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Title: Cutaneous Burn Injury Promotes Shifts in the Bacterial Microbiome in Autologous Donor Skin: Implications for Skin Grafting Outcomes

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Abstract

Introduction: The cutaneous microbiome maintains skin barrier function, regulates inflammation, and stimulates wound healing responses. Burn injury promotes an excessive activation of the cutaneous and systemic immune response directed against commensal and invading pathogens. Skin grafting is the primary method of reconstructing full-thickness burns, and wound infection continues to be a significant complication.

Methods: In this study, the cutaneous bacterial microbiome was evaluated and subsequently compared to patient outcomes. Three different full-thickness skin specimens were assessed: 1.)control skin from non-burned subjects; 2.)burn margin from burn patients; and 3.)autologous donor skin from the same cohort of burn patients.

Results: We observed that skin bacterial community structure of burn patients was significantly altered compared to control patients. We determined that the unburned autologous donor skin from burn patients exhibits a microbiome similar to that of the burn margin, rather than unburned controls, and that changes in the cutaneous microbiome statistically correlate with several post-burn complications. We established that *Corynebacterium* positively correlated with burn wound infection, while *Staphylococcus* and *Propionibacterium* negatively correlated with burn wound infection. Both *Corynebacterium* and *Enterococcus* negatively correlated with the development of sepsis.

Conclusions: This study identifies distinct differences in the cutaneous microbiome between burn subjects and unburned controls, and ascertains that select bacterial taxa significantly correlate with several co-morbid complications of burn injury. These preliminary data suggest that grafting donor skin exhibiting bacterial dysbiosis may augment infection and/or

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graft failure and sets the foundation for more in-depth and mechanistic analyses in presumably "healthy" donor skin from patients requiring skin grafting procedures.

Key Words: skin graft, wound, bacteria, microbiome, infection, burn injury

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Introduction

The cutaneous microbiome exists as a diverse community capable of maintaining skin barrier function, regulating inflammation, and promoting wound healing responses (1-3). The pathophysiology of burn injury to the skin suggests an excessive activation of the cutaneous and systemic immune responses targeted against commensal and invading pathogens post-injury. It is interesting to theorize that, in some patients, a shift in the colonizing microbiota of the skin may provoke and propagate primary and secondary complications in burn patients, leading to increased morbidity and mortality. The identification of a "pathogenic" microbiota could lead to early diagnostic tools that may be able to predict infection risk or wound healing delays in burn subjects. Individuals with substantial burn injuries exhibit more diverse responses, as compared to other types of traumatic injury. For example, burn subjects exhibit greater morbidity than predicted using the injury severity scoring system (4), and they demonstrate a greater prevalence of sepsis and mortality (5, 6). These outcomes suggest that the destruction of the cutaneous barrier caused by severe burn injury may be provoking a unique impact on local and distal tissues, leading to increased morbidity and mortality. These outcomes are partially attributed to disturbances in the skin, including changes in innate immune function and the resident microbiota (7-9).

Skin grafting is the predominant method of reconstructing full-thickness burns. Autologous grafts from distal, unburned skin often exhibit functional deficiencies and tissue breakdown after grafting. Burn wound infection at both the donor and burn site remain a frequent and serious complication of major burn injury and account for over 50% of all deaths related to burn injury (6, 10, 11). We recently determined that epidermal lipid and antimicrobial peptide (AMP) responses are impaired in both donor skin and burn margin from human burn patients (12). These alterations in epidermal barrier function demonstrate that traumatic burn injury elicits a global change in the antimicrobial function of presumably

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normal skin, which would serve as donor skin for burn patients. Thus, after burn injury, the cutaneous microbiota is likely altered in donor skin, and may also be a source of graft failure, burn wound infections, and/or subsequent infectious complications in burn patients.

To our knowledge, the impact of burn injury on the cutaneous microbiome in the context of skin grafting has not been evaluated. In this study, we hypothesize that unburned autologous donor skin from burn patients exhibits a microbiome similar to that of the burn margin, rather than unburned controls, and that features of the cutaneous bacterial microbiome form burn patients statistically correlate with several post-burn complications. We propose that the colonizing microbiota in the skin may be used as a tool to predict morbidity and graft failure in burn patients, or other patient cohorts necessitating skin grafting procedures.

Materials and Methods

Sample Collection and Clinical Information

All protocols were approved by the Institutional Review Board at Loyola University Chicago Health Sciences Campus. A standing approval for discarded skin was used to collect the tissue samples. Briefly, patients admitted to the Burn Intensive Care Unit (BICU) were excluded from the study under the following conditions: age < 18 years, pre-existing skin disease, pre-existing clinically-evident infection, previous transplant recipient, recent major traumatic injury <4 months prior to the burn injury, history of disseminated cancer, and/or pre-existing immunodeficiency. The following clinical characteristics and outcomes were extracted from the electronic medical records and entered into a database: age, gender, % total body surface area (%TBSA) injured, inhalation injury, burn injury mechanism, and subsequent pneumonia, urinary tract infection, graft failure, wound infection, sepsis and/or multisystem organ dysfunction (MODS), and mortality. Injury severity was determined based

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on %TBSA with partial and/or full thickness burns. Initial fluid resuscitation was directed according to the Parkland formula (4 mL / kg / % TBSA with half given during the first 8 hours following injury and the remaining half given over the next 16 hours), per the BICU standard protocol. Discarded skin samples from burn patients undergoing routine excision/debridement and skin grafting were obtained in the operating room. On average, the burn skin samples were obtained during routine surgeries (excision, debridement, and grafting) within 5 days post-burn. The burn margin (partial thickness) was obtained from the skin adjacent to the excised area of the burn and not directly in contact with the thermal source. Following excision of the burn wound, a 5-10 mm margin of grossly normal appearing skin was excised simultaneously with the wound. The wound itself was debrided up to the point of viable tissue to facilitate optimal wound healing in the patients, thus yielding viable tissue near the burn margin that was excised. Donor skin (partial thickness) was taken from a site distal to the original injury (autograft site), per standard surgical protocols. Although two burn patients required multiple surgeries, and thus contributed two samples for this study, none of the patients required repeat use of a specific donor site. Control skin samples were obtained from patients undergoing elective surgeries (*e.g.* breast reduction; panniculectomy).

Wound and Skin Care Prior to Surgery

In general, when burn patients arrive to the hospital, the wounds are immediately washed and manually debrided (with scrubbing) using a 4% chlorhexidine gluconate solution. The wounds are then typically dressed with a topical antimicrobial ointment, such as silver sulfadiazine, and gauze, and the dressings are routinely changed 1-2 times each day until they undergo their surgical debridement; additional washings are not routine and the donor sites are not specifically washed with any solutions. Bacterial cultures from the burn unit are not routinely evaluated. Oral and/or intravenous antibiotics are not routinely administered unless

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a patient develops a clinical infection, which does occasionally happen (**Table 2**). Patients undergoing elective surgery (controls) are routinely asked to wash/shower using the same 4% chlorhexidine solution that is used on the burn patients, the evening prior to their surgery. Pre-operatively (immediately prior to surgery), similar topical antiseptic/antimicrobial products are used on the donor skin prior to harvesting and the burn skin prior to debridement (both are prepped with a solution containing 4% chlorhexidine gluconate), as compared to the skin from controls (prepped with a solution containing 2% chlorhexidine gluconate and 70% isopropyl alcohol).

Bacterial Microbiome Analysis

For all analyses, skin specimens were frozen at -80°C until microbial DNA isolation and sequence analysis. Partial-thickness skin samples were thawed and homogenized in Assay Assure™. DNA was extracted from the cell pellets using a Qiagen DNeasy (Qiagen Inc., Valencia CA) tissue extraction kit. Genomic DNA was eluted in nuclease-free water and stored at 4°C until 16S rRNA PCR amplification and sequencing. The V1-V3 region 16S rDNA PCRs included 2 µl of skin gDNA preparation, Phusion high fidelity DNA polymerase (New England Biolabs, Ipswich, MA) and oligonucleotide primers, as previously described (13). Mothur software (version 1.23.0) was applied to deconvolute the 454 sequence reads into individual samples based on complete matches to the barcode sequences. Primers and barcodes were clipped from each read and clipped sequences shorter than 200 bp were discarded. Low-quality and chimeric sequences were eliminated with default parameters as described in the Mothur's standard operating procedure (http://www.mothur.org/wiki/454_SOP) (14). Taxonomic classification (from phylum to genus level) of the sequence reads was conducted by the Ribosomal Database Project Classifier (version 2.4) with the default 0.8 confidence threshold (15). In total, 37,734 high-quality reads were obtained from 25 samples (1509±683.8). To minimize unequal sampling

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effects, subsampling without replacement was performed to randomly extract 750 reads from each sample. The process was then repeated 10 times and the average taxonomic count was employed for subsequent statistical analysis. Microbial diversity indices were calculated from subsampled sequence data, which was performed by subsampling without replacement of 1000 reads from each sample for 1000 times (if a sample contains less than 1000 reads in total, all of its reads were used for analysis without subsampling) to avoid any bias caused by the various sequencing depths of samples, as described previously (16, 17). Analyses of the bacterial abundance between each cohort was performed using the metagenomeSeq package with a built-in multiple test correction (18). We determined correlations using numerous diversity indices (S. chao1; S. ACE; Shannon; Simpson; Eveness; Inverse Simpson). All statistical tests were performed using the R software environment (http://www.r-project.org). All of the sequences and associated metadata were deposited to the NCBI Sequence Read Archive under the BioProject ID is PRJNA293586.

Mock specimens were processed in parallel with skin specimens to monitor for reagent contamination. PCR Amplicons were purified by Qiaquick gel extraction kit (Qiagen) and quantified by Quant-It HS double stranded DNA assay (Invitrogen, Carlsbad CA). Emulsion PCR and 454 library generation steps were performed according to the manufacturer's protocol (454 Life Sciences). Sequencing was performed on a Roche/454 GS-FLX Titanium system at the Indiana University Center for Genomics and Bioinformatics, Bloomington, IN. All *p* values reported were corrected for multiple tests with the Benjamini-Hochberg procedure. Bray-Curtis dissimilarity was visualized using non-metric dimensional scaling (NMDS), a non-parametric ordination approach based on rank-order. All of the sequences and associated metadata were deposited to the NCBI Sequence Read Archive under the BioProject ID is PRJNA293586.

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Results

Patient Demographics and Clinical Morbidities

Skin samples from 9 BICU patients (including males and females) aged 20-54 years were evaluated (median age: 47). The median total burn surface area (TBSA) in the study group was 35% (range 11-52%). Of the 9 burn patients studied, 44% (n = 4) developed pneumonia, 55% (n = 5) suffered a wound infection of the donor or burn site, and 44% (n = 4) were treated for blood culture positive sepsis. Patients with no cutaneous burn were excluded (**Table 1**). The mortality rate was 20% (n = 1) for all patients in the study group; the individual who succumbed to their injury was 53 years old and had a 52% TBSA burn injury. Of the 9 burn patients studied, 44% (n = 4) were admitted for a scald burn, while 55% (n = 5) were admitted for a flame burn (**Table 2**). Control skin samples were obtained from 9 non-burned volunteers aged 18-51 years (median age: 45). All patients (burn and control) received intravenous antibiotics prior to surgery (**Table 2**), which was determined based on several standard patient/clinical factors (including current/recent infections, allergies, etc.).

Burn Injury Augments Microbial Diversity

Burn subjects colonized with distinct microbiota will presumably develop secondary complications, which may contribute to graft failure or infection. To test this hypothesis, we used non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity to first demonstrate that the bacterial microbiome in both donor skin and burn margin was significantly different than non-burned control skin **(Figure 1A)** (PERMANOVA test $p<0.002$, either with or without considering age, gender, and ethnic group as confounding factors in the test). Based on 16S rDNA sequences, most skin bacteria were classified into four phyla: Actinobacteria, Bacteroides, Firmicutes and Proteobacteria, similar to previous reports of the skin microbiome (3, 19). The results of this phyla level composition is to very

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broadly compare our results with previously published skin microbiome studies (2, 3, 19, 20) and demonstrate that our dataset in burn subjects falls within the expected range of the bacterial phylogeny that are typically present on the skin. However, we do not assume that taxa within a phylum stimulate similar clinical responses. We next determined whether different bacterial taxa are enriched in donor skin and burn margin compared to control skin by analyzing genera abundance using a negative binomial mixed-effect model (taxa abundance was the response variable; skin type was the explanatory variable with age, gender and ethnic group as confounding factors; subjects were treated as the random effect in the mixed model to account for intra-subject correlations). **(Figure 1B)**. Donor skin and burn margin were enriched with several taxa in comparison to control skin, including *Aeribacillus* ($p<0.005$ and $p<0.03$, respectively), *Caldalkalibacillus*, ($p<0.02$ and $p<0.02$, respectively) and *Nesterenkonia* ($p<0.004$ and $p<0.0007$, respectively). These taxa are similar in that they are extremophiles, specifically thermophilic or halophilic (21-23), and have not been extensively associated or studied in the context of pathogenesis in humans. In contrast, *Corynebacterium*, a widespread skin commensal, was significantly lower in both donor skin and burn margin relative to control skin ($p<0.001$ and $p<0.02$, respectively). Of note, since innate differences in community structure/membership of skin sites exist (19), we ensured that the control sites matched the general microenvironment of the donor sites. A summary of the genera determined to be statistically more or less abundant between each cohort is represented in **Table 3**.

Microbial Diversity Correlates with Clinical Outcomes after Burn Injury

We next assessed whether skin bacterial taxa significantly correlated with the following co-morbid complications of burn injury **(Table 4)**: pneumonia (n = 3), wound infection (n = 7) and sepsis (n = 6) using a negative binomial model (response variable being each type of co-morbid complications of burn injury, explanatory variable being the skin

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types, with age, gender and ethnic group as confounding factors). Five taxa in the burn margin were correlated with the development of pneumonia in burn subjects: *Propionibacterium* (negatively correlated, *p*= 0.00134*), Aeribacillus* (positively correlated, *p*=0.0297), *Nesterenkonia* (positively correlated, *p*=0.000358), *Halomonas* (positively correlated, *p*=0.000319), *Sediminibacterium* (positively correlated, *p*=0.00112*)*, with *Nesterenkonia* being the most abundant genera enriched in those patients with pneumonia (14.91%). Three taxa in the burn margin were correlated with wound infection: *Corynebacterium* (positively correlated, *p*=0.00573), *Staphylococcus* (negatively correlated, *p*=0.00112), and *Propionibacterium* (negatively correlated, *p*=0.0261), with *Corynebacterium* being the most abundant genera enriched in those patients with wound infections (15.76%). Two taxa in the burn margin were negatively correlated with the development of sepsis: *Corynebacterium* (*p*=0.0231) and *Enterococcus* (*p*=0.000296), with *Corynebacterium* being the most abundant genera enriched in those patients without sepsis (7.84%).

Discussion

The intricate pathophysiology of burn injury stimulates major local and systemic effects mediated by the initial inflammatory response, thus influencing global skin function and the resident microbiota. In this study, we introduce the first assessment of the cutaneous bacterial community in burn subjects, a cohort of trauma patients with a high risk of morbidity and mortality. We were able to capture distinct features of the microbiome in both donor skin and burn margin from burn subjects, which significantly differ from unburned controls and correlate with infectious outcomes.

We recently determined that epidermal AMP responses (e.g. protein levels and activity) are impaired in both donor skin and burn margin from human burn patients, which

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likely influences, or is influenced by, changes in the resident skin microbiota (12). Several skin pathogens (*e.g. Staphylococcus aureus*) are known to induce antimicrobial molecules and pro-inflammatory cytokine production through cutaneous innate immune receptors, such as Toll-like Receptors (TLRs), and are the predominant species associated with skin wound infections. In parallel, resident commensal microbes (*e.g. Staphylococcus epidermidis*; *Propionibacterium acnes*) help maintain epidermal homeostasis by minimizing pro-inflammatory cytokine release after skin injury (24-26) or by undergoing fermentation to restrict the overgrowth of other commensal bacteria (27). We observed in this study that a lower abundance of skin *Propionibacterium* correlated with a greater risk of pneumonia and wound infection in burn patients. Several skin pathologies and chronic wounds suggest an imbalance of this microbiota, without evidence of a clinical infection (2, 20). *Corynebacterium* and *Propionibacterium*, both prevalent members of *Actinobacterium*, were previously shown to be inversely correlated with non-resolvers and resolvers of pustule-forming skin infections, respectively (28). Thus, these bacterial shifts likely promote subtle changes in skin function and immune defense at the burn site, which precipitate more robust complications observed in our patient population, including pneumonia, wound infection, and sepsis.

Interestingly, we observed that *Aeribacillus*, *Caldalkalibacillus*, *Nesterenkonia*, *Halomonas*, were enriched in the burn margin and/or donor skin. These taxa are analogous in that they are extremophiles, specifically thermophilic or halophilic, and tend to be isolated from soil and water sources (21-23). Of these, only *Halomonas* has been reported as pathogenic, causing bacteremia and peritonitis in dialysis centers (29, 30) . We speculate that enrichment of these taxa may be partially derived from external exposure to hospital water (*e.g.* steam) sources following debridement procedures. We previously determined that burn injury significantly impairs normal skin barrier function in autologous donor skin in mice and

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in humans, which may facilitate invasion by these bacterial taxa during debridement procedures. However, our bacterial microbiome analysis includes full thickness skin samples (*e.g.* epidermal and dermal reservoirs), rather than only an external swab, and control samples did exhibit these taxa, but at a lower abundance. Thus, alternatively, cutaneous shifts in osmolarity caused by disruption of the local ionic environment after burn injury may facilitate their proliferation by providing nutrients that are normally limited in the skin. Both of these scenarios warrant further investigation as a mechanism to explain the positive correlation between these taxa and the development of pneumonia in our burn patient population.

Our findings suggest that the colonizing microbiota may be a useful biomarker to predict morbidity in burn subjects, but must be confirmed in subsequent studies with larger populations and a longitudinal assessment. Due to the relatively small sample size, we cannot consider various cofounding factors such as gender and race and sampling locations. However, even in a cohort of 242 subjects analyzed by the NIH Human Microbiome Project, only 1 taxa at 1 skin site (the antecubital fossa) was found to be differ significantly across races at the substantial FDR of $q<0.2$. Gender and other aspects of host phenotype were not found to statistically correlate with skin taxa in this large cohort (31).

Graft failure due to poor wound healing or infection remains a significant problem for subjects necessitating skin grafts. Because skin grafting is the predominant method of reconstructing a defect in the skin, and is commonly used for the reconstruction of other skin pathologies (*e.g.* chronic ulcers, cutaneous malignancies), these data suggest that the bacterial microbiota in the donor skin may predict how well the graft site heals or resists pathogenic infection. Thus, grafting donor skin exhibiting bacterial dysbiosis may increase the risk for infection and/or graft failure in any patient requiring skin grafting. As such, treatment of donor skin with probiotics or prebiotics prior to grafting may improve patient outcomes. By

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increasing the abundance of "protective" bacteria on the skin prior to grafting, the time needed for the skin to regain its baseline barrier function may be significantly shortened. One study utilized topical *Lactobacillus* after various degrees of burn injury in humans, but the outcomes were not robust in terms of promoting healing and reducing infection (32). Our study indicates alternative bacterial taxa (*e.g. Propionibacterium*), which may be potential targets for topical "probiotics" to improve healing and limit secondary complications in burn subjects. Although the optimal "protective" bacterial profile remains elusive, the identification of novel mechanisms for shifts in the cutaneous microbiota after burn injury, or after traumatic injury in general, could prove rather beneficial. Specifically, further studies can potentially elucidate both the source of the distinct microbiota (*e.g.* steam; topical agents) and the molecular mechanisms by which a shift in the microbiota profile occurs in burn margin and autologous donor skin in burn subjects. There is the possibility that bathing and other hygienic activities conducted during hospitalization may influence the skin microbiome. However, recent work has demonstrated that the skin microbiome is stable over the long term despite these perturbations (33). Specifically, little to no effect on resident microbiota was observed after topical administration of soaps (34). Furthermore, chlorhexidine washes, which are broad spectrum antiseptic treatments, do not select for specific populations in the same way that antibiotics may. In our study, both controls and burn subjects were subjected to similar chlorhexidine compositions. While bacterial load is effectively reduced with these treatments, they do not change the composition or diversity of the skin microbiome. Our burn margin, donor sites, and control samples are partial-thickness samples (rather than skin swabs) comprised mostly of epidermis. Thus, our microbiome analysis will identify bacteria typically found within the epidermis and upper dermis. Future studies are necessary to identify temporal changes in the microbiome in burn patients, which will assess the stability of the cutaneous microbiome over the following year (s), as the

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patient continues to heal from their injuries. It is also possible that the skin microbiome of the burn patients is inherently transferred to their caregivers, and needs further exploration. It would also be of interest to investigate whether the loss of epidermis at the donor site after donor skin harvest will impact the developing microbiome over time, relative to other non-burned sites that contain epidermis, to assess whether it is the absence of epidermis or a local response to the burn/grafting that alters the microbiome.

Because it may take 1-2 days to evaluate a burn patient's cutaneous microbiome by 16S rRNA gene sequencing, this information would not likely be rapidly available for inclusion in a Burn Injury Severity Score (BISS). However, it could potentially be used at a later time as an adjunct to the BISS to provide a modified score for subsequently predicting a patient's prognosis. Expanded culture techniques may also be used to cultivate live bacteria from these patients, as bacterial genomic sequencing and expanded bacterial culture techniques are emerging as critical complementary tools to identify bacterial dysbiosis under pathological conditions (35, 36). Controlling dramatic changes in the skin microbiota immediately after burn injury may have systemic implications, as the burn wound serves as the foundation for most of the secondary immune and wound healing responses and co-morbidities. These preliminary studies suggest that grafting donor skin exhibiting bacterial dysbiosis may augment infection and/or graft failure in patients necessitating skin grafting procedures, and sets the foundation for more in-depth and mechanistic analyses in presumably "healthy" donor skin from burn patients.

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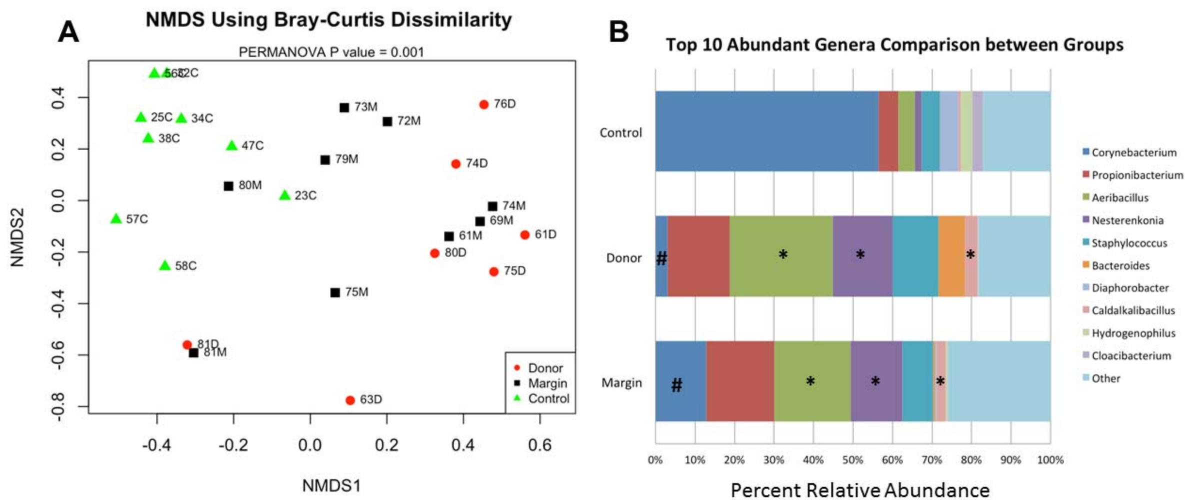
Figure and Table Legends

Figure 1. NMDS plot of control skin vs. donor skin and burn margin **(A)** demonstrates the impact of burn injury on the overall cutaneous bacterial microbiome. Control skin (CS) (green), N=9; donor skin (DS) (red), N=7; Burn margin (BM) (black), N=9. **(B)** The most abundant bacterial genera in control skin, donor skin, and burn margin skin are indicated in horizontal bar graphs. * indicates genera in donor skin and burn margin that are significantly enriched as compared to control skin. # indicates genera in donor skin and burn margin that are significantly deficient as compared to control skin.



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Sample ID#	Sample Subtype	Age	Gender	Race	% TBSA	Pneumonia	Wound infection	Sepsis	Death
61	BM	30	male	hispanic	44.0	No	Yes	Yes	No
*63	BM	30	male	hispanic	44.0	No	Yes	Yes	No
69	BM	31	female	white	35.0	Yes	No	No	No
72	BM	40	female	hispanic	11.0	No	No	No	No
73	BM	30	male	hispanic	17.0	No	Yes	No	No
74	BM	53	male	hispanic	52.0	Yes	No	Yes	Yes
*76	BM	53	male	hispanic	52.0	Yes	No	Yes	Yes
75	BM	20	female	black	35.0	No	Yes	Yes	No
79	BM	54	female	white	14.0	Yes	Yes	No	No
80	BM	54	male	hispanic	32.0	Yes	Yes	Yes	No
81	BM	47	male	white	11.4	No	No	No	No
61	DS	30	male	hispanic	44.0	No	Yes	Yes	No
74	DS	53	male	hispanic	52.0	Yes	No	Yes	Yes
75	DS	20	female	black	35.0	No	Yes	Yes	No
76	DS	53	male	hispanic	52.0	Yes	No	Yes	Yes
80	DS	54	male	hispanic	32.0	Yes	Yes	Yes	No
81	DS	47	male	white	11.4	No	No	No	No
23	CS	49	female	white	N/A	N/A	N/A	N/A	N/A
25	CS	18	female	black	N/A	N/A	N/A	N/A	N/A
32	CS	45	female	black	N/A	N/A	N/A	N/A	N/A
34	CS	45	female	black	N/A	N/A	N/A	N/A	N/A
38	CS	45	female	white	N/A	N/A	N/A	N/A	N/A
47	CS	51	male	white	N/A	N/A	N/A	N/A	N/A
56	CS	32	female	black	N/A	N/A	N/A	N/A	N/A
57	CS	40	female	black	N/A	N/A	N/A	N/A	N/A
58	CS	23	female	hispanic	N/A	N/A	N/A	N/A	N/A

***Note:** Samples 61/63 and 74/76 are from the same patient. Each patient had two surgeries, which are listed separately.

Table 1. Patient demographics and clinical information for burn subjects. DS= donor skin; BM= burn margin; CS= control skin; N/A= not applicable; TBSA= Total body surface area.

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Sample ID#	Gender	Age, years	Type of Burn	%TBSA Burn	Specimen Type	Specimen Site	Post-Burn Day	Antibiotic Usage
61	Male	30	Scald	44%	Margin Donor	Shoulder Chest	7 (OR1)	Topical silver sulfadiazine (burn area only, since admission), clindamycin IV (x1 dose pre-op)
***63**	Male	30	Scald	44%	Donor	Thigh	13 (OR2)	Clindamycin IV (x1 dose pre-op)
69	Female	31	Flame	35%	Margin	Chest	21	Topical silver sulfadiazine (burn area only, since admission), piperacillin/tazobactam IV (x4 days before surgery for pneumonia)
72	Female	40	Scald	11%	Margin	Thigh	9	Topical silver sulfadiazine (burn area only, since admission), ampicillin/sulbactam IV (x1 dose pre-op)
73	Male	30	Flame	17%	Margin	Leg	3	Topical silver sulfadiazine (burn area only, since admission), cefazolin IV and gentamicin IV (x3 days before surgery for open leg fractures)
74	Male	53	Scald	52%	Margin Donor	Hand Shoulder	5 (OR1)	Topical silver sulfadiazine (burn area only, since admission), piperacillin/tazobactam IV (x5 days before surgery for pneumonia)
***76**	Male	53	Scald	52%	Donor	Shoulder	10 (OR2)	Piperacillin/tazobactam IV (x10 days before surgery for pneumonia)
75	Female	20	Scald	35%	Margin Donor	Thigh Abdomen	6	Topical silver sulfadiazine (burn area only, since admission), ampicillin/sulbactam IV (x1 dose pre-op)
79	Female	54	Flame	14%	Margin	Arm	4	Topical silver sulfadiazine (burn area only, since admission), ceftriaxone IV (x2 days before surgery for pneumonia)
80	Male	54	Flame	32%	Margin Donor	Flank Thigh	6	Topical silver sulfadiazine (burn area only, since admission), cefazolin IV (x1 dose pre-op)
81	Male	47	Flame	11.4%	Margin Donor	Chest Thigh	3	Topical silver sulfadiazine (burn area only, since admission), cefazolin IV (x1 dose pre-op)
23	Female	49	n/a	n/a	Control	Chest	n/a	Cefazolin IV (x1 dose pre-op)
25	Female	18	n/a	n/a	Control	Chest	n/a	Cefazolin IV (x1 dose pre-op)
32	Female	45	n/a	n/a	Control	Chest	n/a	Clindamycin IV (x1 dose pre-op)
34	Female	45	n/a	n/a	Control	Chest	n/a	Clindamycin IV (x1 dose pre-op)

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CS vs. DS	Genera	*Corrected P* value	Average relative abundance in CS (standard deviation)	Average relative abundance in DS (standard deviation)

38	Female	45	n/a	n/a	Control	Chest	n/a	Cefazolin IV (x1 dose pre-op)
47	Male	51	n/a	n/a	Control	Abdomen	n/a	Cefazolin IV (x1 dose pre-op)
56	Female	32	n/a	n/a	Control	Chest	n/a	Cefazolin IV (x1 dose pre-op)
57	Female	40	n/a	n/a	Control	Abdomen	n/a	Cefazolin IV (x1 dose pre-op)
58	Female	23	n/a	n/a	Control	Chest	n/a	Cefazolin IV (x1 dose pre-op)

***Note:** Samples 61/63 and 74/76 are from the same patient. Each patient had two surgeries, which are listed separately

Table 2. Select specimen details and antibiotic usage. %TBSA Burn = percent total body surface area of burn. OR = operating room (surgery 1 or 2, if multiple surgeries were performed); Pre-op = 1 dose given in the operating room pre-operatively (before the surgery started); IV = intravenous dosing

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	Cloacibacterium	4.92E-09	2.47% (3.93%)	0.05% (0.06%)
	Corynebacterium	6.53E-06	52.13% (31.66%)	2.91% (2.84%)
	Diaphorobacter	7.53E-06	4.30% (5.00%)	0 (0)
	Nesterenkonia	0.000730168	1.61% (1.80%)	14.43% (10.80%)
	Aeribacillus	0.003681914	3.86% (4.89%)	24.71% (19.31%)
	Hydrogenophilus	0.011783222	2.73% (4.03%)	0.20% (0.48%)
	Caldalkalibacillus	0.018756316	0.48% (0.48%)	3.01% (2.19%)
CS vs. BM	**Genera**	***Corrected P* value**	**Average relative abundance in CS (standard deviation)**	**Average relative abundance in BM (standard deviation)**
	Cloacibacterium	2.10E-20	2.47%(3.9%)	0.18% (0.24%)
	Diaphorobacter	1.71E-05	4.35% (5.00%)	0.31% (0.34%)
	Nesterenkonia	0.005316297	1.61% (1.80%)	11.36% (8.54%)
	Corynebacterium	0.028968988	52.13% (31.66%)	11.16% (11.18%)
	Aeribacillus	0.028968988	3.86% (4.89%)	16.85% (11.01%)
	Hydrogenophilus	0.028968988	2.73% (4.03%)	0.33% (0.48%)
	Caldalkalibacillus	0.028968988	0.48% (0.48%)	2.04% (1.83%)
DS vs.	**Genera**	***Corrected P* value**	**Average relative abundance in DS**	**Average relative abundance in BM**

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BM			(standard deviation)	(standard deviation)
	Lactobacillus	5.86E-06	0.18% (0.33%)	1.03% (1.40%)
	Corynebacterium	0.010124178	2.91% (2.84%)	11.16% (11.18%)

Table 3. The bacterial genera demonstrating a statistically significant difference in the relative abundance between different skin sites are shown. CS= control skin; DS= donor skin; BM= burn margin. Pairwise comparisons were performed between these three locations. A negative binomial mixed effect model was applied with age, gender, and race as confounding factors. The multiple test correction was applied to the P values with Benjamin-Hochberg procedure. The average relative abundance and the standard deviation from different skin communities of each interested genera are shown in the table.

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	Genera	*Corrected P* value	Average relative abundance in Pneumonia+ (standard deviation)	Average relative abundance in Pneumonia- (standard deviation)
Pneumonia+ vs. pneumonia- (BM)	*Nesterenkonia*	0.000407149	14.91% (9.36%)	8.52% (7.60%)
	Halomonas	0.000407149	3.55% (3.28%)	0.17% (0.22%)
	Propionibacterium	0.001468212	3.86% (0.91%)	23.96% (34.23%)
	Sediminibacterium	0.001433633	2.86% (3.00%)	0.79% (1.03%)
	Aeribacillus	0.035263334	21.20% (12.26%)	13.37% (9.77%)
Wound infection+ vs. Wound infection- (BM)	**Genera**	***Corrected P* value**	**Average relative abundance in Infection+ (standard deviation)**	**Average relative abundance in Infection-(standard deviation)**
	Staphylococcus	0.001029655	5.00% (3.97%)	9.15% (15.30%)
	Corynebacterium	0.007617801	15.76% (12.46%)	5.41% (6.84%)
	Propionibacterium	0.028735336	5.79% (5.31%)	26.58% (38.79%)
Sepsis+ vs. sepsis- (BM)	**Genera**	***Corrected P* value**	**Average relative abundance % in Sepsis+(standard deviation)**	**Average relative abundance % in Sepsis-(standard deviation)**
Sepsis+ vs. sepsis- (BM)	*Enterococcus*	0.000249211	0.32% (0.32%)	1.12% (1.12%)
	Corynebacterium	0.027060777	7.84% (12.08%)	13.81% (13.81%)

Table 4. Significant correlations between genera within the skin bacterial community structure of burn margin (BM) and patient co-morbidities (i.e. pneumonia, wound infection, and Sepsis). A negative binomial mixed effect model was applied with age, gender, and race as confounding factors. The multiple test correction was applied to the P values with Benjamin-Hochberg procedure. The average relative abundance and the standard deviation of the interested genera in different patient co-morbidities cohorts are shown in the table.

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