

Evidence Summary: Wound Infection: Biofilms defined and described

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Author

Wound Healing and Management Node Group

Question

What is the best available definition and description of biofilm in wounds?

Clinical Bottom Line

An understanding of the relationship between chronic wound infection and the presence of biofilm is gradually increasing. The bioburden of an infected wound that fails to respond to treatment and progresses to a chronic wound is likely to involve one or more biofilm/s.¹ Single or multiple species of microorganisms rapidly multiply to form a wound biofilm community that is encased in a self-secreted matrix. The matured biofilm colony is resistant to antimicrobial intervention and if disrupted will quickly reform. An understanding of the physiology and function of biofilm organisms and the community they form is essential to guide clinical application of multimodal therapeutic intervention in chronic wounds.²

- Along with other bacteria, free-floating, single-cell planktonic bacteria are present on the skin surface in a non-pathogenic relationship with the host. A biofilm is created when bacteria (multiple or single species) adhere to a wound surface and secrete a protective extracellular polymeric substance (EPS) that encapsulates and structures the biofilm community.^{1,3} (Levels II & III resp.)
- Through this process a previously non-pathogenic and mutually interdependent relationship between the human host and commensal bacteria undergoes a parasitic transformation that results in a self-sustaining cycle of chronicity causing harm to the human host.³ (Level III)
- The formation of biofilm communities follows a complex and well-coordinated sequence of molecular events designed to maximise the microorganism community's survival and sustainability. *In vitro* tests* have identified the following events associated with the formation of biofilm:^{1,3} (Level II & III resp.)
 - o Free-floating and non-motile, single cell planktonic (bacterial) cells migrate to the wound surface and form adhesions to available (biological) surfaces.^{1,3} (Level II & III resp.). Their presence initiates the inflammatory response; the exudate released during the response supplies nutrition to the biofilm.
 - o Two stages are associated with the process of attachment; (i) reversible adhesion (bacterial cells

can revert to the planktonic state) and (ii) irreversible adhesion that commences the formation of bacterial microcolonies and biofilm. Bacterial cells grow and divide forming cell clusters or microcolonies.^{1,3,4} (Level II, III & III resp.)

- o The size of the microcolonies is regulated by 'quorum sensing' (signalling) molecules (autoinducers) produced by the dividing cells. Once an appropriate 'quorum' has been reached, these bacterial communities produce a protective EPS matrix that encapsulates and structures the community within a three-dimensional biofilm.^{1,3} (Level II & III resp.)
- o The protective matrix commonly consists of polymers, extracellular DNA, polysaccharides, lipids, proteins, nucleic acids and enzymes which facilitate bacterial adhesion to the wound bed and provide the pathogen with a nutrient rich source that sustains the community and its structural integrity.^{1,3,5} (Level, II, III & IV resp.)
- o The biofilm community retains a dynamic process that regulates the cyclical release of selected planktonic cells back to the wound surface to recommence the cycle of bacterial colonisation in a previously uncolonised site (some cells however may also become integrated into an established biofilm community).^{1,3,4} (Level II, III & III resp.)

* Research has noted structural congruence between the features of biofilms formed from single species *in vitro* and those formed by mixed species in nature.⁶ (Level III)

- Changes at the molecular level of cell structure alter the genetic expression of cells from previously non-pathogenic to pathogenic biofilm-forming cells. An example of this is the alteration of free-floating planktonic cells into surface-attached (pathogenic) phenotypes; furthermore, some of these cells can then revert, detaching themselves once again in order to spread infection to other tissues and form additional biofilm colonies.^{3,4} (both Level III)
- The molecular changes that occur during the formation of biofilm equip the microcolony with a range of defensive strategies which include:
 - o An increased resistance to environmental stress and substances including antibiotics, biocides and human immunity.¹ (Level II)

- o An increased metabolic efficiency ⁷(Level III) and substrate accessibility (access to underlying wound tissue and wound bed).
 - o An increased ability to cause local tissue damage and infection by interfering with wound healing and preventing wound closure.¹ (Level II)
 - o Promoting growth of anaerobic bacteria by limiting oxygen diffusion to the inner core of the biofilm while the outer biofilm membrane consumes oxygen and maintains a metabolically active layer of bacteria.⁸ (Level IV)
 - o Transforming biofilm cells from metabolically active to inactive “persister cells.” This defence strategy protects the bacterial biofilm cell from antibiotic infiltration and therefore cell death. This characteristic exploits the function of antibiotics to inhibit the bacterial enzymes; a function that bacterial cells require to maintain metabolic activity.⁸ (Level IV)
- In an established biofilm, prevention and eradication through the use of antibiotics and topical antimicrobials is largely ineffective due to the protective EPS² (Level III); the use of povidone iodine however is showing some promise,⁹ (Level IV), as is silver-containing hydrofibre.¹⁰ (Level III)
 - Aggressive debridement of the wound slough and the underlying tissue that contains biofilm is recognised as a key intervention at the beginning of treatment and as a continuing maintenance strategy as biofilm cells reform quickly once disrupted; this intervention represents an important prevention strategy to stop regrowth biofilm.⁸ (Level IV). Currently, non-sharp forms of aggressive debridement (for example, low-frequency ultrasound) are also being explored.¹¹ (Level IV)
 - Following debridement the wound is dressed with an appropriate antimicrobial dressing.^{1, 3, 4, 8} (Level, II, III, III & IV resp.)
 - The first 24 hours after sharp debridement or the first 24 hours of an initial biofilm development, provides a therapeutic window for the application of topical antimicrobials. Cells involved in early biofilm (re)formation demonstrate increased sensitivity to antimicrobials and anti-biofilm agents at this time. These findings have been demonstrated *in vitro* using porcine and mouse models and also *in vivo* using venous leg ulcer samples from humans.^{4, 12} (Level III & II resp.)
 - Interventions designed to inhibit cell to cell communication present the possibility of blocking the quorum sensing signal thus preventing cells from further biofilm development.⁴ (Level III)

Detecting biofilm:

- Accurate detection and identification of the bacterial bioburden in a biofilm is complex and requires more advanced technological methods for accurate reporting than the standard, routine clinical culture tests are able to deliver.¹ (Level II). Routine culture tests will only pick up the surface free-floating planktonic bacteria and therefore return a false negative result for biofilm.² (Level III)
- Wound biofilm is microscopic; scanning electron microscopy and light microscopy of wound biopsies facilitates detection. Biofilm however is not distributed evenly throughout the wound bed or surface; a biopsy could feasibly return a false negative result if the sample happens to be taken from an area that is currently without biofilm.^{1, 8} (Level II & IV resp.)
- Molecular methods of identification reserved for laboratory research include the use of fingerprinting using 16S rRNA, fluorescence *in situ* hybridisation (FISH), pyrosequencing and quantitative PCR (Q-PCR).⁴ (Level III). This form of research has revealed two important characteristics of biofilm bacteria:-
 - o The location of bacteria within a wound is dependent upon the bacterial phenotype e.g. *P. aeruginosa* is found at the deepest part of the wound, while *S. aureus* is located near the wound surface.³ (Level III)
 - o Cells within bacterial communities work synergistically to produce chronic infection; the term “functional equivalent pathogroups” (FEPs) is used to describe this characteristic.³ (Level III)

Biofilm management strategies

A number of approaches designed to disrupt biofilm infrastructure are currently being investigated, amongst these are:

Characteristics of the Evidence

This evidence summary is based on a structure search of the literature and selected evidence-based health care databases. The evidence in this summary comes from:

- A study of biofilm-based wound management in 190 subjects with critical limb ischaemia.¹ (Level II)
- A paper summarising the relevance of biofilm model to the treatment of chronic infections.² (Level III)
- A paper that presents a hypothesis that attributes wound biofilm formation to an impotent initial immune response that perpetuates inflammation and chronicity.³ (Level III)
- A paper that advances knowledge of the relationship between biofilms and wound chronicity with an emphasis on early biofilm diagnosis in wounds.⁴ (Level III)
- An expert panel discussion at a conference focusing on biofilms.⁵ (Level IV)
- A paper that translates the ecological characteristics of microbial biofilm into a model that describes the inherent molecular genetics.⁶ (Level III)
- A review of research on bacterial extracellular polysaccharides involved in biofilm formation.⁷ (Level III)

- A video that presented an expert summary to date of biofilm-based wound care.⁸ (Level IV)
- An educational summary of the application of iodine in wounds.⁹ (Level IV)
- A lab-based study that reported the eradication of wound biofilm using a silver hydrofibre wound dressing.¹⁰ (Level III)
- Expert opinion from the Wound Healing and Management Node Expert Reference Group.¹¹ (Level IV)
- A laboratory controlled study that examined the relationship between sharp debridement and time-dependent therapeutic intervention.¹² (Level II)

Best Practice Recommendations

The same practice and principles of good wound care are applicable to biofilm-based wound care. Biofilm-based wound care emphasises the importance of:

- Debriding the wound to remove wound slough and the underlying tissue that contains the biofilm. (Grade A)
- Following debridement, dress the wound with an antibacterial barrier dressing that prevents planktonic bacteria from rapidly reforming biofilm colonies on the wound. (Grade B)
- Debride on a regular basis in order to create an optimal molecular environment in the wound. (Grade B)
- Prevent the reformation of biofilms by instigating antimicrobial intervention within the first 24 hours of biofilm (re)formation. (Grade B)
- Remain informed of the latest developments in the emerging knowledge and treatment of biofilms. Currently investigations indicate promising results with the following two approaches:

- o The use of nanocrystalline silver dressings in the prevention of biofilm formation. (Grade B)
- o The use of sustained release cadexomer iodine to kill bacteria biofilm. (Grade B)

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