

# **BIS TIME management by medicinal larvae**

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**Original publication:**  
**TIME management  
by medicinal larvae**  
**Pritchard D.I., Cerovsky  
V., Nigam Y., Pickles S.F.,  
Cazander G., Nibbering P.H.,  
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**International Wound Journal.**  
**doi: 10.1111/iwj.12457,**  
**published online 15 July, 2015**

**The review “TIME  
management by medicinal  
larvae” describes the role  
of larval therapy in the  
context of wound bed  
preparation (WBP).**

Wound bed preparation is an integral part of the care program for chronic and hard to heal wounds. The acronym TIME describes four barriers to healing, which are removed when the wound bed is prepared for healing. **T** stands for tissue, namely dead tissue, **I** for infection or inflammation, respectively. **M** stands for moisture imbalance (too much or wrongly composed wound fluid) and **E** stands for edge, meaning the epithelial wound edge, which is not moving towards wound closure in chronic wounds.

The efficacy of larval therapy in regard to the removal of dead tissue and the associated biofilm (debridement) has been proven by clinical studies. In addition, pre-clinical studies and a long-standing, successful clinical use, involving a large number of patients, confirm the anti-infective and anti-inflammatory effect, which indirectly also leads to a normalisation of fluid imbalance. Moreover, larval therapy supports the formation of granulation tissue and wound closure.

Larval therapy has been successfully used for the treatment of chronic wounds for many years. Scientific interest in the mechanisms of action of larval therapy has led to intense research. One target of this research was to isolate active components, which might be used as pharmaceuticals. Thus, two enzymes were isolated and characterised which led to the laboratory production of these proteases, ***Lucilia sericata* Trypsin** and ***Lucilia sericata* Chymotrypsin** (insect specific proteases, ISP). They are primarily responsible for the degradation of dead tissue and debris (Debridement).

In addition, one important antibacterial substance, ***Lucifensin*** (*Lucilia sericata* Defensin), could be isolated and structurally characterised.

The structure as well as the action of the above molecules are depicted in detail in the review. However, taking into account the complex mixture of the larval secretions, it seems highly unlikely that isolated components have a clinical effect equivalent to that complex mixture. Moreover, larvae as living organisms have the possibility to adapt to their environment and produce those active substances, which are needed in a given situation. This is not possible with isolated substances.

In addition to the above active substances there are more components, which, although not fully identified and elucidated yet, contribute to the overall efficacy of larval therapy. The review details the mechanisms, which lead to improved wound healing, and the respective literature is listed. In the following, these mechanisms are briefly outlined. Also, “the four pillars” are summarised in table 1 and the clinical effect of larval therapy is depicted in figure 1.

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# T

## Tissue:

### Removal of dead tissue and debris (Debridement)

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Debridement encompasses the removal of dead tissue, including biofilm, slough and fibrinous material, and all kinds of foreign bodies, with the objective to improve wound healing. The efficacy of larval therapy in the removal of the so-called "bioburden" has been proven in clinical studies and constitutes the official indication for larval therapy.

In principle, two proteolytic enzymes, which are secreted into the wounds by larvae, are responsible for this effect: larval trypsin and larval chymotrypsin (Insect Specific Proteases, ISP). They are similar but not identical to human, endogenous trypsin and chymotrypsin, which is manifested in a different response to tissue proteinase inhibitors. This is why they are called ISP. Within the wound, the so-called extra-corporal digestion takes place. Proteins contained in wound slough and bioburden are diluted by the secreted enzymes and the resulting liquid is sucked up by the larvae, which use it as their nutrition. The pharmacological action – enzymatic digestion of dead tissue – is thus complemented by a physical action, the sucking-up of liquefied debris by the larvae.

One important effect of debridement is the removal of biofilm. It is often associated with slough and debris and may contribute to chronic infection and inflammation. The potential of larval secretions to remove biofilm has been proven. The already mentioned insect specific proteases (ISP) are responsible for this effect, as well as other enzymes contained in larval secretions, e.g. a specific nuclease.

# I

## Infection/inflammation:

### Reduction of infection and inflammation

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Debridement logically leads to a reduction of bioburden and thus reduces the risk of infection. However, several other mechanisms add to the anti-infective and anti-inflammatory effect of larval therapy. Firstly, an elevation of pH, which is caused by the presence of ammonium carbonate, allantoin and urea in larval secretions, leads to the reduction of bacterial counts in the wound. Secondly, specific antimicrobial substances, which are produced to defend themselves in a contaminated environment, have been found. This effect is inducible. One of these antimicrobial peptides (AMP), named Lucifensin, has been fully identified and produced in a laboratory. Another anti-fungally active peptide, named Lucimycin, has been found but not fully elucidated yet.

The anti-inflammatory effect of larval therapy, which can be observed in clinical practice, may be attributed to the reduction of bioburden. On the other hand, it is also being investigated if the complement system, which plays an important role in the regulation of inflammatory processes, is directly influenced by components of larval secretions. Moreover, there are scientific hints for a "direct" anti-inflammatory effect of larval therapy, in that the migration of inflammatory cells is enhanced and the secretion of pro-inflammatory cytokines is reduced.

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# M

## Moisture:

### Normalising of Wound Fluid

In chronic wounds, the moisture balance is disturbed. This relates to the amount as well as to the composition of wound fluid. Some barriers to healing, e.g. dead tissue **(T)**, infection and inflammation **(I)**, directly influence the moisture balance. The essential first step to restore moisture balance therefore is effective debridement.

Based on our current knowledge, larval therapy does not have a direct effect on the restoration of moisture balance, however, an indirect effect is attributed to effective debridement, anti-infective and anti-inflammatory properties, as described above.

# E

## Epithelial Edge:

### Wound Closure

Due to the chronicity of wounds, the function of cells necessary for wound healing is disturbed. For instance, fibroblasts, which are responsible for the production of intracellular matrix, or keratinocytes, in charge of the epithelial coverage of the wound, do not function properly. In-vitro investigations have shown, that the motility of those cells, as well as the secretion of wound healing factors such as VEGF, bFGF and HGF can be increased by larval therapy.

In summary, larval therapy can be said to have a positive impact on all four pillars of wound bed preparation, which are symbolised by the TIME acronym: By the effective removal of dead tissue/debridement **(T)**, an anti-infective and anti-inflammatory effect **(I)**, an indirect influence on moisture balance **(M)** as well as a stimulating effect on those cells which are responsible for wound closure **(E)**.

The comprehensive number of references, which lists all evidence cited in the above text, can be found in the original article.

**Figure 1.** An 81-year-old male patient with a venous leg ulcer, which has been in existence for several years, was treated with BioBags.

(a) Yellowish, sloughy wound before the onset of treatment



(b) The foam surrounding the wound protects the larvae when compression therapy is used.



(c) Fully granulated wound after 2 treatment cycles of 4 days each



**Table 1**

The impact of larval therapy (Larval Debridement Therapy, LDT) on the individual components of TIME, with indications of the active molecules responsible for these effects

<b>T</b>	<b>I</b>	<b>M</b>	<b>E</b>
<b>Tissue</b> (dead tissue)	<b>Infection/ Inflammation</b>	<b>Moisture</b> (too much or wrongly composed wound fluid)	<b>Edge</b> (wound margin, not leading to wound closure)
<b>Enzymatic effect of LDT</b>	<b>Multi-factorial effect of LDT</b>	<b>Impact on T and I leading to normalisation of moisture balance</b>	<b>Promotion of cellular activity which supports wound healing and closure</b>
<ol style="list-style-type: none"> <li>1. Removal of dead tissue thus reducing the bioburden</li> <li>2. Removal of biofilm</li> <li>3. Removal of tissue docking sites for bacteria</li> </ol> <p>Active molecules: - Serin proteinases - DNase - Glycosidases</p>	<ol style="list-style-type: none"> <li>1. Raising of wound pH</li> <li>2. Anti-bacterial and anti-fungal effects</li> </ol> <p>Active molecules: - Lucifensin - Lucimycin - Lucilin</p> <ol style="list-style-type: none"> <li>3. Inhibition of the complement system and support of anti-inflammatory cells</li> </ol> <p>Active molecules: - Lucilia hypodermins</p>	<ol style="list-style-type: none"> <li>1. Removal of dead tissue thus reducing the bioburden</li> <li>2. Anti-bacterial and anti-fungal effects</li> <li>3. Promotion of cell motility and angiogenesis (granulation)</li> <li>4. Inhibition of the complement system und support of anti-inflammatory cells</li> </ol>	<ol style="list-style-type: none"> <li>1. Promotion of cell motility and angiogenesis (granulation)</li> </ol> <p>Active molecules: - Serin proteinases - Amino acids</p> <p>LDT-induced growth factors (HGF)</p>

HGF: Hepatocyte Growth Factor

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## About the author



### Wilhelm (Willi) Jung, PhD

Willi's academic background is biochemistry. He has worked in several pharmaceutical and medicinal product-producing companies, in marketing as well as medical scientific positions. He spent most of his professional time, over 25 years, in the field of wound care, on an international basis. Willi has supported the foundation of the European Pressure Ulcer Advisory Panel (EPUAP) and the development of Wound Bed Preparation (WBP) as a clinical concept.

In 2008 he received a lifetime achievement award from the World Union of Wound Healing Societies (WUWHS), recognizing his contribution to the development of wound care as a speciality. During his last working years, Willi was committed to the scientific and clinical aspects of larval debridement therapy as a Director of Scientific Affairs at BioMonde.