Transfer of skin microbiota between two dissimilar autologous microenvironments: A pilot study

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Abstract

Dysbiosis of skin microbiota is associated with several inflammatory skin conditions, including atopic dermatitis, acne, and hidradenitis suppurativa. There is a surge of interest by clinicians and the lay public to explore targeted bacteriotherapy to treat these dermatologic conditions. To date, skin microbiota transplantation studies have focused on moving single, enriched strains of bacteria to target sites rather than a whole community. In this prospective pilot study, we examined the feasibility of transferring unenriched skin microbiota communities between two anatomical sites of the same host. We enrolled four healthy volunteers (median age: 28 [range: 24, 36] years; 2 [50%] female) who underwent collection and transfer of skin microbiota from the forearm to the back unidirectionally. Using culture methods and 16S rRNA V1-V3 deep sequencing, we compared baseline and mixed (“transplant”) communities, at T = 0 and T = 24 hours. Our ability to detect movement from one site to the other relied on the inherent diversity of the microenvironment of the antecubital fossa relative to the less diverse back. Comparing bacterial species present in the arm and mixed (“transplant”) communities that were absent from the baseline back, we saw evidence of transfer of a partial DNA signature; our methods limit conclusions regarding the viability of transferred organisms. We conclude that unenriched transfer of whole cutaneous microbiota is challenging, but our simple technique, intended to move viable skin organisms from one site to another, is worthy of further investigation.

Introduction

Until recently, skin microbiota research has been primarily descriptive. Foundational studies in healthy subjects have revealed remarkable topographical diversity [1] and temporal stability [2]. Increasingly, we are recognizing associations between microbial dysbiosis and inflammatory skin conditions. Most clearly elucidated with the role of *Staphylococcus aureus* in atopic
dermatitis [3,4], important microbial trends of dysbiosis are also emerging in acne [5,6] and hidradenitis suppurativa [7], among other conditions.

The clinical promise of transferring microbiota has been demonstrated with fecal microbiota transplantation, which has shown curative potential on the individual level (C. difficile colitis) in addition to its benefit to the greater biosphere with enhanced antimicrobial stewardship [8,9]. In the emerging field of cutaneous bacteriotherapy, studies have focused on applying a single species to target sites to treat atopic dermatitis, given these species’ ability to inhibit Staphylococcus aureus growth [10–12].

No studies to date have explored the feasibility of performing a skin microbiota transplant that moves the entire cutaneous bacterial community, with its complex web of metabolic interactions. The mechanistic significance of transferring a community rests upon the fact that many microbes need their community partner, ie some microbes make associations of obligately mutualistic metabolism, sometimes termed syntrophy, or cross-feeding mode of living [13]. In humans, research in this area has focused on pathogens that evolve co-dependent iso-
genic variants, acting like a multicellular organism to produce functional antibiotic resistance [14,15] However, in human gut microbiome research, there is emerging evidence of cross-feeding of commensal bacteria to produce bioactive short chain fatty acids in the healthy host [16–18]. Given this growing body of evidence for syntrophy in microbial systems of the healthy human host, we believe that transferring the naive microbial community without species bias introduced by an enrichment step in vitro, is a valid investigational approach for the treatment of inflammatory skin disease.

Within this context, our study asks whether moving superficial cutaneous microbial communities is feasible. Our experimental design relies on the topographical variation of skin microbiota within a single host. We selected sites with a contrasting composition of microbes, the antecubital fossa and the upper back [1]. Using both sequencing and traditional microbiological culture, we took the advantage of the differences in baseline populations to distinguish a signal of successful transfer. Here, we aim to follow the signal of these transferred species and demonstrate that a simple and inexpensive method for moving superficial skin microbiota can create a viable and representative transplant.

Materials and methods

The study was approved by Seattle Children’s Institutional Review Board. Written consent was obtained for study participants. The study was conducted at Seattle Children’s Hospital from January-March 2017.

Recruitment of study participants

Healthy medical students 23–37 years of age were recruited for the study from the University of Washington School of Medicine, screened with exclusion criteria by questionnaire, and consented at the time of the screening swab. Exclusion criteria were no antibiotics in the last six months; generally healthy; no skin disease other than acne, keratosis pilaris, or dry skin; no soaping/scrubbing of arms and back when bathing; no bathing with antibacterial soap.

Because our preliminary trials revealed that skin microbiota biomass varies considerably between individuals, volunteers’ antecubital fossae were screened for a minimum bioburden. To assess bioburden, a moistened swab (BD, ESwab) with 0.85% sterile saline; (Remel) was vigorously rubbed on a 2cm x 2 cm area of antecubital fossa. This is the same saline we use throughout the experiment, including for collection of baseline samples, collecting bacteria for transfer pellet, and recovering the transferred pellet. The swab was placed in 1 mL of modified liquid Amies medium (BD) and vortexed for 30 seconds. A blood agar (BA) plate (Remel) was
inoculated with 0.1 mL of the Amies medium and incubated aerobically at 35˚C for 48 hours. We calculated cutaneous biomass and evaluated each volunteer’s bioburden. We set a limit of >1000 colony forming units per milliliter Amies medium (CFU/mL) for inclusion criteria. Using cutaneous bacterial biomass as inclusion criteria ensured there was sufficient burden for our subsequent analyses. We screened nine volunteers, all of whom gave written informed consent. Of them, two men and two women (median age: 28 [range: 24, 36]) had sufficient biomass for inclusion. The individual in this manuscript (identifiable in S1 Photo) has given written informed consent (as outlined in PLOS consent form) to publish these case details.

**Collection of baseline samples**

Study participants did not bathe for at least 24 hours prior to sampling. On the day of sampling, the subject’s arms and back were fitted with pre-constructed, raised grids of waterproof medical tape (Nexcare Absolute Waterproof, 3M; S1 Photo; Fig 1). Baseline samples (Ba, Bb; Fig 1) from the arms (Ba) and back (Bb) were obtained by vigorously rubbing the designated 2.5 cm x 3.0 cm grid-squares for 30 seconds with dampened swabs. For all adjacent samples, swabs of one grid-square in went in 1 mL Amies for culture, and the other grid-square in 0.5 mL of PowerBead solution (Qiagen) for PCR. Culture and PCR methods are outlined in detail in the following sections.

**Moving the arm microbiota to the back**

To create the bacterial transfer pellets, the donor sites (D; Fig 1) were vigorously rubbed with dampened swabs. We then submerged each swab in 1 mL saline and vortexed for 30 seconds. Next, we transferred the saline to a DNA-free microcentrifuge tube and centrifuged at 2,000 x g for 5 minutes, followed by a second, equivalent centrifugation with the tube rotated 180 degrees [19]. This created a pellet in the apex of the tube. We removed all but 50 μL of supernatant, and resuspended the pellet in the remaining supernatant, creating a solution with the consistency of thick mucus. This solution was pipetted directly onto the appropriate recipient site (T0, T24; Fig 1), and spread with a disposable inoculating loop (Fisherbrand). There was no pre-treatment of the recipient sites prior to transfer.
Assessing the efficacy of our microbiota transfer technique

To assess the efficacy of our technique, we collected transferred pellet samples immediately and 24 hours after we spread the pellet across the recipient sites (T₀, T₂₄; Fig 1). The T₀ samples were collected with the same method used for obtaining the baseline samples as described above (Fig 2).

After 24 hours, we recreated the tape grids in exactly the same position on the subject’s back (marked on day one with surgical pen). Study subjects were instructed not to bath.
between placement and harvest of the bacterial pellet. We then collected the transferred pellet samples (T<sub>24</sub>; Fig 1) and baseline back samples (Bb<sub>24</sub>; Fig 1). All the T = 0 and T = 24 samples were analyzed by both bacterial culture and 16S rRNA deep sequencing (Fig 2).

In total, there were eight replicates of the entire experiment: one on each anatomical side of the four participants (one replicate being right arm + right upper back; second replicate being left arm + left upper back). For every replicate, culture and 16S deep sequencing each owned an adjacent grid-square at each time point.

**Analyzing microbiota composition with 16S rRNA sequencing**

The swabs were placed into 0.5 mL of PowerBead solution (Qiagen) and vortexed for 30 seconds. The samples were transferred to bead tubes provided with the DNeasy PowerSoil Kit (Qiagen), and 0.06 mL of C1 solution was added to each tube. The tubes were briefly vortexed and incubated at 70˚C for 10 minutes. The samples were lysed with a Precellys24 (Bertin Technologies) operated at 5000 RPM for 30 seconds. The manufacturer’s instructions were followed for the remaining extraction and purification steps.

A negative (reagent-only) control and a positive control of five organisms—*Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 49619, *Pseudomonas aeruginosa* ATCC 27853 and *Haemophilus influenza* ATCC 49247—were included with each set of extractions. Negative environmental control swabs (swabs that were opened and exposed to the air of the sampling room for about 15 seconds) were collected for each subject (both at T = 0 and T = 24) and extracted concurrently with the experimental swabs.

All amplification and deep sequencing was completed by the University of Minnesota Genomics Center (UMGC), with the V1-V3 region of the 16S rRNA gene amplified using the UMGC dual-indexing protocol, as previously described [20]. Sequencing was completed on the Illumina MiSeq using the 300 base pair, paired end approach.

Fastq files were uploaded to One Codex [21] and taxa assigned according to the targeted loci database (closed reference). The read counts for each sample were analyzed using Calypso v8.20 [22], without read filter or removal of rare taxa, using total sum normalization without transformation, and the Greengenes taxonomy database (v13.8). Shannon Index was used for beta diversity analysis, and PCoA plot with Bray-Curtis index for comparing community structure.

**Analyzing microbiota composition with traditional culture methods**

Swabs were placed in 1 mL of modified liquid Amies medium and vortexed for 30 seconds. A BA plate, mannitol salt agar (MSA) plate (BD) and phenylethyl alcohol agar (PEA) plate (Remel) were each inoculated with 0.1 mL of Amies medium. An additional BA plate was inoculated with 0.1 mL of a 1:10 dilution of Amies medium and a third BA plate was inoculated with 0.1 mL of a 1:100 dilution of Amies medium. A 2 mL aliquot of Reasoner’s 2A (R2A) broth (Teknova) containing a vancomycin disk (30 μg, BD) and 0.05 mL of amphotericin B (250 μg/mL, Fisher) [23] was inoculated with 0.5 mL of Amies media.

The BA and MSA plates were incubated aerobically at 35˚C for 48 hours and screened for growth. Each unique morphotype was subcultured to a BA plate and identified via matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics Inc.). Colony counts, measured in CFU/mL, were obtained for each morphotype. The PEA plate was placed in a sealed box with an AnaeroPack System (MGC) and incubated at 35˚C for 120 hours. The R2A broth was incubated at 32˚C at a constant shaking of 150 RPM for 48 hours. A BA plate was inoculated with 0.2 mL of R2A broth and incubated aerobically at 35˚C for 48 hours. As with the BA plates, for the PEA and R2A-inoculated plates each unique morphotype was identified via MALDI-TOF MS.
We classified two bacterial isolates as the “same” only when both the MALDI identification and the pattern of morphological properties (by size, shape, pigment, texture, etc.) of the two organisms were identical. Colony morphologies and MALDI identifications were compared between plates grown from all sites on the same side of each study participant’s body (plates compared within each replicate of the experiment). Use of colony morphology to identify different species is a common tool in microbiology; colony morphology has also been shown to distinguish different strains of the same bacterial species [24].

Results
At baseline with the 16S deep sequencing data, we found the microbial community of the ante-cubital fossa (Ba) was more diverse than the back (Bb) in all four subjects. This is reflected by the number of distinct species found at each site (median: 232 species unique to Ba [range: 120, 363]; 57 unique to Bb [28, 103]; and 155 shared between Ba and Bb [123, 252]) and also in the increased Shannon diversity of the arm as compared to the back (significant in 5/8 replicates) (Fig 3A).

Comparisons of relative abundance of bacteria in the arm and back samples also demonstrate the differences in microbial signature between the two anatomical sites (Fig 4). Although Cutibacterium accounted for the majority of reads in most back samples, this was not true for the much more diverse antecubital fossae. Fig 4 also demonstrates that while antecubital fossae...
across individuals show commonalities (Staphylococcus, Streptococcus, and Corynebacterium species playing prominent roles along with Cutibacterium), we also see differences between study subjects that set them easily apart. Subject 2 hosts notable quantities of Fusobacterium; subject 3, Simonsiela (commonly found in the oral cavity of dogs); and subject 4, Micrococcus. These differences form the basis for the growing field of microbiome forensics [25].

We saw unique, transferred, arm species (absent in Bb samples) appear in all T₀ and T₂₄ samples. By sequencing, a median of 34 arm-only species [range: 18,85] appeared in the T₀ samples, with a median of 4 arm-only species [range: 1,16] persisting in the T₂₄ samples. The
most common of these organisms were *Gardnerella vaginalis*, *Brachybacterium faecium*, *Janithobacterium lividum*, and unclassified species of *Actinomyces*, *Anaerococcus*, Microbiaceae, and *Dermabacteriaceae* (Table 1). By culture, we also saw a limited number of bacteria unique to the arm (absent in *Bb* samples) appear in *T*<sub>0</sub> and *T*<sub>24</sub> samples (Table 2). Our difficulty in identifying the movement of unique live bacteria through culture techniques is best appreciated in supplementary data (S1 Dataset), which details the >900 subtyped colonies from our four study subjects. These data show that the majority of species which we grew were the *Staphylococcus*, *Streptococcus*, Corynebacteria, and *Roseomonas* that reside at baseline on both the arm and the back. Despite our attempts to incubate in R2A with vancomycin to inhibit overgrowth of *Staphylococcus*, we were unable to cultivate the rare, primarily gram negative species that are unique to the arm. Nevertheless, three arm-only species in *T*<sub>0</sub> samples were identified by both sequencing and culture (in bold italics in Tables 1 and 2). Although very limited in number, these three species offer some support for the movement not only of DNA but viable organisms. Further evidence for the movement of viable organisms are the unique colony morphotypes of species common to both sites that we demonstrated moving from the arm to the back in our study subjects (Table 2).

Besides identifying specific “arm” bacterial DNA moved to the back, we assessed the transfer of DNA signature by comparing community compositions with diversity analysis and PCoA Bray-Curtis plot. The *T*<sub>0</sub> samples were more diverse than *Bb* samples in 7 of 8 replicates, although this trend was not significant (Fig 3B). Also, in a projection of community composition (PCoA Bray-Curtis plot), five of eight *T*<sub>0</sub> samples shift towards the *Ba* cluster and away from the *Bb* cluster (Fig 5). Specifically, three of the *T*<sub>0</sub> samples plot between their baseline back samples (this is what we would expect if the *T*<sub>0</sub> samples were not impacted by the community composition of the transfer pellet). Five of the *T*<sub>0</sub> samples have moved to the right of both of their respective back samples, towards the arm samples, showing a qualitative impact of the transfer pellet on the structure of the community.

Not all bacterial DNA from the arm was moved to the back with our transplant process. The sequencing data show a median of 16% of unique arm bacterial species were recovered from *T*<sub>0</sub> samples [range: 10% - 25%]. This result shows our incomplete success in moving the entire arm skin microbiota DNA signature.

Our positive controls (one with each of four DNA extraction runs) were consistent with each other and showed that *Staphylococcus aureus* was underrepresented in our final results, either because of incomplete extraction of DNA or because of bias in the PCR-sequencing pipeline. We were reassured by the result of negative controls (environmental and reagent), which showed read counts ten times lower than experimental samples. As expected, negative reagent controls showed read counts for only a limited number of species.

We include here one further result from preparatory trials for our study, a simple measurement of whether the process of pelleting the bacteria by centrifugation (the process of preparing bacteria for transfer) resulted in loss of viability. From two adjacent sites (of equal surface area) of the antecubital fossa, we saw equivalent growth on blood agar from bacterial pellets (created with the centrifuge technique, as described above in Materials and Methods, and resuspended in Amies solution) and baseline swabs (mixed directly into an equivalent volume of Amies solution) (Table 3).

**Discussion**

Current investigations in skin microbiota transplantation show promise in the application of single strains of bacteria to lesional skin. Myles, et. al. showed that certain Gram-negative species, particularly *Roseomonas mucosa* collected from the skin of healthy volunteers, have
Table 1. List of species identified by sequencing that were present in the baseline arm [Ba], absent in baseline back [Bb], and present in the recipient site sample [T0].

| Subjects 1 and 2; left [L] and right [R] side | 1L | 1R | 2L | 2R |
|---------------------------------------------|----|----|----|----|
| Actinomyces sp.*                           | Agathobaculum butyriciproducens | Gardnerella vaginalis | Brachybacterium faecium |
| Anaerococcus unclassified                  | Atopobium parvulum               | Janthinobacterium lividum | Gardnerella vaginalis |
| Brachybacterium faecium                    | Oxalobacteraceae unclassified    | Microbacteriaceae unclassified | Actinomyces turicensis |
| Janthinobacterium lividum                  | Peptontophilus indolicus         | Alphaproteobacteria unclassified | Eggerthella sinensis |
| Microbacteriaceae unclassified             | Pseudomonas fluorescens          | Candidatus Peptoniphilus massiliensis | Enteroabacter ludwigii |
| Actinomyces odontolyticus*                 | Pseudomonas synxantha            | Dialister propionicificiens | Gordonibacter pamelaeae |
| Agathobaculum butyriciproducens            | Sphingomonas melonis             | Eggerthella sinensis | Intrasporangiaceae unclassified |
| Alphaproteobacteria unclassified            | Chryseobacterium halperniae      | Rhizobiales unclassified | Chitinophagaceae unclassified |
| Micrococcus unclassified                   | Chryseobacterium indolgenes      | Roseomonas mucosa | Corynebacterium coniforrheum |
| Peptostreptococcus anaerobius               | Clostridiales Family XIII.       | Simonsiella muelleri | Corynebacterium matruchotii |
| Pseudomonas synxantha                      | Incertae Sedis unclassified      | Triticum aestivum | Fusobacterium nucleatum* |
| Lactobacillus jensenii                     | Candidatus Microthrix calida     | Pseudomonas unclassified | Bacillus sp. N6 |
| Lysobacter unclassified                    | Chryseobacterium halperniae      | Rhizobiales unclassified | Chitinophagaceae unclassified |
| Micrococcus unclassified                   | Chryseobacterium indolgenes      | Roseomonas mucosa | Corynebacterium coniforrheum |
| Peptostreptococcus anaerobius               | Clostridiales Family XIII.       | Simonsiella muelleri | Corynebacterium matruchotii |
| Pseudomonas synxantha                      | Incertae Sedis unclassified      | Triticum aestivum | Fusobacterium nucleatum* |
| Serratia liquefaciens                      | Eikenella corrodens              | Actinomyctetaceae unclassified | Microbacterium esteromaticum |
| [Clostridium] saccharolyticum               | Janibacter sanguinis             | Amycolatopsis orientalis | Mycobacterium asiaticum |
| Anaerococcus prevotii                      | Leptotrichia goodfellowii        | BOP clade unclassified | Pseudomonas fluorescens |
| Atopobium unclassified                     | Mobiluncus curtisi              | Corynebacterium minutissimum* | Rhizobiales unclassified |
| Campylobacter gracilis                     | Mogibacterium unclassified       | Delftia unclassified* | Sphingomonadaceae unclassified |
| Capnophytophaga granulosa                  | Ottowia beijingsis              | Dialister unclassified | Streptococcus cristatus |
| Chryseobacterium lathyri*                  | Peptonanaerobacter stomatis     | Flaviflexus salisbiostrolatica | |
| Citrobacter freundii                       | Porphyromonas endodontalis      | Gordonia unclassified | |
| Collinsella aerofaciens                    | Prevotella micans               | Lactobacillus acetolerans | |
| Coprococcus eutactus                       | Prevotella timonensis*          | Massilia aurea* | |
| Cupriavidus metallidurans*                 | Rhizobiales unclassified         | Massilia unclassified | |
| Deinococcus unclassified                    | Sphingomonas phyllospheareae*    | Negativicutes unclassified | |
| Dermacoccus unclassified                    | Streptococcus pneumoniae        | Paraeggerthella hongkongensis | |
| Dialister pneumosintes                     | Treponema vincentii*            | Peptoniphilus asaccharolyticus* | |
| Dysgonomonas mossii                        | Varibaculum anthropi            | Peptoniphilus lacrimalis | |
| Enterobacteriaceae unclassified             | Varibaculum cambriense           | Rhodococcus erythropolis | |
| Gammaproteobacteria unclassified*           | Rothia mucilaginosa              | | |
| Geobacillus steinotherophilus               |                       | Sphingobium yanoikuyae | |
| Lleibacterium massilense                    |                       | Streptomyces cingwhensis | |
| Libanococcus massilensis                   |                       | | |
| Luteolibacter unclassified                  |                       | | |
| Microbacterium oxydans                      |                       | | |
| Ottowia unclassified                        |                       | | |
| Parvimonas unclassified                     |                       | | |
| Peptococcus sp. feline oral taxon 012       |                       | | |
| Prevotella melaninogenica                   |                       | | |
| Prevotella shahii                           |                       | | |
| Prevotella sp. oral taxon 292               |                       | | |

(Continued)
Table 1. (Continued)

| Subject and Side | 3L | 3R | 4L | 4R |
|------------------|----|----|----|----|
| Actinomyces sp.  | Actinomyces sp. | Anaerococcus unclassified | Anaerococcus unclassified |
| Brachybacterium faecium | Dermbacteriae unclassified* | Dermbacteriae unclassified | Janthinobacterium lividum |
| Dermbacteriae unclassified | Gardnerella vaginalis' | Actinomyces odontolyticus' | Anaerococcus hydrogenalis |
| Microbacteriae unclassified | Actinomyces neuii | Actinomyces turicensis | Bacillales unclassified |
| Actinomyces neuii | Atopebium parvulum | Anaerococcus hydrogenalis | Brevundimomas vesicularis |
| Bacillales unclassified | Prevotella veroralis | Betaproteobacteria unclassified | Candidatus Peptoniphilus massiliensis |
| Enterobacteriales unclassified | Rhizobiales unclassified | Brevundimomas nasdae | Corynebacterium mucifiicans |
| Flavobacteriae unclassified | Arabidopsis thaliana' | Brevundimomas vesicularis | Enterobacter ludwigii |
| Helcobacillus massiliensis | Corynebacterium macginleyi | Corynebacterium mucifiicans | Enterobacteriales unclassified |
| Mesangiospermae unclassified | Glutamicbacter ardleyensis | Dialister propionicificaciens | Firmicutes unclassified |
| Micrococcus unclassified | Hydrogenophilus islandicus | Friedmanniella spumicola | Friedmanniella spumicola |
| Peptostreptococcus anaerobius | Lachnospiraceae unclassified | Helcobacillus massiliensis | Intrasporangiaceae unclassified |
| Streptococcus parasanguinis | Lactobacillus delbrueckii | Lactobacillus gasseri | Lactobacillus gasseri |
| Triticum aestivum | Leuconostoc garlicum | Lactobacillus jensenii | Macroccus equiperccicus |
| Actinomyces oris | Microbacterium paraoxydans | Lyobacter unclassified | Methylbacterium unclassified |
| Bergeyella cardium | Micrococcus luteus | Macroccus equiperccicus | Mycolicibacterium iranicum |
| Bergeyella unclassified | Nesterenkonka halotolerans | Mesangiospermae unclassified | Neisseria unclassified |
| Brachybacterium unclassified* | Roseomonas riguloci | Methylbacterium unclassified | Rhodobacteraceae unclassified |
| Campylobacter concisus | Mycolicuterium iranicum | Sphingomonas desiccabilis* |
| Chryseobacterium hominis | Neisseria unclassified* | Staphylococcus haemolyticus |
| Chryseobacterium unclassified | Peptoniphilus indolicus | Actinomyces mediterranea |
| Corynebacterium accolens | Pseudomonas fluorescens | Amricoccus macauensis |
| Gemella sanguinis | group unclassified | Burkholderiales Genera | incertae sedis unclassified |
| Microbacterium unclassified | Pseudomonas unclassified | |
| Parvimonas micra | Rhodobacteraceae unclassified* | Caulobacter vibrioides* |
| Parvimonas sp. oral taxon 110 | Roseomonas mucosa | Devosia neptuniae |
| Pentapetaleae unclassified | Serratia liquefaciens | Gemella haemolysans |
| Poaeeae unclassified | Simonsiella muelleri* | Gemmobacter caeni* |
| Prevotella histicola | Sphingomonas desiccabilis | Granulicattela para-adiakens |
| Prevotella salviae | Staphylococcus haemolyticus | Janibacter unclassified |
| Pseudogracilibacillus auburnensis | Streptococcus parasanguinis | Lactobacillus reuteri* |
| Stenotrophomonas maltophilia* | Acinetobacter septicus | Leptotrichia trevisanii |
| Agrobacterium fabrum* | Luteimona unclassified |
| Agrobacterium tumefaciens | Macroccus canis |
| Altererythrobacter salegens | Macroccus unclassified |
| Aridibacter kavangonensis | Mesorhizobium loti |
| Blastocellaceae unclassified | Methylbacterium radiotolerans |
| Brachybacterium congomeratum | Methylosinus trichosporium |

(Continued)
| Family                     | Genus                          |
|---------------------------|--------------------------------|
| Burkholderiaceae unclassified | Microbacterium saccharophilum |
| Burkholderiales unclassified    | Mycobacterium austroafricanum* |
| Caulobacteraceae unclassified | Nakamuraella sp.               |
| Chryseobacterium gleum       | Neisseria meningitidis         |
| Chryseobacterium hispanicum  | Nioella sediminis              |
| Chryseobacterium taiwanense* | Paraburkholderia tropica       |
| Clostridiales unclassified   | Paracoccus siganidrum          |
| Deinococcus sp.              | Paracoccus yeei                |
| Dermacoccus nishinomiyaensis| Peptoniphilus coxii            |
| Dietzia maris               | Porphyromonas bennonis*        |
| Fenollaria massiliensis      | Roseomonas gilardii            |
| Gordonia sputi              | Sphingomonas echinoides        |
| Granulicatella elegans      | Staphylococcus equorum*        |
| Haemophilus influenzae      | Staphylococcus saprophyticus    |
| Kouleothrix aurantiaca      | Stenotrophomonas rhizophila    |
| Lactobacillus johnsonii     | Streptococcus oralis*          |
| Massilia alkalitolerans     | Streptococcus salivarius       |
| Methyloburum extorquens*    | Veillonella parvula            |
| Nakamuraella multipartita  | Vicinamibacter silvestris      |
| Neisseria flavescens       | Neorhizobium huautlense        |
| Nocardiaeae unclassified    | Nocardioides oleivorans        |
| Nocardioiides sp.           | Nocardioides sp.               |
| Nocardioiides unclassified* | Nocardioides unclassified*     |
| Nonspecific*                | Oryza sativa                   |
| Pantoea agglomerans*        | Pantoea vagans                 |
| Paracoccus marinus*         | Paracoccus versutus            |
| Phenlobacterium unclassified| Propionibacteriaceae unclassified |
| Proteobacteria unclassified | Pseudomonas putida             |
| Pseudomonas stutzeri        | Riemerella anatipestifer       |
| Sphingobacterium sp.        | Sphingobacterium sp. enrichment culture clone* |
| Sphingobium unclassified    | Sphingomonadales unclassified   |
| Sphingomonas guangdongensis | Sphingomonas hengshuiensis     |
| Variorox paradoxus          | Xanthomonadales unclassified   |
| Xanthomonas axonopodis      | Zhizhongheella caldifontis     |

(Continued)
antimicrobial activity against *Staphylococcus aureus* [11], and in a phase 2 clinical trial, application of *R. mucosa* to active atopic dermatitis was associated with decreased disease severity, topical steroid requirement, and *S. aureus* burden [12]. Similarly, Gallo and Nakatsuji identified *Staphylococcus epidermidis* strains with antimicrobial activity against *S. aureus* [10]. In animal models of atopic dermatitis, the application of Nakatsuji’s *S. epidermidis* eliminated *S. aureus* colonization. In the context of these studies, our investigation reflects a slightly different goal: to move interconnected communities of microbes, with their web of metabolic interactions, from healthy individuals to the skin of patients with inflammatory skin disease.

Tables 1 and 2 summarize our evidence supporting the feasibility of transferring a partial DNA signature from one site to another, listing species that were present in the baseline arm [Ba], absent in baseline back [Bb], and present in the recipient site sample [T₀]. These unique-to-arm species likely represent the tip of a larger transplant iceberg, i.e. they could serve as a proxy for the majority of successfully transferred organisms that are species shared between the two sites, and which we could not detect with 16S sequencing. We also interpret the shift of community structure between Bb and T₀ as evidence that our intervention made the recipient back sites more “armlike” in their community composition (Figs 3B and 5).

Despite the viability of the pelleted bacteria in trials and our success in growing some unique arm organisms from T₀ samples, our results most clearly show the movement of DNA, with only limited corroboration that the DNA is recovered from live organisms. We explore this limitation, and how future studies can better assess the viability of transferred bacteria in “Limitations”, below.

While the DNA of several of the unique, rare arm bacteria persisted at 24 hours in their new back environment, we saw a steep drop in this signal. Given their new microenvironment we cannot say what dynamics led to the failure of these bacteria to colonize the recipient site. If

**Table 1.** (Continued)

| Species                          | Zoogloe a oryzae |
|----------------------------------|------------------|

Species listed in blue cells occur in >2 replicates, species listed in orange cells occur in >1 replicates, and species listed in white boxes occur only once across replicates. Species in bold italic are examples where the culture data (derived from a sample taken centimeters away on the same individual) corroborates the sequencing data (present in the baseline arm [Ba], absent in baseline back [Bb], and present in the recipient site sample [T₀]). Species from T₀ that persist in the T₂₄ site (and remain absent at Bb₂₄ site) are annotated with a (‘).

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**Table 2.** List of unique morphotypes of species identified by culture and MALDI-TOF that were present in the baseline arm [Ba], absent in baseline back [Bb], and present in the recipient site sample [T₀].

| Subjects 1 and 2; left [L] and right [R] side | 1L | 1R | 2L | 2R |
|---------------------------------------------|----|----|----|----|
| None                                        |    |    |    |    |
| Staphylococcus epidermidis                   |    |    |    |    |
| Micrococcus luteus                          |    |    |    |    |
| Staphylococcus sp[1]                        |    |    |    |    |
| Subjects 3 and 4; left [L] and right [R] side| 3L | 3R | 4L | 4R |
| Staphylococcus epidermidis                   |    |    |    |    |
| Corynebacterium mucifaciens (x2)            |    |    |    |    |
| Staphylococcus capitis’                     |    |    |    |    |
| Staphylococcus hominis                      |    |    |    |    |
| Actinomyces neuii                           |    |    |    |    |
| Staphylococcus hominis                      |    |    |    |    |

Species listed in bold italic are those where the culture and sequencing data both show movement of the same unique arm species not present on the back. Species from T₀ that persist in the T₂₄ site (and remain absent at Bb₂₄ site) are annotated with a (‘).

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Fig 5. PCoA Bray-Curtis Plot which relates the similarity in community structure between samples by plotting each sample as a point in two dimensions. The shapes and color allow us to compare the baseline arm [Ba], baseline back [Bb], and recipient site samples [T0] from each side of each individual. There is a trend in five of eight T0 samples (orange), showing a shift “rightwards” of both of their corresponding back samples (same shape, only yellow), towards their corresponding arm samples (same shape, but red). These T0 samples are denoted with a (●).

Table 3. Viability of resuspended transfer pellet vs. standard skin swab, measured in colony forming units on blood agar (48 hrs).

|               | Centrifuged transplant pellet (resuspended in Amies solution) | Standard skin swab (mixed in equivalent volume of Amies solution) |
|---------------|---------------------------------------------------------------|---------------------------------------------------------------|
| Replicate 1   | 1600 CFU                                                      | 1560 CFU                                                      |
| Replicate 2   | 1950 CFU                                                      | 1840 CFU                                                      |

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we strictly interpret the persisting signal of unique arm bacterial DNA at $T_{24}$ (ignoring our pilot trials that showed the viability of transfer pellets, and our modest success at culturing unique arm species and colony morphotypes from the $T_0$ and $T_{24}$ samples), we cannot say whether it is merely residual from dead transferred bacteria 24 hours prior, whether there was a die-off from competition against resident bacteria, or whether the transferred bacteria didn’t survive because they were poorly adapted to their new, sebaceous microenvironment. Another possibility is that growth and establishment of transferred bacteria takes more time to detect.

Investigations in fecal microbiota transplantation show an incremental shift towards the donor microbiota signature that takes months to reach its fullest extent, with only partial engraftment detectable several days after transplant [26]. Within the context of previous literature, our finding that the antecubital fossa is significantly more diverse than the back is consistent with other descriptions of the skin microbiome; in one previous study where 20 distinct skin sites were ranked by evenness, the back was the least diverse, while the antecubital fossa was the 18th most diverse; when ranked by richness the back was the second least diverse and the antecubital fossa the 17th most diverse [1].

Limitations

Limitations of our study begin with our difficulty culturing the bacterial species (unique to the arm) whose DNA we demonstrated moving with 16S sequencing. It was our intention to use culture to demonstrate the viability of this transplant “signal”, but we did not effectively culture these organisms from baseline samples of the arm, nor the $T_0/T_{24}$ samples. Retrospectively, we were overly optimistic that we would be able to culture organisms that had been largely unrecognized prior to deep sequencing survey of the skin, even with our incorporation of special methods to grow gram negative species. We were also limited by the MALDI-TOF library, which has developed to identify clinically relevant isolates and was unable to define a number of the cultured isolates of commensal skin microbiota.

Another crucial limitation in our design was the lack of a control arm with heat-killed transfer samples. As an alternative to heat-treatment, we could have generated transfer pellets in ethanol at the centrifugation step instead of saline. If the non-viable transfer pellet (recovered at $T_0$ and $T_{24}$), showed less robust culture growth and a steeper drop-off in the persistence of unique DNA at 24 hours, we would have a much stronger claim that we had not just transferred a partial DNA signature, but viable organisms.

Other limitations of the study include the small number of participants (underpowered analysis), our focus on bacteria and exclusion of fungi and viruses, and the fact that our transplant is superficial, excluding the rich microbial habitats of appendageal structures (follicles and glands).

One unexpected finding was the number of species found exclusively in the $T_0$ samples. The $T_0$ samples showed a median of 45 unique species [range: 20,79] not found in the $Ba$ or $Bb$ samples of the same side of the study subject. We attribute this finding primarily to sample bias. Our sampling grids spanned an area from the antecubital fossa proper into the edge of volar forearm and the medial upper arm. Adding this slight geographical variability to the natural variability inherent in any two adjacent samples, we suspect that some of the bacteria in the pellet were not sampled from the arm at baseline, resulting in a number of species that appeared novel in the $T_0$ samples. A supporting fact is that many species unique to $T_0$ samples were found on the contralateral arm of the same study subject at baseline.

Future directions and conclusions

With our pilot serving as a proof of concept that it is possible to transfer a partial DNA signature, the next step is to investigate the viability and colonization efficiency of transferred skin
microbiota between the same site of two different individuals. Using whole genome sequencing, we could follow strains of identical species from one individual to another. Without question, we would incorporate a heat or ethanol-treated control with each replicate. Longitudinal swabs, including at 24 hours and 240 hours, would give meaningful information about the persistence of a transplant, and by using the same body site between donor and recipient individuals, we can examine colonization efficiency without the confounding factor of a new microenvironment for transplanted bacteria.

We conclude that unenriched transfer of whole cutaneous microbiota is challenging, but our simple technique intended to move viable skin organisms from one site to another shows the first transfer of a partial DNA signature, and is worthy of further investigation and refinement. There still remain many questions in skin microbiota transplant including 1) whether a community of microbes, not any single, offer advantage in ensuring colonization at the recipient site, 2) whether there is one or a few particular organism(s) essential in restoring eubiosis, and thus skin health, and 3) how host immunity facilitates or inhibits colonization of a transplanted community.

Supporting information
S1 Photo. Supplementary photos. (a) we placed wax/parchment paper over a template, wiped it with bleach, and constructed the grid over it with waterproof medical tape, which had been cut into strips (~0.63 cm wide, which is \(\frac{3}{4}\) the width of the tape); (b) the transplant grid was easily removed like a sticker from its backing and placed on a study subject for sampling. (TIF)

S1 Dataset. Culture data. Excel spreadsheet includes legend and data that document colony counts and subtyped cultures from each sample with their corresponding MALDI results. (XLSX)

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