaligenaceae	194	0.0379	40	0.0020	153	0.0502
Paenibacillus	293	0.0364	79	0.0429	214	0.0342
Brevibacterium	222	0.0325	44	0.0070	177	0.0414
Comamonadaceae	321	0.0193	84	0.0227	236	0.0178
Finegoldia	245	0.0151	64	0.0111	181	0.0165
Helcococcus	207	0.0125	46	0.0144	160	0.0119
Pseudomonas	110	0.0109	20	0.0014	90	0.0142
Actinomycetales	151	0.0103	33	0.0048	118	0.0123

Table S3. Summaries of taxonomic composition by community type. The values shown are the mean relative abundance for each community type. Only the top 25 taxa are shown. Species belonging to the same genera are combined for convenience.

	CT 1	CT 2	CT 3	CT 4
Streptococcus	0.0466	0.0528	0.6175	0.0275
anginosus	0.0048	0.0083	0.0032	0.0000
unclassified	0.0418	0.0445	0.6143	0.0275
Staphylococcus	0.2759	0.1710	0.0975	0.3688
aureus	0.1205	0.1012	0.0918	0.3479
pettenkoferi	0.0880	0.0374	0.0026	0.0152
sciuri	0.0025	0.0001	0.0000	0.0000
haemolyticus	0.0001	0.0004	0.0001	0.0000
unclassified	0.0649	0.0320	0.0030	0.0057
Corynebacterium	0.1586	0.0885	0.0269	0.1191
simulans	0.0024	0.0042	0.0010	0.0001
stationis	0.0000	0.0000	0.0000	0.0000
unclassified	0.1563	0.0843	0.0259	0.1190
Anaerococcus	0.0370	0.0884	0.0623	0.1759
Planococcaceae	0.0539	0.0345	0.0050	0.0442
Brevibacterium	0.0385	0.0346	0.0016	0.0419
Paenibacillus	0.0582	0.0233	0.0058	0.0206
Alcaligenaceae	0.0479	0.0495	0.0061	0.0008
Helcococcus	0.0079	0.0114	0.0079	0.0440
Finegoldia	0.0114	0.0197	0.0095	0.0254
Comamonadaceae	0.0245	0.0246	0.0020	0.0009
Peptoniphilus	0.0054	0.0134	0.0095	0.0204
Actinomycetales	0.0056	0.0185	0.0076	0.0106
Porphyromonas	0.0023	0.0141	0.0123	0.0107
Prevotella	0.0101	0.0032	0.0135	0.0026

Table S4. Clinical factors associated with the DFU microbiome at baseline.

Association/correlation between clinical factors and features of the DFU microbiome as measured at initial study visit. Spearman rank correlations were used for continuous variables.

Significant values ($p < 0.05$) are bolded.

	CRP		Depth		HbA1C		Tissue Oxygen		Area		Duration		WBC	
	rho	p-value	rho	p-value	rho	p-value	rho	p-value	rho	p-value	rho	p-value	rho	p-value
Anaerobes	-0.057	0.594	0.210	0.048	-0.049	0.648	0.102	0.349	0.031	0.777	-0.112	0.298	0.131	0.222
Anaerococcus	0.040	0.713	0.204	0.055	0.036	0.736	-0.016	0.886	0.087	0.416	-0.037	0.734	0.131	0.223
Corynebacterium	0.099	0.355	-0.065	0.544	0.038	0.726	0.029	0.791	0.053	0.621	0.099	0.357	-0.144	0.179
Firmicutes	-0.051	0.635	-0.033	0.759	0.144	0.179	0.138	0.203	-0.084	0.436	-0.091	0.398	-0.028	0.799
Phylogenetic Diversity	0.044	0.682	0.047	0.661	-0.061	0.568	0.090	0.406	0.063	0.556	0.091	0.394	0.011	0.923
Proteobacteria	-0.065	0.543	-0.129	0.228	-0.150	0.161	-0.056	0.610	0.097	0.366	0.139	0.193	-0.043	0.689
OTU Richness	-0.059	0.582	-0.005	0.967	-0.122	0.253	0.218	0.043	-0.053	0.624	0.035	0.746	-0.048	0.655
S. aureus	0.088	0.411	-0.080	0.457	-0.048	0.653	0.036	0.743	-0.095	0.378	-0.114	0.290	-0.130	0.228
Unclassified Staphylococcus	-0.018	0.869	-0.109	0.307	0.152	0.154	0.112	0.303	-0.283	0.007	0.044	0.684	-0.168	0.118
S. pettenkoferi	-0.017	0.878	-0.165	0.123	0.016	0.885	0.183	0.089	-0.286	0.007	-0.104	0.331	-0.119	0.269
Shannon Diversity	0.051	0.634	-0.008	0.942	-0.146	0.172	0.081	0.454	0.076	0.481	-0.051	0.632	0.010	0.925
Staphylococcus	0.070	0.517	-0.208	0.050	0.034	0.752	0.065	0.553	-0.186	0.081	-0.108	0.313	-0.182	0.089
Streptococcus	0.163	0.126	0.173	0.105	0.221	0.037	-0.002	0.984	0.018	0.871	0.289	0.006	0.037	0.729

Table S5. Estimated Markov chain parameters of DFU CT transitions. The values shown are 1) the stationary distribution, which describes the expected frequencies of CTs to be for a Markov chain, and 2) the expected recurrence time, which estimates the average time it would take to encounter the same CT again. Both show marked changes in the stability and frequency of CT3 and CT4 between DFUs that heal within 12 weeks and those that do not.

	Stationary Distribution		Expected Recurrence Time (weeks)	
	<12 weeks	>12 weeks	<12 weeks	>12 weeks
CT1	0.5460	0.4330	3.66	4.62
CT2	0.3240	0.2012	6.17	9.94
CT3	0.0343	0.1341	58.28	14.91
CT4	0.0958	0.2317	20.89	8.63