Microbiology, Biofilms and Factors affecting Wound Healing

Professor Val Edwards-Jones
Director of Research
Manchester Metropolitan University
Lecture overview

- Types of chronic wounds
- New techniques
- Typical pathogens
- Infection/colonisation
- Biofilms
- MRSA
Chronic wounds

Diabetic foot

Venous ulcer

Pressure sore
Acute wounds
Chronic wounds - why do they not heal??

- Static
- Matrix metalloproteases
  - Endogenous – cellular
  - Exogenous – bacterial
- Inhibition of growth factors
- critically colonised??
  - what does this really mean?
- Role of the Biofilm
Lag phase (Adaptation) – production of adhesins

Log phase
(Exponential Multiplication)
Production of competitive factors

Stationary phase production of toxins and invasive enzymes

Quorum sensing
$10^6$ cfu/ml

Contamination → Colonisation → Critical Colonisation/Infection

This is a dynamic process and a number of factors can alter the process.
Biofilm

Cell-Cell Communication

Stoodley, P.
Role of the Biofilm?

Definition

• ‘A biofilm is a structured community of microorganisms encapsulated within a self-developed polymeric matrix and adherent to a living or inert surface.
• A complex aggregation of microorganisms marked by the excretion of a protective and adhesive matrix.
• A coating or covering on the surface of a living or nonliving substrate composed of organisms like bacteria, protozoa, algae, and invertebrate.
• Adherent layer of bacteria and/or other microorganisms on a solid surface bound together in a bacterially-derived polysaccharide matrix.
• A cooperative community of single-cell organisms (bacteria) that builds an extracellular matrix of slime or fibrous material.
• An aggregate of microbes with a distinct architecture.
Facts about biofilms

- Biofilms form on the surface of catheter lines, pacemakers, heart valve replacements, artificial joints and other implants.
- Major cause of HAI’s
- Bacteria growing in a biofilm are up to 1,000 times more resistant to antibiotics and antiseptics. Standard antibiotic therapy often useless and necessitates the removal of the implant.
- Fungal biofilms also frequently contaminate medical devices.
- Biofilms are involved in numerous diseases e.g. *Pseudomonas* sp. infections in CF lungs - often result in antibiotic resistant biofilms protected by a alginate slime layer.
Strategies for biofilm management


- 60% chronic wounds characterised as containing biofilms
- Chronic wound specimens revealed diverse polymicrobial communities including strict anaerobes
- Many chronic bacterial infections which are not easily eradicated by conventional antibiotic therapy, involve biofilms.
- There are recurring symptoms, after cycles of antibiotic therapy, until the sessile population is surgically removed from the body.
- Significantly greater proportion of biofilms in chronic than in acute wounds
Normal wound sampling procedures using a moist swab
Sample the surface layers

For example
MRSA +++
Should we just sample the infected looking areas?
Perhaps using curettage or biopsy?
Tissue culture
mixed coliforms ++
mixed anaerobes++
Conventional Techniques
Baird-Parker
Ciprofloxacin agar

Mannitol Oxacillin salt agar

ORSAB medium

CHROMagar MRSA
Typical wound pathogens

- *Staphylococcus aureus* (MRSA…GRSA)
- *Pseudomonas aeruginosa*
- Gram-negative bacilli (*Acinetobacter sp, Klebsiella sp, E.coli*)
- *Streptococcus pyogenes*
- other streptococci
- *Enterococci (VRE)*
- *Candida sp and Aspergillus sp*
- Anaerobes (dependant upon site)
Typical ‘Normal’ Skin Flora

- highly diverse – hundreds of bacterial species amongst 6 individuals
- only a few bacteria are common among the individuals.
- These included
  - Propionibacteria,
  - Corynebacteria,
  - Staphylococcus spp,
  - Streptococcus spp.

Molecular Techniques

• Population analysis studies
  – **PCR**: Amplify 16s ribosomal DNA
  – separate using **Denaturing Gradient Gel electrophoresis** (DGGE)
  – sequence the gene fragments

• James *et al.* (2008) - Biofilms in Chronic Wounds *Wound Repair and Regeneration* 16: 37-44
Polymerase chain reaction (PCR)

Increase the number of copies of the genes
DGGE
DNA Sequencing

Different coloured bases (ATCG)-capillary sequencing
Chronic wounds

- Study by Citron *et al.*, (2007)
- Bacteriology of diabetic foot infections
  - Tend to be polymicrobial (83.8%)
    - 48% aerobes only
    - 43.7% aerobes and anaerobes
    - 1.3% anaerobes only
Aerobes v Anaerobes

- MSSA (14.3%)
- MRSA (4.4%),
- CNS (14.3%)
- *Streptococcus* sp (15.5%)
- *Enterococcus* sp (13.5%)
- *Corynebacterium* sp (10.1%)
- Enterobacteriaceae (12.8%)
- *Pseudomonas aeruginosa* (3.5%)

- Gram pos cocci (45.2%)
- *Prevotella* sp (13.6%)
- *Porphyromonas* sp (11.3%)
- *Bacteroides fragilis* gp (10.2%)
Venous
Diabetic
Pressure

Dowd et al. BMC Microbiology 2008 8:43
Could debridement help with removal of the biofilm?

- Biofilms are notorious difficult to remove from surfaces
  - Physical removal with cleaning agents
  - High pressure wash
  - Sonication

- Debridement will remove dead tissue from which the bacteria gain their nutrition and also disrupt the physical structure of the biofilm.
DEBRIDEMENT METHODS

- Autolytic
- Sharp
- Larval
- Enzymatic
- Mechanical

The cleaner way to operate.
Bio-Surgery (Lucilia Sericata)

nature’s little surgeons!
Comparison of three therapies for treatment of MRSA-infected foot ulcers

- Qualitative vs. Quantitative
- Patient removed from study – MRSA negative; clinically improved wound

![Bacterial Profile - Patient 1 (Biopsy)](image1)

![Bacterial Profile - Patient 1 (Swab)](image2)
• MRSA still present in both biopsy and swab at week 12.

• However, wound clinically improved
In-vitro study
Versajet

• Porcine model
  – Wounds – deep, surface, sinus
  – Infected with MRSA, *Pseudomonas aeruginosa*, *E.coli* and washings from chronic wound
  – Sampled before, after incubation and after versajet
  – Histology, microbiology, EM
What about topical antimicrobial treatments?

- Dressings and creams/ointments
  - silver
  - Iodine based dressings and creams
  - Chlorhexidine dressing
  - Stabilised hydrogen peroxide
- PHMB (polyhexamethylene biguanide)
- Honey
  - Manuka honey
- pH alteration
- VAC

These could create an imbalance of the bacterial biofilm and help resolve infection?
Thank you Collaborators

CMHT
Prof Andrew Boulton
Mr Frank Bowling
(funded Diabetes UK)

MMU
Monika Stuczen
Anne Leahy-Gilmartin
Helen Duxberry
Allan Surrey

Burns SMUHT
Mr Ken Dunn
Jackie Edwards
(Framework 7 EU funded)