Future management strategies for wound infection:
Where is the science taking us?

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“The only way to manage an infection is to understand the process;
• who (microorganisms) is there
• what are they or it capable of doing
• Who (microorganisms) is actually doing what
• what is the host doing
• what is the best therapeutic approach

“The only way to understand the process is by having appropriate tools”

“Conventional culture has taken us this far, but genomics can lead us into the future”.

How do we apply genomics to wound infection?
Historic/Current analysis of microbiology for wounds
Revascularization
Offloading
Compression therapy
Compliance
Nutrition

Microbial

Understanding the wound environment
Genome sequencing

16S rDNA sequencing
Whole genome sequencing (Shotgun sequencing)
RNA Transcriptome
Nanopore sequencing
Can molecular DNA-based techniques unravel the truth about diabetic foot infections?

Abstract

Diabetic foot infections (DFI) are a common condition and a major cause of lower extremity amputation. Identification of causative pathogens is vital to directing appropriate therapy. Historically, bacteria have relied upon culture-dependent techniques that are now acknowledged as both being selective for microorganisms that thrive under the physiological and nutritional requirements of the microbiology laboratory and that greatly underestimate the microbial diversity of a sample. The amplification and sequence analysis of the 16S rRNA gene has revealed a diversity of microorganisms in DFIs, exceeding the view of the diabetic foot microbiome. The interpretation of these findings and their relevance to clinical care remains largely unexplored. The advent of molecular technologies that are culture-independent and employ massively parallel DNA sequencing technology represents a potential ‘game changer’. Management and its change approach to sampling all DNA within a sample (whole genome sequencing) affords the possibility to characterize not only the microbial diversity within a DF (i.e., which microorganisms are present) but the ecological function of the community such as virulence and pathogenicity (i.e., what are the microorganisms capable of doing), moving the focus from single species as pathogens to groups of species. This review will examine the new molecular techniques for the exploration of the microbe of infected and uninfected diabetic foot ulcers, elucidating the potential of these new technologies and pondering how they could translate to improved clinical care. Copyright © 2016 John Wiley & Sons, Ltd.

Keywords: microbe; diabetic foot infection; DNA sequencing; metagenomics

Introduction

One of the causal pathways to lower extremity amputation in a person with diabetes is the development of a diabetic foot ulcer (DFU) that becomes infected (Diabetic foot infection or DFI) [1]. In a person with diabetes, the development of a DFU is most commonly associated with loss of protective sensation (peripheral neuropathy), altered foot architecture and some form of trauma. These factors allow a physical break in the protective barrier of the skin to go unnoticed and provide an ideal environment for colonization with various opportunistic microorganisms [2]. Factors including a reordered host immune response and pathogens-related dynamics (such as virulence or pathogenicity), may predispose the DFU to further microbial replication.
Next Generation DNA sequencing platforms
16S rDNA sequence work flow
Next Generation DNA sequencing
Conventional Culture vs Molecular?
MinION from Oxford Nanopore is a portable, real-time DNA/RNA sequencing device.
Where can technology take us
The role of genomics

- Everyone responds to stress and the environment differently, and they also respond to disease and to treatments differently.

- Different people may have small variations in specific genes, and some people may have genes that others do not. These may increase susceptibility to a specific disease or provide protection from that illness.

- Scientists continue to discover new ways that subtle gene differences cause large differences in health. This understanding can lead to better ways to prevent, diagnose, and treat many types of health conditions.
As we find out more about our genes and how we react differently to diseases and to treatment, personalised medicine becomes increasingly important.

Genomic medicine is the use of genomic information in clinical decision-making and patient care.

This type of personalised medicine can be used: to support more accurate diagnosis and prognosis, to identify patients at a greater risk of disease or complications, to select and prioritise therapy in the prevention and control of outbreaks of infections.

To tailor therapy
The field of **pharmacogenomics** seeks to understand these differences. For some medications, identifying individual gene differences can help customize both the selection of medications and dosing for the best response.
Genomic Medicine is already a reality

Examples of Genomic Medicine

Translational

- The causes of intellectual disability are often unknown, but a team in the Netherlands has used diagnostic exome sequencing of 100 affected individuals and their unaffected parents in order to uncover novel candidate genes and mutations that cause severe intellectual disability. *Neur. 2012.* [PubMed]

- Colorectal cancers with a particular mutation can benefit from treatment with aspirin post-diagnosis. Aspirin (and other non-steroidal anti-inflammatory drugs) decrease the activity of a signaling pathway called PI3K. Between 15 and 20 percent of colorectal cancer patients have a mutation in a gene called PIK3CA that makes a protein that is part of the PI3K pathway, and it has been discovered that regular aspirin treatment is associated with increased survival compared to colorectal cancer patients who have the non-mutated version of PIK3CA. *NeJM. 2012.* [PubMed]

- Currently, every baby born in the United States is tested at birth for between 29 and 50 diseases. Inherited treatable genetic diseases through a public health program called newborn screening. Whole genome sequencing would enable clinicians to look for mutations across the entire genome simultaneously for a much larger number of diseases or conditions. Rapid whole genome sequencing has been shown to provide a useful differential diagnosis within 61 hours for children in the neonatal intensive care unit. *Science. 2012.* [PubMed]

- Researchers at Stanford University in California have been developing a new test to detect when a transplanted heart may be rejected by the recipient. Currently, the only way to detect the onset of rejection is by performing an invasive tissue biopsy. This novel approach only requires blood samples, and detects the levels of cell-free circulating DNA from the donor organ in the recipient’s blood stream. This circulating DNA from the donor can be elevated for up to five months before rejection can be detected by biopsy, and the level of DNA correlates with the severity of the rejection event (i.e., more circulating DNA signals a more severe event). *Sci Transl Med. 2014.* [PubMed]

- Cell-free circulating DNA is also being explored as a biomarker for cancer. As tumor cells die they release fragments of their cellular DNA into the bloodstream. Sequencing this DNA can give insights into the tumor and possible treatments, and even be used to monitor tumor progression (as an alternative to invasive biopsies). *Sci Transl Med. 2014.* [PubMed]

Clinical

- Pharmacogenomics involves using an individual’s genome to determine whether or not a particular therapy, or dose of therapy, will be effective. Currently, more than 100 FDA-approved drugs (90% of all) have pharmacogenomics information in their labels, in diverse fields such as analgesics, anti-arrhythmics, cardiovascular drugs, and anti-cancer therapeutics.

- FDA has also cleared or approved (30% of all) 40 human genetic tests, and more than 100 nucleic acid-based tests for microbial pathogens.

- DNA sequencing is being used to investigate infectious disease outbreaks, including E. coli virus, drug-resistant strains of Staphylococcus aureus and Klebsiella pneumonia, as well as food poisoning following contamination with Escherichia coli. Sequencing has also recently been used to diagnose loco pesticidal encephalopathy, rapidly identifying the correct therapeutic agent for the patient.

- Cystic fibrosis is one of the most common genetic diseases, caused by mutations in a gene called CFTR. More than 1,000 different CFTR mutations that cause cystic fibrosis have been identified to date. Approximately four percent of cases are caused by a mutation known as ΔF508, and now a drug called ivacaftor has been developed that is extraordinarily effective for patients with this particular mutation.
How can we use genomics in wound research/clinical care?

- Laboratory *in vitro* experiments have been the mainstay of wound research – product development – wound biology.
- After the past century of mostly *in vitro* experiments, we now have detailed knowledge of bacterial behavior in standard laboratory conditions.
- Poor understanding of bacterial functions and behaviors during human infection. It is well-known that the growth and behavior of bacteria are largely dictated by their environment, but how bacterial physiology differs in laboratory models compared with human infections is not known.
How can we use genomics in wound research?
P. aeruginosa in Human Samples and in vitro Samples Have Distinct Transcriptomes

Quorum-Sensing Genes Are Expressed at Lower Levels in Human Infection.

P. aeruginosa Antibiotic Tolerance Determinants Are Highly Induced During Human Infection compared to in vitro.
How can we use genomics in clinical research?

Abstract:
Chronic, non-healing wounds are a major complication of diabetes associated with high mortality and healthcare expenditures estimated at $9-13 billion annually in the U.S. Though microbial infection and critical colonization is hypothesized to impair healing and contribute to severe outcomes such as amputation, antimicrobial therapy is not efficacious and the role of microbe in tissue repair regeneration and healing remains unclear. Here, in a longitudinal prospective cohort study of 100 subjects with non-infected neuropathic diabetic foot ulcer (DFU), we performed metagenomic shotgun sequencing to elucidate microbial temporal dynamics at strain-level resolution, to investigate pathogenicity and virulence of the DFU microbiome with respect to outcomes, and to determine the influence of therapeutic intervention on the DFU microbiota. Slow healing DFUs were associated with signatures of bottleneck formation, host invasion, and virulence. Though antibiotic resistance was widespread in the genetic level, disruption, rather than antibiotic treatment, significantly shifted the DFU microbiome in patients with more favorable outcomes. Primary clinical isolates of S. aureus, C. striatum, and A. faecalis induced differential biological responses in keratinocytes and in a murine model of diabetic wound healing, with the S. aureus strain associated with non-healing wounds eliciting the most severe phenotype. Together these findings implicate strain-level diversification of the wound pathogens S. aureus in chronic wound outcomes, while revealing potential contributions from skin commensals and other previously underappreciated constituents of the wound microbiota.

New Results:
The microbial basis of impaired wound healing: differential roles for pathogens, “bystanders”, and strain-level diversification in clinical outcomes
Lindsay Kulk, Jerzyko S. Mei, Michael A. Laschke, Joseph Honwinski, Irena Szolka, Xiaoxuan Chen, Sue E. Gordon, Mattiah A. Grace
doi: 10.1111/1477-9560.13754
Higher taxanomic resolution
Genes identified
Using genomics to better understand wound microbiome
Genome analysis relies heavily on bioinformatics
Networks of interacting objects exist across many systems ranging from ecological communities, social networks, infrastructure and communications. Networks are composed of two elements: nodes and edges.

**Nodes** are the objects that compose the system, such as species, genes, Facebook profiles or computers.

**Edges** are the Interactions between the nodes, such as mutualistic ecological relationships, similar gene-expression profiles, Facebook friendships or a connection between computers via the internet.
How could network analysis be useful?

- In the context of the microbiome, networks have emerged as a sophisticated tool to visualise the interactions between species and to determine the drivers of microbial community patterns.

- This can be used to identify modules or clusters of highly associated taxa.
Keystone taxa

• These properties can be harnessed statistically to identify keystone taxa - organisms central to structuring the microbiome and indicative of particular states – healing or chronic infection.

• Removal of these taxa tends to lead to failure of the system as a whole or wholesale shifts in microbiome structure and function.
How do we translate genomics into personalized medicine in the wound arena?
Translation of Research

Wound infection
Infection resolution
Wound healed
Thank you