The human (mostly skin) microbiome

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The Skin Microbiome

1. Human Microbiome
   • Looking back to move forward: Historical perspectives of skin microbiology
   • Definitions & Methodology: Entering the molecular microbiology era
   • Impact on Health & Disease

2. Microbiome of Healthy Human Skin
   • Ecological features
   • How/when are we colonized?
   • Temporal and topographical diversity of healthy adult
   • Relationship to other body sites

3. Microbiome Studies in Skin Diseases
   – Atopic dermatitis, atopy, psoriasis, chronic wounds
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Dermatology and microbiology: A long and rich partnership

Fig. 1. Regional differences in the total numbers of organisms. Values represent the geometric mean value.

Kligman et al 1976, JID. Bacteriology
“In the soil the densest populations of microorganisms are in the rhizosphere, the region that surrounds plant roots. The comparable region in the skin is the hair follicle.” —Mary Marples 1969.

Homunculus illustrating headquarters and range of *P. acnes* skin colonization
Isolation of *Propionibacterium acnes* in culture is associated with acne vulgaris

Leyden et al 1975, *JID.*

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*Fig.* Forehead and cheek densities of *P. acnes* in acne and nonacne subjects. *Bars* represent the 95% confidence limits of the standard error of the mean.
Age-related changes in the resident bacterial flora of the human face

Leyden et al 1975, JID.

**Fig. 1.** Quantitative determinations of *P. acnes* levels on the forehead of normal subjects. *Bars* represent the 95% confidence levels of the standard error of the mean; the *number on each column* represents the number of subjects studied in that age group.
Examining microbial diversity in culture

- Traditional approaches rely on isolating bacteria in pure culture
- The majority of bacterial species do not grow in culture = “the great plate count anomaly”
- Culturing favors lab weeds--not necessarily the most dominant or influential species
- Excludes microbes that rely on community interactions
MICROBIOME:
The totality of microbes and their respective genomes in a given environment

METAGENOMICS:
A lens by which to study microbiomes directly in their natural environment, bypassing isolation and culturing.
Why the Human Microbiome?

- We are a composite of species: eukaryotic, bacterial, archaeal, viral;
- Our microbial census exceeds the total number of our own human cells by approximately 10-fold;
- In the intestine, the microbiome may contain 100-fold more genes than our ‘own’ genome;
- The microbiome is an integral part of our genetic landscape.
Our natural flora and its role in protecting our bodies

- Synthesis and excretion of vitamins
- Nutrition
- Prevents colonization by pathogens—fills a niche
- Antagonizes other bacteria—production of antimicrobial peptides
- “Educates” the immune system: Skin commensals autonomously control local inflammatory milieu and tune resident T-cell — Naik et al. 2012 Science
The 16S ribosomal RNA gene enables rapid, accurate bacterial identification

- Present in all prokaryotic genomes
- Hypervariable regions -> species-specific sequence signatures
- Conserved regions -> molecular clock and PCR priming sites
- Eliminates culturing biases
A more powerful lens for identifying bacteria

• >1 million annotated reference sequences in database (Ribosomal Database Project).

• Align query sequences to reference databases to identify and place in phylogeny.

• Advances in sequencing technology, computation are key drivers of metagenomic studies
Skin microbiome diversity survey workflow

1. Obtain superficial skin sample containing mixed bacterial population
2. Isolate DNA from skin sample
3. Amplify bacterial 16S rRNA gene with primers encompassing variable regions of interest
4. Sequence 16S rRNA genes
5. Perform data processing, quality control and analysis of bacterial 16S rRNA sequences:

Derived from H. Kong (2011) Trends in Molecular Medicine
The Human Microbiome Project: a reference of the healthy microbiome

- 16S rRNA gene sequence data compiled for n=242 “healthy” adults
- High interpersonal variation
- Site-specific signatures preserved

The Human Microbiome Project: Applications to human disease

- Skin
  - Atopic dermatitis
  - Acne
  - Psoriasis
- Urogenital
  - Vaginosis
- Gastrointestinal
  - Crohn’s disease
  - Ulcerative colitis
  - Obesity
- Oral
  - Gingivitis
- Nasal
  - S. aureus infection
The gut microbiome: an example of impact on human health

- Mice with leptin gene mutation (ob/ob)
- 16S rRNA gene sequencing of gut microbes
- Obese microbiome characterized by more efficient nutrient-harvesting

Ley et al. PNAS 2005
Turnbaugh et al. Nature 2006
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1.8 meters$^2$ of diverse habitat for microbes

- Barrier function
- Acidic, cool surface that is continually sloughed via terminal differentiation
- Appendages and invaginations
  - Pilosebaceous unit
  - Sweat (eccrine and apocrine) glands
- Varied topography
  - Folds
  - Thickness
  - Hair and gland density
  - Occlusion
Factors contributing to skin microbiome variation

Grice and Segre (2011) *Nature Reviews Microbiology*
Sequencing vs. Culturing Skin Microbes

Alar Crease

Umbilicus

Actinobacteria
- Corynebacterineae
- Propionibacterineae
- Micrococcineae
- Other Actinobacteria

Bacteroidesetes

Cyanobacteria

Firmicutes
- Other Firmicutes
- Staphylococcaceae

Proteobacteria

Divisions contributing < 1%

Unclassified

Sequence vs. Culture
How does delivery mode affect colonization of skin by microbiota?

- Initially, undifferentiated microbiota across all body habitats
- Vaginal birth = mothers vaginal microbiota colonizes baby’s skin
- C-section birth = mother’s skin microbiota colonizes baby’s skin

Dominguez-Bello et al. *PNAS* 2010
The skin microbiota changes during the first year of life

- Diversity and evenness of species increases with time
- *Staphylococcus* decreases
- Sites = arm, forehead, and buttock
- N = 31 healthy infants

Capone et al 2011 JID
Topographical variation of healthy human skin microbiota

Grice and Segre (2011) Nature Reviews Microbiology
Data from Grice et al (2009) Science
Predominant Bacteria Depends on Microenvironment

Sebaceous sites:
Predominance of Staphylococcus and Propionibacteria

Moist sites:
Predominance of Corynebacteria

Dry sites:
Greater prevalence of Flavobacteriales and Betaproteobacteria

Applying ecological diversity statistics to compare skin sites:

The Shannon Diversity Index

Least diverse:
- Back
- Retroauricular crease
- Toeweb
- Alar crease
- External auditory canal

Most diverse:
- Popliteal crease
- Plantar heel
- Antecubital crease
- Interdigital space
- Volar forearm

Diversity, evenness, richness largely dependent on site, but sebaceous sites are on the low end.

Site inter-personal variation

- Generally, same **dominant** bacteria are present between individuals
- The remainder is highly variable

Grice and Segre (2011) *Nature Reviews Microbiology*
Data from Grice et al (2009) *Science*
Diversity of the human microbiome is strongly determined by microbial habitat.

Human Microbiome Project
N= 242 individuals
Skin sites:
- Antecubital crease
- Retroauricular crease

C Huttenhower et al. Nature 2012
Bacterial community variation in human body habitats across space and time

- Skin microbiota is **more diverse** than gut or oral
- Skin microbiota is **distinct** from gut and oral
- Temporal variation is minimal

Costello et al., *Science* 2009
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Skin microbiota: the role in dermatological diseases

- Acne
  \((P.\ acnes)\)

- Atopic Dermatitis
  \((S.\ aureus)\)

- Psoriasis
  \((Streptococcus)\)
• **S. aureus** colonization/infection status
  – *S. aureus* infection linked with AD flares
  – >90% AD skin colonized by *S. aureus*

• Low expression of anti-microbial peptide

• Flares associated w/ low bacterial diversity
• Treatments increase diversity

Kong et al., *Genome Research* 2012
S. aureus colonizes during AD flares

Kong et al., Genome Research 2012
Greater severity correlates to \textit{S. aureus} relative abundance

Kong et al., \textit{Genome Research} 2012
Chronic Wounds
An escalating health care problem

- Diabetic foot ulcers (DFU)
  - 19 million persons w/ diabetes in US → ~25% will get DFU
  → ~15% end in amputation
- Effective treatments lacking
- Bacterial burden considered a contributing factor

**Hypothesis:**
Profiles of microbial colonization can predict wound outcomes and/or suggest best treatment modality.
Comparing bacterial load and diversity in diabetic foot ulcers: genomics vs. cultures

Gardner et al. In press Diabetes
Diabetic foot ulcers are heterogeneous, despite similar etiology.
Correlation of microbiome features with DFU clinical factors

Spearman rank correlation; *P<0.05, FDR-adjusted

Clinical factors measured at sampling

Dark red= Positive correlation

White= Negative correlation

Species Richness
Species Diversity
Proteobacteria
Anaerobes
Streptococcus
Staphylococcus

Gardner et al. In press Diabetes
Relationships among environmental biodiversity, skin microbiota, and atopy

Hanski I et al. PNAS 2012

A
Species diversity of flowering plants

Number of uncommon flowering plant species

Total number of plant species

B
Generic diversity of gammaproteobacteria

Number of genera in gammaproteobacteria

Total number of bacterial genera

Atopic sensitization
Solid circles = atopic individuals
Open circles = healthy individuals

N = 118 adolescents
All living in 100 x 150 km area
Effect of atopy is highly significant
IL-10 expression against the relative abundance of *Acinetobacter*

Solid circles = atopic individuals
Open circles = healthy individuals

- IL-10 expression correlated with abundance of *Acinetobacter* only in **healthy** individuals (a Gram-negative gamma-Proteobacteria)
- IL-10 has central role in immunologic tolerance to harmless substances
- *Acinetobacter* widely present in soil, environment

Hanski I et al. PNAS 2012
Psoriasis

- Antimicrobial peptides are highly expressed in lesions
- Guttate psoriasis: respiratory *Streptococcus* infection
- Increased Firmicutes, decreased *Corynebacterium* in lesions (N=6 plaque psoriasis patients)
- Increased Proteobacteria, decreased *Staphylococcus* and *Propionibacterium* in lesions (N=10 plaque psoriasis patients, 12 controls).
- No solid evidence so far for fungal component
Skin Microbiome Summary

- We are not alone! Microbial cells outnumber human cells in/on our bodies by a factor of 10;
- These microbes have a critical role in maintaining health: nutrition, controlling pathogenic species, priming immune system, etc;
- Genomics/metagenomics is a lens that provides a clearer pictures of those microbes present;
- Skin is a multi-niche habitat that supports the colonization and growth of microbes;
- Skin site determines dominant colonizing bacteria, diversity, stability;
- Bacterial diversity clusters based on body site, and skin is more diverse than gut or mouth;
Future questions

• How do antibiotics, cosmetics affect skin microbiota?
• What about fungi? Other microeukaryotes (Demodex)? Viruses? Bacteriophage?
• Are microbial communities associated with disease causative? If so, what is the mechanism of pathogenesis?
• What are the complex interactions between host and microbial genomes and how do they contribute to disease and aging?
• How can the microbiome be therapeutically manipulated? Probiotics? Prebiotics?
• The microbiome as a biomarker: can we use microbiome to diagnose disease or predict best treatment modality?
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