

Summary

How do maggots operate?

The underlying mechanisms of action of maggot debridement therapy

The aim of this thesis was to provide insight into the underlying microbiological and immunological mechanisms of action of Maggot Debridement Therapy. The three areas in which MDT is supposed to work, were investigated: disinfection, debridement and stimulation of wound healing.

Chapter 1

MDT is an ancient and successful method to treat severe, infected wounds. In these times of increasing antibiotic resistance and resulting impaired healing of infections, it is important to search for potential new treatment methods. Basic mechanisms of wound healing, debridement, biofilm formation and complement activation are described in *Chapter 1*. Inappropriate complement activation after dermal tissue injury and/or (bacterial) infection can lead to a prolonged inflammatory phase in the process of wound healing, which impairs the physiological process and results in the formation of chronic wounds. The inflammatory phase is characterized by debris on the wound bed. In biofilm-associated infected wounds, biofilm is part of the debris. Debridement, including biofilm reduction, is essential to allow the process of wound healing to continue. Maggots are successful in debriding and healing chronic wounds and in this thesis, controlled in-vitro experimental studies were performed to investigate the various possible interactions of larvae and/or their ES with free-living bacteria, antibiotics, biofilm formation and complement activation.

Chapter 2

One of the most important hypotheses about MDT is that maggots possess direct antibacterial activity. In this study, the susceptibility of strains of six bacterial species, which are regularly found in infected wounds, to live maggots and/or their excretions/secretions (ES) was investigated to find proof supporting direct antibacterial activity. The results reveal that live maggots increase bacterial growth of

all bacteria. A turbidimetric assay was performed with maggot ES which shows no inhibition of bacterial growth. In contrast, for all bacteria, except *Pseudomonas aeruginosa*, their growth in presence of ES increased. The study concludes that there is no direct antibacterial effect of maggots and/or ES in-vitro, however in clinical observations, MDT is successful. It is possible that the disinfection of the wound is caused by indirect, immunological antibacterial activity.

Chapter 3

During MDT, faster healing of wounds in combination with antibiotics has been observed. Therefore, in-vitro experiments were performed to study whether maggot ES influence the antibacterial activity of different antibiotics. Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) were determined of nine combinations of antibiotics and bacterial species by checkerboard titration. The results show a dose-dependent increase of the antibacterial effect of gentamicin in the presence of ES on *S. aureus*. The MBC of flucloxacillin with addition of ES against *S. aureus* is also decreased. The other antibiotic and ES combinations define an indifferent effect. Again, ES alone do not have any antibacterial effect. The synergism between gentamicin and maggot ES could be of direct importance in clinical practice, because it could permit the use of lower doses of gentamicin and thus minimize the risk of gentamicin related side-effects.

Chapter 4

This chapter is divided in two parts. The first part describes the research results of the effect of ES on *Pseudomonas aeruginosa* (PAO1) biofilms on titanium, stainless steel and polyethylene in-vitro and the second part shows the results of biofilm formation by five other bacteria and the effect of ES on biofilms of *Staphylococcus aureus* and *Staphylococcus epidermidis*. Biofilm-associated infections on biomaterials are difficult to treat, because the bacterial pathogens in the biofilms are resistant to antibiotics and host defenses. It is therefore necessary to focus on new treatments for the healing of severe biofilm-associated infections. In current MDT, sterile maggots are captured in small permeable bags that allow free exchange of maggot excretions and secretions to the wound surface. Observations of wounds that were suspected for biofilm formation showed resolution of the debris with larvae in bags. Therefore, it was hypothesized that ES could interfere with biofilm formation.

Biofilms were formed in a specially developed, new experimental design with comb-models of titanium, stainless steel and polyethylene, suspended in a 96-well microtiter plate. The highest amount of biofilm formation is reached within approximately 7 days. The least biofilm of *P. aeruginosa* was formed on stainless steel, in contrast to *S. aureus* and *S. epidermidis* biofilms that have the weakest attachment to titanium. The biofilms of the other tested bacteria occurred in insufficient quantity to be measured. So, the amount of biofilm formation is dependent on the biomaterial as well as on the bacterial specie creating the biofilm. Maggot ES prevent and inhibit biofilm formation and even break down existing biofilms. A maximum of 92% of biofilm reduction was measured. ES from full-grown maggots are more effective than ES from maggots that had just hatched from the egg. ES still have considerable biofilm reduction properties after storage at room temperature for one month. Based on these research results, maggot ES could provide a new treatment of biofilm-associated infections of orthopaedic biomaterials in the future.

Chapter 5

The inflammatory response, which takes place during the process of wound healing is the result of complement activation and is necessary for successful tissue repair. However, inappropriate complement activation can also cause injury and contribute to severe tissue damage. In the impaired wound healing process, the inflammatory phase is prolonged, which is the result of disturbed complement activation. Perhaps maggot ES stimulate wound healing by affecting inappropriate complement activation. In this study, two different methods, that are also clinically used to determine complement activation in patients, were tested. This research shows that ES inhibit complement activation in healthy and in post-operatively gained, immune-activated human sera up to 99.9%, via all three pathways of the complement system. ES showed the best complement-inhibiting properties upon boiling at 100°C and even after storage for a month at room temperature, ES inhibited CA. Degradation of proteins in ES reduced the complement-inhibiting effect. This study shows the first pathway independent complement-inhibitor, probably a small molecular weight, boiling stable protein affecting either C3 conversion or MAC formation, that is already successfully used in clinical practice and explains part of the improved wound healing caused by maggot therapy. Furthermore, the complement-inhibitor(s) in maggot ES

could form a new treatment for all kind of diseases, resulting from an (over)active complement system.

Chapter 6

The clinical indications for the use of maggots are reviewed, and the prevalent hypotheses about the underlying mechanisms of action of MDT are discussed, including the results from this thesis, except for those from the complement study that will be discussed in *Chapter 7*. Finally, some possible future prospects are suggested. Part of the underlying mechanism of MDT could be explained by modification of extracellular matrix components and by biofilm reduction. Maggots and/or their ES do not possess direct antibacterial properties, as described in *Chapter 2*. The review suggests an immune-related effect of maggot ES, which results in the inhibition of the pro-inflammatory response. This hypothesis is confirmed in *Chapter 5* of this thesis. Together with basic research, randomized controlled multicenter clinical trials are needed to come to evidence based medicine for MDT. Furthermore, ES seem to play an essential role in the wound healing process. Future research has to investigate the composition of ES and determine the effective substances in order to develop a possible new treatment for (some) inflammatory diseases.

Chapter 7

In this chapter, the main question of this thesis is (partially) addressed: How do maggots operate? During larval colonization of wounds, the maggots produce ES. These ES, which contain various proteins, reduce the (biofilm) debris on the wound surface and inhibit the complement system of the infested organism. The complement inhibition causes a decrease of the inflammatory response, which practically means that chemotaxis, e.g. of neutrophils, is reduced. A suppressed inflammatory response results in less wound debridement, however the debridement is now 'adopted' by the maggots and/or their ES. Since the inflammation is reduced, further tissue damage will be prevented and the wound healing process can advance. ES also influence proteolytic activity in the wound. During the proliferative phase of wound healing, ES modify fibroblast adhesion and spreading across the extracellular matrix. ES also provide proliferation of endothelial cells and thereby

stimulation of angiogenesis. Furthermore, ES have a motogenic effect on keratinocytes that cover the wound surface. Finally, the wound will be closed.

This thesis shows that maggot ES have a high potency as therapeutic biofilm- and/or complement-inhibitors. Finally, the principle aim of future research is to develop a new pharmacological agent for the treatment of infectious and/or immune-mediated diseases, isolated from the ES of *Lucilia sericata* larvae.