Chapter 7

General discussion: How do maggots operate?

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This thesis has investigated the underlying mechanisms of action of maggots and/or their excretions and secretions in MDT. The principal findings are that larvae and/or excretions and secretions do not inhibit bacterial growth, that excretions and secretions reduce bacterial biofilm formation on biomaterials and that excretions and secretions are able to inhibit the human complement system via each of the three pathways of complement activation. These results explain part of the beneficial effects of larval therapy on the process of wound healing and, moreover, could provide new insights in the role of complement activation and inflammatory response in chronic wounds.

How do maggots operate on wounds? During larval colonization of the wound beds, the maggots produce excretions and secretions. These excretions and secretions, which contain various (poorly defined) proteins, reduce the (biofilm) debris on the wound surface and inhibit the complement system of the infestated organism. The complement inhibition causes a decrease of the inflammatory response, which practically means that chemotaxis, e.g. of neutrophils, is reduced.[1] A suppressed inflammatory response results in less wound debridement, however the debridement is now 'adopted' by the maggots and/or their excretions and secretions. Since the inflammation is reduced, further tissue damage will be prevented and the wound healing process can advance. Excretions and secretions also influence proteolytic activity in the wound.[1,2,3] During the proliferative phase of wound healing, excretions and secretions modify fibroblast adhesion and spreading across the extracellular matrix[2] and provide proliferation of endothelial cells and thereby stimulation of angiogenesis.[3] Furthermore, excretions and secretions have a motogenic effect on keratinocytes that cover the wound surface.[2] Finally, the wound will be closed.

The role of bacteria during wound treatment with MDT is unclear. Excretions and secretions do not have antibacterial properties, but perhaps there is an indirect antibacterial effect as a consequence of the interaction between maggots and/or excretions and secretions and humans. However, it is also possible that the wounds are able to heal despite bacterial colonization if the inhibition of complement activation and inflammatory response are sufficient to advance the process of wound healing.

Is it useful to apply antibiotics during MDT? This question cannot completely be answered, however some antibiotics, such as gentamicin and flucloxacillin, do have synergistic effects with excretions and secretions against *Staphylococcus aureus*. Moreover, antibiotics do not influence the viability of larvae.[4] During MDT there are no contra-indications for the use of antibiotics.

After all the cases that are described about the beneficial effects of maggots, why do they sometimes still fail in wound healing? For example, gram-

negative infected wounds are barely influenced by MDT and therefore, are a relatively contra-indication. A possible explanation for the failure of the treatment could be the toxic effects that some Gram-negative bacterial products have, e.g. the quorum-sensing regulated virulence factors in biofilms.[5] Their toxic products could interfere with the maggots and/or their excretions and secretions. Furthermore, in 2009, a randomized controlled trial has been published that found no difference in the healing rate of venous leg ulcers between maggots and hydrogel application, although it showed very effective debridement by larvae (debridement reached within 14 days) as compared to the hydrogel (debridement reached within 72 days).[6] These results cannot be explained with the current knowledge of basal mechanisms of action of MDT and the wound healing process, in which debridement is essential to progress from the inflammatory phase to the proliferative phase[7] and so, should increase the healing rate of the wound. The trial had unclear inclusion criteria, which could explain the contrary findings. For example, different sizes of wound areas and percentages of slough were included, which finally resulted in a median larger ulcer area in the larval group comparing to the hydrogel group and a quantity of debris that varied from 26% up to 100%. Patients with immune-related diseases and malignancies were not excluded from the trial, however these underlying diseases could significantly interfere with the process of wound healing. In conclusion, more in vivo research is needed to investigate the effect of maggots on the healing rate of wounds.

In this thesis, excretions and secretions from maggots proved to prevent, inhibit and break down bacterial biofilm formation on biomaterials. Probably, excretions and secretions disturb the structure of the biofilm matrix, because excretions and secretions do not possess direct bactericidal or bacteriostatic activity. Since biofilm-associated infections on prosthetic materials are difficult to treat, future research has to investigate the possibilities and in vivo efficacy e.g. to flush operation wounds before closure with sterile substance(s) from excretions and secretions to reduce the peri-operative risk of infection or to impregnate medical devices with the anti-biofilm substance(s) from excretions and secretions.

This thesis further shows for the first time that maggot excretions and secretions have pathway independent complement-inhibiting effects, probably caused by small molecular weight boiling stable proteins, which affect both healthy and post-operatively gained, immune-activated human sera.

Maggots are not the only symbiotic organisms that inhibit the human immune system. All symbionts in nature survive on their host by using immuno-suppressive strategies and sometimes antibacterial strategies. However, in humans, most symbionts are commensals or parasites, which do not benefit the human survival. Therefore, in these cases of colonization, humans will attempt to destroy the infestating organism. Consumption of raw herring, for example,

can cause intestinal infections by *Anisakis simplex*, the herring worm. This worm possesses complement-inhibiting properties to evade the human immune defense, although *Anisakis simplex* also excretes biochemical substances that harm the intestins.[8] Therefore, the human immune system evolutionary developed (undefined) strategies against this parasitic infection resulting in death of the herring worm in all immunocompetent patients. Other examples of natural complement inhibitors are *Borreliae* species, causing borreliosis (Lyme disease). These bacteria can evade the innate immune system by binding of a borrelial surface protein to complement factor H to escape AP activation and by binding to complement inhibitor C4b-binding protein to avoid CP activation.[9,10] However, *Borreliae* appear to have specific effects on the complement cascade which finally do not result in a decrease of the inflammatory response. Adversely, aggravated inflammation is observed during borrelial infection. In contrast to these natural (parasitic) complement-inhibitors, maggots do benefit humans and their mutualistic symbiosis results in non-specific complement inhibition by excretions and secretions and reduction of the inflammatory response.

Larval ES are the first pathway independent complement-inhibitors that are currently used in clinical practice. Up to date, there are very few complement-directed therapeutics available.[11] The complement drugs that are used, target one specific key in the cascade and only cover some rare diseases. Controlled therapeutic intervention in the complement system, especially at the core of complement activation (C3), could be a treatment for many ischemic, inflammatory and autoimmune diseases.[12,13] Scientists attempted to develop specific (synthetic) molecular drugs to inhibit the complement system, but the clinical success is limited, because of lack of potency and short half-lives.[11] Maggot excretions and secretions have a high potency as they are the first pathway independent complement-inhibitors that are already applied in humans and do not show any side-effects.

In conclusion, this thesis provides the first evidence that maggot excretions and secretions possess biofilm-reducing capacity on medical devices and complement-inhibiting capacity on the (activated) human complement system. Furthermore, the underlying mechanisms of action of MDT, especially caused by excretions and secretions, are partially elucidated by the results from this research. Possibly, excretions and secretions can replace the live maggots for wound treatment, as excretions and secretions proved to accelerate wound healing in various studies.[2,6,14] More in vitro and in vivo research into the specific effects of excretions and secretions is needed to examine this possibility, e.g. to determine their half-life time. Furthermore, the exact composition of excretions and secretions needs to be investigated to identify the excretions and secretions constituents responsible for the observed effects in this research. Following studies will further investigate the role of bacteria in the process of wound

healing during MDT and the biofilm-reducing and immunomodulatory effects by excretions and secretions. Finally, the principle aim of future research is to develop a new pharmacotherapeutic agent for the treatment of infectious and/or immune-mediated diseases, isolated from the excretions and secretions of *Lucilia sericata* larvae.

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