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The risks of amputation: Methods currently in use to reduce these risks with a focus on larval therapy as a stimulant for wound healing – Critical Literature Review

Abdullah Arabe[1]; Amira Alrshood[1]; Salima Enaiba[2] and Adel Almangoush[2]
1- Ministry of Health Kingdom of Saudi Arabia.
2- Faculty of Medical Technology– Misurata – Libya

Abstract
Objectives: *the purpose of this literature review is to discuss the current literature surrounding wounds, the risks of amputation, and methods currently in use to reduce these risks with a focus on larval therapy as a stimulant for wound healing.*
Search methods: *The following resources were searched: the Cochrane Bone, Joint and Muscle Trauma Group and Cochrane Rehabilitation and Related Therapies Field specialised registers, the Cochrane Controlled Trials Register, The MEDLINE, EMBASE, and CINAHL.*
Data collection: *This review discusses current literature using research articles from within the last 10 years, these contain a mix of studies and reviews of research articles.*
Main results: *There is limited amount of research to definitively give an answer as to what treatment option is the best when it comes to chronic non-healing wounds, although there is some research into ulcers of the lower limb and foot.*

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Glossary
DFU – Diabetic Foot Ulcers
DM – Diabetes Mellitus
CLI – Critical Limb Ischaemia
LT – Larval Therapy
MDT – Maggot Debridement Therapy
ABPI – Ankle Brachial Pressure Index
LLU – Lower Limb Ulcers
PAD – Peripheral Arterial Disease
HGF - Hepatocyte Growth Factor
PA - Pseudomonas aeruginosa
SA - Staphylococcus aureus

I. Introduction and Relevance to Podiatry

Chronic non-healing wounds and ulcers are a problem that is getting more prevalent and burdensome (Cowan et al, 2013). There is an ageing population in the UK (Smith et al, 2017), easier access to fast food contributing to an increase in obesity and Type 2 Diabetes Mellitus (DM) (Swaney, J. 2017) diagnoses which bring with it increased risk factors. Although prevention in the first place is ideal there are circumstances where prevention is unavoidable and as a result it is important to look at different ways of treating ulcers and chronic wounds.

Lower Limb Ulcers (LLU) and diabetic foot ulcers and the ability of them to heal are of great relevance and importance to Podiatrists whose duty of care is to treat the foot and lower limb. *"Podiatrists are health care professionals who have been trained to prevent, diagnose, treat and rehabilitate abnormal conditions of the feet and lower limbs"* (NHS.co.uk, 2015). Many patients who are at risk of ulceration particularly of the lower limb have type 2 DM or some form of lower limb venous or arterial insufficiency (Tweedie, B. and Vig, S. 2014).

There are many factors that contribute to the formation of an ulcer, including venous or arterial insufficiency, prolonged pressure and/or diseases such as diabetes mellitus. These factors that contribute to the formation of ulcers also affect the ability of a wound to heal (Vowden, K. 2014). In-order for wounds to heal sufficiently they need to be able to go through the four main stages of healing with no disruptions: Haemostasis,

Inflammation, Proliferation and Maturation (Cowan et al, 2013). Acute wounds generally take one – three weeks to heal by going through this process and anything that takes longer than this may be classified as chronic (Tweedie, B. and Vig, S. 2014).

Chronic wounds tend to become 'stuck' in the inflammatory stage which can be due to the formation of a bacterial biofilm (Nishijima et al, 2017). When a biofilm begins to form it can attract other bacteria to expand into a diverse species of bacteria that have numerous defences (Cowan et al, 2013). Biofilms can be successful in defending themselves against antibiotics or other skin cleansing products, which leads to delayed healing as the wound is unable to progress to the proliferation stage (Nishijima et al, 2017).

A wound that is sloughy and necrotic or has evidence of biofilms present will need to be debrided before successful healing can start (Wilasrusmee et al, 2014). Larval therapy (LT) or Maggot debridement therapy (MDT) has been used throughout history as a debridement technique for chronic wounds/ulcers, after being discovered 'accidentally' by American Civil War surgeon Dr John F. Zacharias, however since the introduction of antibiotics and the 'yuck factor' now associated with maggots, the use has diminished since the 19th Century (Cowan et al, 2013).

Larval therapy is the use of sterile green bottlefly larvae (Lucilia sericata) to remove slough and dead tissue from the surface of wounds (Stang, D., and O'Brien, J.J., 2014). The larvae can be applied free-range with a dressing in place to prevent them escaping, or within a biobag (Tyrer, J. 2014). LT is widely used as a last resort (Vowden, K., 2014) when the threat of amputation is already a possibility, especially in diabetic patients or those with Peripheral Arterial Disease (Tweedie, B. and Vig, S., 2014). However, it has shown to be very effective, especially in wounds that have a heavy bacterial load (Cowan et al, 2013) and there are strong indications that the proteolytic secretions made by the larvae assist with the healing process. There is no harm to the patient as maggots do not excrete anything harmful into the wound, and only remove necrotic materials or bacteria (DellAntonio, J. 2017).

Currently there is very little evidence to support one method of treating chronic wounds and ulcers over another (Bennett et al, 2013), therefore the purpose of this literature review is to discuss the current literature surrounding wounds, the risks of amputation, and methods currently in use to reduce these risks with a focus on larval therapy as a stimulant for wound healing.

II. Literature Review

Many studies have reported that Larval Therapy (LT)/Maggot Debridement Therapy (MDT) has assisted with wound healing as well as removing necrotic tissue, however, the sole focus of these studies has generally been 'time to debridement, with comparisons to other debridement techniques'. This review discusses current literature using research articles from within the last 10 years, these contain a mix of studies and reviews of research articles.

Chronic Wounds and the Costs

Chronic wounds, as well as being hugely debilitating and painful to patients, are costly to the NHS, with an estimated £2.3-£3.1 billion spent annually on treatment and rehabilitation (Bennett et al, 2013) therefore there is a strong need for quick, safe, and cost-effective treatments. In 2013, Bennett et al created a model based on previous literature in-order to provide an analysis of the cost-effectiveness of chronic wound debridement using Larval Therapy (LT) as a comparator against: mechanical, hydrogel, honey, surgical, sharp and hydro-surgical debridement. Not all these forms of debridement are appropriate for all patients, therefore they expressed that if one method of debridement was unsuccessful patients may have been moved on to another method the next month or requested surgical intervention at this point (Bennett et al, 2013). The analysis concluded that LT may be a more dominant intervention compared with the other treatments in terms of cost savings (Bennett et al, 2013), however the analysis was done using retrospective literature rather than a current randomised controlled trial. The research was funded in full by BioMonde who are a manufacturer of larval debridement therapies, therefore it is unclear how much bias there may be involved with the conclusions. This analysis was conducted in 2013; it may be more beneficial to do an up to date randomised controlled trial with groups, randomisation and controlled variables in-order to gain more reliable statistics and through an independent funding source.

The UK isn't the only place that chronic wounds are a significant health problem, with the USA estimating in 2013 a $20 billion cost annually for the treatment and management of chronic wounds (Cowan et al, 2013). One of the key factors that is contributing to this increase in non-healing is the build-up of bacterial biofilms that disrupt the healing processes; these biofilms require debridement to allow the normal stages of healing to continue successfully within the wound. In-Vitro experiments using pigskins were conducted by Cowan et al (2013) to evaluate the effectiveness of LT as a debridement technique to remove biofilms.

Three pigskin explants were inoculated with Pseudomonas aeruginosa (PA) and another three with Staphylococcus aureus (SA35556) bacteria and maintained on soft agar for three days in-order to model a

biofilm environment. Each were given an antibiotic wash treatment and the bacterial colonies measured before and after. Larvae were then introduced to the pigskins and the bacterial colonies measured again after 24 and 48 hours. Both the PA and SA pigskins were tolerant to the antibacterial wash treatment after 24 hours and had bacterial counts of 3.4 million in the PA pigskins with 300,000 within the biofilm and 11.3 million in the SA pigskins with 430,000 being in the biofilms before the introduction of maggots. After 24 hours with larval therapy both counts had dropped to 1.7 million and 3.3 million respectively, however after another 24 hours the bacterial load on both was down to 0 indicating that the larval therapy had completely removed the biofilms and bacterial loads (Cowan et al, 2013).

The bacterial loads were completely removed within 48 hours indicating larval therapy to be a hugely successful and quick treatment, as well as Cowan et al (2013) specifically commenting that the larvae did not cause any damage to the pigskins or each other therefore it is also safe. However, the trial was conducted using animal hosts therefore there is no guarantee that the same experiment in human hosts would be as successful; the researchers identified this as a limitation to the study themselves and that plans were in place to conduct the study again using human hosts.

One main group of patients that are especially at risk of chronic wounds and non-healing ulcers are those with Diabetes Mellitus (DM) and as a result there have been studies that have specifically looked at the use of larval therapy in Diabetic Foot Ulcers (DFU). A retrospective study conducted by Wilasrusmee et al in 2014 aimed to discuss the effectiveness of larval therapy in comparison with hydrogel dressings by reviewing past medical records of DFU patients treated at the Bang Yai Hospital, Thailand between January 2008 and December 2009. Only patients who met the following criteria were included in the study: one single wound on the foot, able to walk without assistance, data that was available for 6 months post treatment, and patients that did not have gangrenous wounds, necrotising fasciitis, an abscess or osteomyelitis (Wilasrusmee et al, 2014). One hundred and eleven patients were included in the cohort with 59 receiving treatment with maggots and 52 with normal saline or hydrogel dressings. Wounds with an area of over $28cm^2$ took longer to heal (15 weeks) than those smaller than $28cm^2$ (9 weeks), and those who had a duration of less than 20 days were found to heal quicker than those of over 20-day duration. The maggot treated group had a statistically significant lower time to healing than the control group ($P<0.001$) with an average time of 9 weeks compared with 29 weeks. Mean ages were similar in both groups and HbA1c levels were also similar at baseline, however those in the maggot therapy group had smaller sized wounds than those in the control group and had, had the ulcers for a shorter duration. There were also less participants with an abnormal ABPI in the maggot treated group than the control group (Wilasrusmee et al, 2014).

These differences in wound size, duration and ABPI within the groups may contribute to the results of the study. There does not appear to be any reason suggested in the study as to why this was, and as patient files were chosen retrospectively it is unclear how and why each patient was selected for the type of treatment by the original practitioners.

Use of Larval Therapy in Vascular wounds

Vascular LLUs can either be venous or arterial in aetiology with ischaemia being caused by microvascular and/or macrovascular disease (Tweedie, B. and Vig, S., 2014). Venous ulcers are generally surrounded by oedema, have a redness to them and can be quite moist. This is due to an insufficient venous return, therefore there is a build-up of toxic substances that lead to a breakdown in the tissues. They require compression therapy to help them heal as well as suitable dressing methods (Ricci, E and Chadwick, P. 2014). With arterial wounds there is generally a blockage in an artery that is impeding the ability of oxygen and nutrient rich blood to reach the lower limb. As a result there is a breakdown of tissues and this can lead to open wounds which are then at risk of infection (Tweedie, B. and Vig, S., 2014). Factors that contribute to Peripheral Arterial Disease (PAD) are being overweight, diabetic, smoking and those with cardiovascular problem such as hypertension and high cholesterol (Tweedie, B. and Vig, S., 2014).

A full vascular assessment is required before treatment can be initiated for a patient as the results will determine the treatment that can be used (Vowden, K. 2014) for example patients with Critical Limb Ischaemia (CLI) are generally not advised to undertake Maggot Debridement Therapy as the insufficient blood flow to the area is usually the cause of the wounds inability to heal (Nishijima et al, 2017).

However, a study was conducted in mid-foot amputation patients after angioplasty to assess whether LT can assist wound healing for improved post-amputation survival compared with a conventional therapy (Nishijima et al, 2017). The study was conducted between January 2013 and October 2016 and patients were CLI patients whose wounds were not clean enough to close post operation. All patients had ABPI, Skin Perfusion Pressure and blood tests taken before treatment was determined. Of the 39 patients included in the study, 7 were treated with LT and the other 32 were treated with a conventional therapy (ointment applied daily after cleansing), however patients who received LT were those where conventional therapy had failed.

Nishijima et al (2017) concluded that although wound healing that avoided any further amputations was higher in the LT group, there was no statistically significant differences between the LT group and the

control group other than a higher blood albumin result in the LT group. It is probable that the large difference in samples in the groups is the reason for this statistical result, as wound healing itself appears to have been quicker in the LT group when looking at individual cases.

It is important to note that all patients within this study had undergone a revascularisation procedure before amputation (Nishijima et al, 2017), therefore although there is some success in treating CLI patients with LT in these cases, it won't always be suitable for all future CLI patients. More research appears to be required into this before any definite answers can be given.

The ideal situation for a patient with PAD or CLI is that any wounds that present can be fully healed and the patient able to continue with a good quality of life, however there are situations where there is a need for amputation (Breeze, K. 2016). In severe cases like this, larval therapy is less likely to be a method of treatment as even if the necrotic tissue is removed by the larvae there is insufficient blood to the area to allow normal healing. However, those who have already had an amputation are more at risk or further amputation being needed (Breeze, K. 2016), this could be as a result of the surgery site getting infected, continued pressure on the area or a deterioration in the patient's condition (Nishijima et al, 2017). Especially for infected post-surgical sites larval therapy could be a good way of preventing the need for further amputation.

Katy Breeze (2016) discusses a patient case study where maggot therapy had a favourable outcome for a 77 year old male patient who had already had a below-knee amputation and was at risk of a further amputation after a fall onto the surgical site that resulted in exposed bone. The patient was informed by his vascular consultant that there was most likely a need for an above-knee amputation due to the extent of the infection present and the size of the two wounds. However, he was referred to a community nurse setting for regular wound dressings and recommended the use of larval therapy by a practitioner, which he agreed to. Two applications of larvae were applied to the larger wound, and only one to the smaller of the two wounds. After these applications there was a marked improvement in the healing of the wounds, and the end decision was that no further amputation was needed (Breeze, K, 2016). For this patient this was a positive outcome, however there is no further data available to determine whether the patient ended up with any further amputation at a later date. One-off case studies are useful in demonstrating situations such as mentioned above, however there is no statistical analysis to back up the findings which is essentially what is needed for research to be considered valid and reliable.

Larval Therapy as a Debridement Technique in Diabetic Foot Ulcers

As previously mentioned one major predicator of foot wounds is diabetes, with diabetic patients having a 12-25% lifetime risk of developing a foot ulcer (Tian et al, 2013). Neuropathy is usually a main factor in DFUs as patients are unable to detect any damage done to the foot due to the reduced pain and sensation (Haycocks, S. and Chadwick, P. 2012). Debridement is generally considered to be an essential part of wound management as a wound is less likely to be able to heal if there is an excess of necrotic tissue and exudate (Haycocks, S. and Chadwick, P. 2012). The vascular status of the patient and the site of the wound will determine what kind of debridement is considered to be most effective. In these cases larval therapy can be used as a quick solution to debridement, however there is the added complications of wounds existing on pressure areas which may not be ideal site for the larvae to thrive successfully (Tyrer, J. 2014).

In 2013 Tian et al conducted a meta-analysis of maggot therapy as a debridement technique for diabetic foot ulcers using seven databases such as PubMed, Web of Science and the Cochrane Library to search for studies. They found 4 studies that met their criteria and had a total of 356 patients from these studies, 180 of which were treated with MDT and 176 with a standard treatment. This standard treatment is not specified within this article therefore it is unclear exactly what the treatment consisted of and whether or not all those patients were treated using the same method. The analysis concluded that there was a lack of consistency between the articles and that overall there was no statistical differences to suggest that MDT or the standard treatment was better over the other. However, they did have a greater proportion of patients in the MDT group achieve complete healing, and suggest that MDT reduces the time to healing and reduces the rate of amputation (Tian et al, 2013).

Diabetic Foot Ulcers were estimated to cost the NHS £17 million in 1994 (Tian et al, 2013), and as the prevalence of diabetes has become greater, there can be an assumption that this figure has significantly inflated in the last 20 years. Therefore, finding a cost-effective treatment as well as quicker time to healing is in the interests of both the NHS and for the quality of life of patients.

Use of Larval Therapy to Promote and Initiate Wound Healing

As mentioned previously larval therapy has long been used as a debridement technique in order to remove necrotic tissue and excess exudate (Arabloo et al, 2015), and although references to wound healing have often been made, there does not appear to be any specific trials conducted looking at wound healing in patients other than those who are in an 'at-risk' category.

Although LLUs generally occur as a result of the conditions mentioned previously such as venous or arterial insufficiency, diabetes and other systemic diseases (Haycocks, S. and Chadwick, P. 2012); there are wounds that occur on the lower limb that are not as a result of these factors. It is clear that ABPI, HbA1c and size/duration of wounds are important factors to healing (Wilarusmee et al, 2014) and as such those with results classified as normal can be assumed to heal quicker than those with abnormal results (Nishijima et al, 2017).

Maggots ingest the necrotic tissue found in wounds, but they also excrete enzymes that have antimicrobial factors that help fight infection (Nigam, Y., and Morgan, C., 2015) as well as promoting the formulation of healthy granulation tissue through encouraging the release of Hepatocyte Growth Factor (HGF) (Nishijima et al, 2017). Many practitioners choose to withdraw the larval therapy once the wound begins to enter the final stages of the healing process, favouring a cheaper dressing alternative (Nigam, Y., and Morgan, C., 2015); and although there are contra-indications to using larval therapy, for example, wounds that have blind channels leading to internal organs, or necrosis around major blood vessels (Cowan et al, 2013) there is much evidence to suggest that larval therapy could reduce healing time in all chronic wounds, not just DFUs or venous/arterial wounds.

III. Discussion

It is evident that there is a significant cost to the NHS in the UK from chronic wounds (Abbade, E.B. 2017), whether they are lower limb ulcers, diabetic foot ulcers, or trauma induced wounds with delayed healing. There are many factors that influence the healing ability of these wounds such as the health of the patient both physically and psychologically (Dumville,C. 2009). Physical factors such as cardiovascular disease, peripheral arterial disease, diabetes and even previous treatments can all influence the ability of a wound to heal (Cowan et al, 2013). Larval therapy has proven to be effective at removing necrotic tissue and excess exudate quickly as well as stimulating wound healing through the excretion of enzymes that initiate normal healing factors within wounds (Arabloo et al, 2015). However, there are many studies that reference time to debridement of larval therapy but not many that look specifically at how larval therapy can initiate wound healing and reduce time to complete healing (Nigam, Y. and Morgan, C. 2015).

Bennet et al (2013) estimated with their analysis that larval therapy is a cost-effective method of debridement when compared with other debridement techniques, however, the analysis was done using a model that was based on previous literature that is quite possibly out of date now. It was also funded in full by BioMonde who are a worldwide manufacturer of larvae for larval debridement therapy which indicates some level of bias may be present. A newer randomised controlled trial would be beneficial in order to fully evaluate the cost effectiveness both short term and long term of larval therapy compared with other treatment types

Cowan et al (2013) were able to identify the effectiveness of the use of larvae against biofilms present in two types of bacteria (PA and SA) and had complete removal of bacterial loads within 48 hours. However, the studies were conducted using In-Vitro pigskins which does not guarantee the same success in a human host, although future trials were mentioned there are potential ethical complications surrounding these. A retrospective study was able to identify that size of wound, duration and factors such as ABPI and HbA1c results were influential in the healing time of wounds (Wilasrusmee et al, 2014). However, within this study many patients were excluded for not meeting the criteria which meant that only a small sample of participants were analysed. Patients who had necrotising fasciitis, an abscess or osteomyelitis were excluded from the study, however these patients may have had a positive outcome from treatment with LT but this information is not included. The conclusion was that LT had a statistically significant time to healing than those who had dressings with saline or hydrogel, however this may have been influenced greatly by the reduced size and duration of the wounds in this group (Wilasrusmee et al, 2014). Those who were treated with saline or hydrogel dressings generally had a poorer ABPI score than those in the LT group which again has a large influence on the healing capabilities. There are suggested contra-indications to the use of LT (Cowan et al, 2013) however there is no mention of this being the reason for the group not being treated with LT, as well as no random sampling with the study being retrospective.

CLI patients are generally not advised to undertake LT however a study by Nishijima et al (2017) looked at LT on the healing rate of patients who had already had a mid-foot amputation after angioplasty. The wounds were not considered to be clean enough to be closed therefore they needed some form of treatment before a skin graft could be applied. Only 7 of the 39 patients were treated with LT and it was only used after conventional application of ointment had failed, therefore it wasn't a controlled trial, and as such results may have been affected by unknown confounding variables. Therefore, it may be worth future trials being conducted specifically regarding the effects of LT on healing of patients post amputation that have had successful angioplasty, with controlled variables determined.

Individual case studies appear to be in favour of LT as a successful treatment to reduce the risk of further amputations, however they do not have the statistical significance required to fully be considered valid and reliable research. A future meta-analysis of all these case studies may be useful, by grouping similar wound

types, durations and health of patients together to determine any differences, however this would be a time-consuming process and not necessarily of huge benefit other than to see if any patterns occur.

Diabetic foot wounds have had successful outcomes with LT, however there are difficulties when applying larvae to the areas as many are pressure areas that may have a detrimental effect on the ability of the larvae to debride the area, resulting in death of larvae (Tyrer, J. 2014). However, due to the costs involved with the management and treatment of DFUs more research is required to determine if there is any benefit to LT treatments being started earlier in the management plan, rather than waiting until the risk of amputation is there (Nigam, Y. and Morgan, C. 2015).

IV. Conclusion

Since the first accidental finding by American Civil War surgeon Dr John F. Zacharias during the war of the 'cleaning' properties maggots can have in wounds (Cowan et al, 2013), LT became a popular method for wound cleaning and stimulation of healing. However, with the introduction of antibiotics this practice was reduced and a more disgusted attitude towards maggots became apparent (DellAntonio, J. 2017). There is limited amount of research to definitively give an answer as to what treatment option is the best when it comes to chronic non-healing wounds, although there is some research into ulcers of the lower limb and foot. Many studies have found that LT is a useful debridement technique and that it promotes healing, however there is limited statistically significant results that determine if LT is better when compared to other debridement techniques and also to other dressing types. It has been found that LT is very successful at removing bacterial biofilms (Cowan et al, 2013) which can be a major factor as to why a wound is not healing.

It is evident that there is room for more research to be done into the area of larval therapy and wound healing.

V. Recommendations

There is a justification for future studies that use LT earlier in treatment as a wound healing factor, not solely for debridement and as a method of reducing the risk of amputation. Therefore, some more specific research questions that could be utilised for future studies are outlined below:

Research Questions

1) What is the optimum time a larval dressing should be kept on for, to result in the quickest and most effective healing time, and should it only be used in cases of infection? – Many practitioners recommend keeping dressings on for 4 days, but what if they were to be kept on longer? Would there be positive effects on patients healing times i.e. quicker healing times? Reduction in infection risks?

2) Are larval therapy dressings more effective in assisting the wound healing process compared with other dressings? – Honey, Inadine, Aquacel etc. A larger RCT is required perhaps with more health professionals involved in order to devise a standardised treatment method.

3) Can larval therapy be considered earlier in the treatment of non-healing ulcers that are at risk of amputation? – Cost effectiveness - not just in diabetic or PAD patients, but for trauma induced wounds.

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