Microbial biofilms and chronic wounds: facts and speculation

Tim Holm Jakobsen[1], **Kendra Rumbaugh**[2], **Tom Coenye**[3], **Thomas Bjarnsholt***[1,4]

[1] Costerton Biofilm Center, Department of Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark
[2] Department of Surgery, Texas Tech University Health Sciences Center, Texas, United States
[3] Laboratory of Pharmaceutical Microbiology, Ghent University, Ghent, Belgium
[4] Department for Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark

*Corresponding author email tbjarnsholt@sund.ku.dk

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Today we know from numerous *in vitro* and *in vivo* biofilm related observations that persistent wound infections contain microbial aggregates of varying sizes, as well as single cells (Figure 1). We know that the microorganisms in these aggregates are in close proximity and possibly surrounded by extracellular polymeric substances (EPS). We also know that these aggregates withstand very high doses of antibiotics which kill planktonic cells (fast growing cells in laboratory cultures). Furthermore, they demonstrate an enhanced ability to evade host immune defenses compared to planktonic cells. These characteristics are well-recognised hallmarks of biofilms, as defined in the literature.

However, chronic infections present complex medical challenges and extrapolation of their characteristics and pathophysiology from *in vitro* observations fails to fully explain the bacterial behavior in these infections. Our understanding of the role of biofilms to the sequelae of

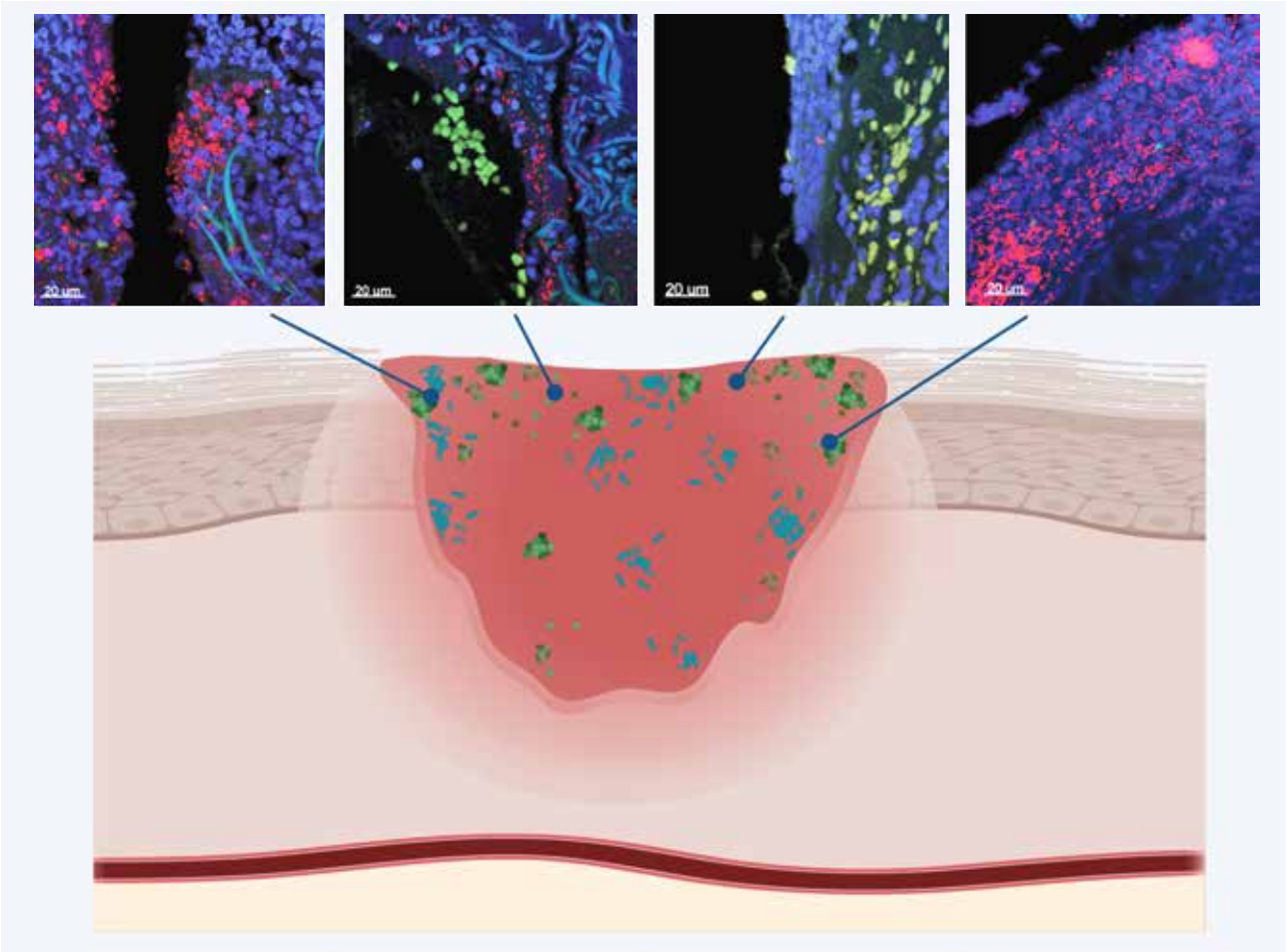

Figure 1: Schematic drawing of a chronic wound with different species of bacteria heterogeneously distributed in various sizes of biofilms and as single cells from the top to the deeper layers of the wound. Four confocal microscopy images from patients with a chronic leg wound. Images show biofilms of different sizes with scattered single cells. Bacteria were stained with a universal 16S PNA-FISH probe (red), and eukaryotic cells were counter-stained with DAPI (blue).

infection is incomplete, the composition of the biofilm matrix, including distinguishing between components produced by the microorganisms themselves and those derived from the host environment. Additionally, we still lack a comprehensive understanding of the metabolism, growth states, and signaling of bacteria over the course of infection.

Here we present a list of current assumptions about biofilm in wounds that are and are not supported by direct observations and/or quantifiable data (Figure 2). We also list key literature reporting direct *in vivo* observation as well as free to use schematic drawings (https://ewma.org/resources/biofilm-library/). We expect this will be an evolving inventory of supporting studies and hope it will stimulate objective debate and evidence-based research within the field of chronic wounds, with the ultimate goal of benefiting patients.

Substantiated facts:

- We know from direct observation of patients tissues that microorganisms (bacteria and fungi) are present in chronically wounds, both as single cells and as aggregates.[1]
- Multiple genera and species can be detected within the same wound.[2–5]
- The microbiome composition and ratio of different organisms (bacteria and fungi) vary across different wounds.[6,7]
- Chronic wounds are arrested in a non-healing trajectory.
- Bacterial load does not necessarily correlate with infection severity.[8,9]
- Microorganisms are not only present in the superficial areas of wounds but also in deeper tissues.[10]
- Attachment to an inert surface is not needed for bacteria to demonstrate a biofilm phenotype.[11]
- Development of chronic wounds infections typically requires comorbidities.[12,13]
- 'Slough' is not the same as biofilms, you can observe inflammation but not biofilm with the naked eye.[14]
- The tolerance of microorganisms in chronic infections to antimicrobial agents can be due to many factors, but the exact mechanism(s) involved varies between the infections and organisms.[15,16]

More research needed:

- Bacteria and other microorganisms interfere with the healing process, but the mechanisms remain unclear.[17,18]
- The single cells observed in wounds do not behave similar to fast-growing planktonic cells observed in shaken laboratory cultures.
- It is unknown if wound senescence is influenced by microorganisms.
- The altered microenvironment in chronic wound infections influences microbial phenotypes.
- The role and importance of quorum sensing during wound infections is not known.
- *In vitro* bacteria in biofilms produce their own matrix components but it is unknown if or to what extent this occurs during wound infection.
- The persistence of infections in chronic wounds is attributed to biofilms, yet definitive proof remains elusive.
- It is not fully understood which bacterial species or virulence factors impact wound severity.
- The concept of 'critical colonisation', meaning the threshold for microbial load to cause infection in wounds is debatable.[8]

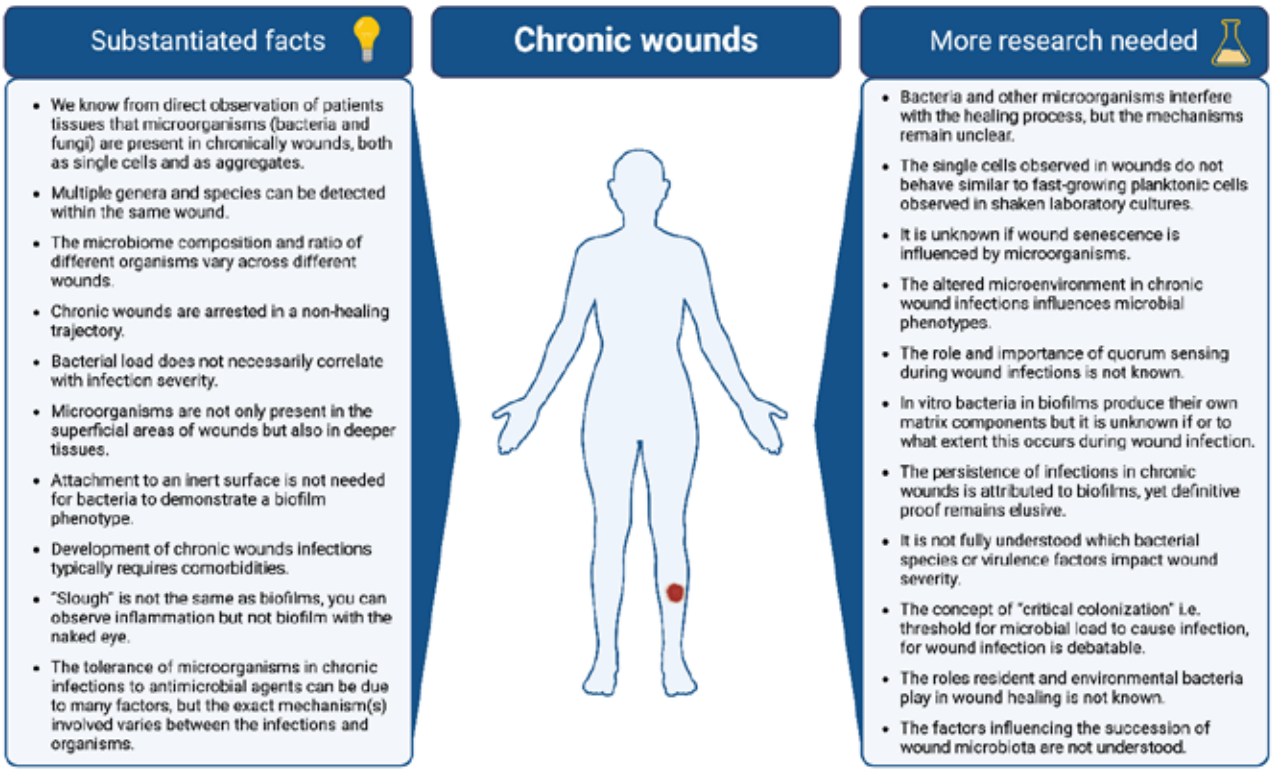

Figure 2. List of current assumptions regarding biofilms in wounds.

- The roles resident and environmental bacteria play in wound healing is not known.
- The factors influencing the succession of wound microbiota are not understood.
- There is not enough *in vivo* evidence that removing biofilm leads to improved wound healing.

CONCLUSION

Globally chronic wounds are on a dramatic rise, however, effective treatment strategies and the precise role of microorganisms and biofilms remain elusive. The scientific and medical fields need more focused research to alleviate this. With this short perspective we highlight important facts and identify areas that require more research. We encourage the field to build further on existing research, identify unsubstantiated assumptions and learn from the advances that have been made in other fields, such as cancer research, to move the understanding and treatment of chronic infections forward.

On behalf of the EWMA Antimicrobial Stewardship (AMS) Group.

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