RESEARCH PAPER

The antimicrobial activity of maggots: in-vivo results

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In the literature maggot therapy is discussed as a promising and potent form of debridement therapy. The number of maggots needed to debride a wound is estimated at 10 per cm^2, and more in case of a higher percentage of necrosis or slough. In the authors' hospital, from March 1999 to May 2002, 16 patients were successfully treated with maggot therapy. The average maggot treatment time was 27 days, with an average of seven maggot changes. Most patients were treated for osteomyelitis, with trauma being the leading aetiological factor. In accordance with in-vitro findings, maggot therapy was found to be more effective in Gram-positive infected wounds. Gram-negative bacteria are cultured more often after maggot treatment than before it (p=0.001). The opposite effect was found for Gram-positive infected wounds (non-significant p=0.07). In vivo maggots seem to be less effective against Gram-negative infected wounds. The authors believe that a higher number of maggots is needed not only for a larger wound or a wound with a higher percentage covered with slough, but also for a wound infected with Gram-negative bacteria.

Key words: maggot, wound, clinical, in vivo, bacteria

The beneficial effects of maggots have been known for hundreds of years[1], however, maggots were only introduced to clinical practice in the late 1920s[2]. Maggots were used extensively in hospitals during the 1930s and 1940s, especially in the United States. Problems with non-sterility of maggots, with consequent tetanus and infection, together with the introduction of penicillin in the 1940s and better aseptic wound dressings, almost completely removed maggots from the therapeutic arsenal[3,4]. It was in the 1980s with the appearance of antibiotic-resistant bacteria, like methicillin-resistant *Staphylococcus aureus* (MRSA), that maggots made a comeback in the hospital setting[5–7]. Clinical observations indicate that maggot therapy accelerates cleansing, combats infection, hastens the removal of necrotic tissue without damaging the healthy tissue beneath and can prevent (further) amputation[8]. Secretions of larvae of the common greenbottle (*Lucilia sericata*) have, in vitro, been shown to be most effective against Gram-positive bacteria, like streptococcus A and B and *Staph. aureus*. Gram-negative bacteria, especially *Escherichia coli* and *Proteus* spp., and to a lesser extent *Pseudomonas* spp., are more resistant to maggot secretions[9,10].

This article reports the in-vivo results of the use of maggots (*Lucilia sericata*) to treat Gram-positive and Gram-negative infected wounds.

Material and methods

The protocol for maggot treatment in the authors' hospital requires a wound swab of every treated wound on every maggot change. A swab is sent for culture (using Stuart medium) for aerobic and anaerobic organisms. Because all maggots in the hospital are sterile before application to the wound, new emerging bacteria in the wounds do not result from the application of the maggots. Antibiotic therapy is given when there are signs of systemic infection, which is always directed at the cultured micro-organism. Wound cultures are always taken as a superficial wound swab and never as a deep tissue biopsy culture. Although microbiological assessment of chronic diabetic patients is probably more sensitive[11], the (sometimes small) size of the wounds and the need to sedate non-diabetic patients for deep tissue cultures stopped the authors from using deep tissue biopsies.

An analysis of all wound cultures taken 1 month before, during the whole maggot treatment period, and 1 month after treatment with maggots was undertaken. A wound culture can either be sterile, show growth of a Gram-positive or a Gram-negative bacteria, or both. If, for example, before maggot treatment three wound cultures were taken and two of these showed a Gram-positive bacteria, the chance of culturing a Gram-positive bacteria is 0.66 (see *Table 1*, patient 1). These wound cultures were then analysed for Gram-positive (*Table 1*) and Gram-negative bacteria (*Table 2*) for 16 consecutive patients treated with maggots in the hospital from March 1999 until May 2002.

The data were analysed using Spearman's rho, which is a measure of association between two variables measured on at least an ordinal scale. An association of p=0.05 was considered a significant effect.

Results

Maggot therapy was first used in the Netherlands in the authors' hospital in 1999. Between then and May 2002, 16 patients have been successfully treated with maggots (*Table 3*). All wounds eventually responded to the therapy and healed within 6 months of commencing maggot therapy. Three patients died, one as a result of an accident, and two as a result of underlying disease (progression of cancer and a haematological disorder). There were two methods for applying maggots to the wound. Initially, with the first three patients, maggots were put freely on the wound covered by a net, but after 3–4 days, when maggots grow up to 8–10 mm, the treatment can become painful. Therefore the last 13 patients of this group were treated with the biobag technique. The larvae are incorporated in a small polyvinylalcohol (PVA) biobag (Polymedics Bioproducts,

TABLE 1.
The chance of culturing a Gram-positive bacteria

Patient no.	Before maggots (1 month)	Maggot therapy	After maggots (1 month)
1	0.66 (3)	0.62 (13)	0.38 (13)
2	0.8 (5)	1 (2)	1 (1)
3	–	1 (3)	1 (4)
4	0.5 (2)	0.3 (23)	0 (7)
5	0.75 (8)	0 (8)	0.66 (3)
6	0 (1)	0 (3)	–
7	0 (1)	0.2 (10)	0.2 (5)
8	2 (1)	0.5 (4)	0 (1)
9	1 (2)	0.33 (15)	0 (1)
10	0.6 (5)	0.1 (29)	2 (1)
11	0 (4)	0 (9)	–
12	0 (2)	0.17 (6)	1.25 (4)
13	0.55 (11)	0.33 (9)	–
14	0.8 (5)	0.1 (10)	0 (5)
15	1 (2)	1.5 (2)	–
16	0 (4)	0 (13)	0 (2)
Median	0.66	0.20	0.20
Average	0.54	0.36*	0.41

*Non-significant Spearman's rho (p=0.07); figure in brackets is the number of wound cultures; – = missing value. Sometimes in one wound culture more than one bacterial species can be found. The average number of cultured bacterial species can therefore be higher than one

TABLE 2.
The chance of culturing a Gram-negative bacteria

Patient no.	Before maggots (1 month)	Maggot therapy	After maggots (1 month)
1	1 (3)	1.38 (13)	1.53 (13)
2	0.2 (5)	0.5 (2)	0 (1)
3	–	0 (3)	0 (4)
4	0 (2)	0 (23)	0.14 (7)
5	0.38 (8)	1.25 (8)	0.33 (3)
6	0 (1)	0 (3)	–
7	1 (1)	0.9 (10)	1 (5)
8	0 (1)	0 (4)	0 (1)
9	0 (2)	0.6 (15)	2 (1)
10	0.8 (5)	1.38 (29)	1 (1)
11	0 (4)	0.77 (9)	–
12	0 (2)	0 (6)	0 (4)
13	0 (11)	0.11 (9)	–
14	1 (5)	0.9 (10)	0.4 (5)
15	0 (2)	0 (2)	–
16	0.25 (4)	0.38 (13)	0 (2)
Median	0.25	0.60	0.33
Average	0.29	0.51*	0.4

*Significant Spearman's rho (p=0.001); figure in brackets is the number of wound cultures; – = missing value. Sometimes in one wound culture more than one bacterial species can be found. The average number of cultured bacterial species can therefore be higher than one

Peer, Belgium). Incorporated in this bag they still act as necrophages, but this is less painful.

Average treatment time with maggots was 27 days (range 12–83 days), with an average of seven dressings applied (range 3–21 dressings). In total almost 15 000 maggots were used (average per patient 925 maggots, range 100–2900). Most patients were treated for osteomyelitis (*Table 3*). Trauma was the cause in 50% of the cases,

TABLE 3.
Characteristics of patients treated with sterile maggots

No.	Sex	Age (yrs)	Diagnosis	Region	Underlying condition(s)	Period of maggot therapy (days)	Dressing used*	Total number of maggots applied	Number of maggot changes
1	M	50	Osteomyelitis	Lower leg	Vascular insufficiency	32	Biobag	800	9
2	M	60	Osteomyelitis	Knee joint	Vascular/DM	12	Net	1000	4
3	M	41	Osteomyelitis	Both feet	Trauma	28	Net	2900	7
4	M	81	Osteomyelitis	Femur	Trauma/steroid/DM/ vascular insufficiency	28	Biobag	550	8
5	F	62	Osteomyelitis	Lower leg	Trauma/vascular	20	Biobag	360	6
6	M	70	Osteomyelitis	Lower leg	Trauma/DM	25	Biobag	260	6
7	M	33	Osteomyelitis	Lower leg	Trauma	37	Biobag	500	10
8	M	59	Osteomyelitis	Elbow	Trauma	24	Biobag	240	6
9	M	38	Osteomyelitis	Heel	DM	83	Biobag	780	21
10	M	50	Fasciitis necroticans	Neck and head	RA/trauma	13	Biobag	560	4
11	M	46	Fasciitis necroticans	Abdomen and perineal region	Scrotal abscess	19	Biobag	1200	5
12	F	88	Soft tissue infection	Upper leg	Trauma	27	Biobag	450	8
13	M	51	Soft tissue infection	Upper leg	Trauma/vascular insufficiency	13	Biobag	100	4
14	M	54	Gangrene	Stump lower limb	Vascular insufficiency/DM	11	Net	2000	3
15	M	16	Gangrene	Both hands and feet	Meningococcal sepsis	27	Biobag	2100	8
16	M	61	Ulcus cruris	Lower leg	Venous insufficiency/ DM/RA/steroid treatment	34	Biobag	1000	10
Average		54				27		925	7

DM = diabetes mellitus; F = female; M = male; RA = rheumatoid arthritis; * net = a loose nylon mesh wound dressing over free maggots; biobags = porous, polyvinylalcohol bags containing maggots followed by diabetes mellitus (38%), arterial vascular insufficiency (38%), rheumatoid arthritis (13%), steroid use (13%), fasciitis necroticans (13%), venous insufficiency (6%) and meningococcal sepsis (6%).

In *Table 1* the result for Gram-positive cultures are presented. Gram-positive bacteria are cultured less often after maggot treatment than before. Using Spearman's rho this is a non-significant effect (p=0.07). Gram-negative bacteria (*Table 2*), on the other hand, are cultured more often after maggot treatment than before (p=0.001).

Discussion

It is still not clear how maggot therapy works. It is probably more complicated than the mere washing out of bacteria by the serous exudate or than the simple crawling of the larvae in the wound. 'Maggots move over the surface of the wound, secreting proteolytic enzymes that break down dead tissue, turning it into a soup, which they then ingest'[12]. Maggots are capable of destroying bacteria in their alimentary tract. They also produce substances with healing properties, such as allantoin and urea. There is also a change in the wound pH, from acidic to alkaline, as a result of the ammonia and calcium carbonate excreted by the maggots[13].

In the 1930s Robinson and Norwood were able to show that Gram-positive bacteria (β-haemolytic *Streptococcus* and *Staph. aureus*) are ingested and killed completely as they pass through the gut of the larvae[14,15]. More recently the direct killing of Gram-negative bacteria (*E. coli*) by maggots was studied. Most of the bacteria were killed, but 17.8% of the hindgut still harboured live bacteria[16]. In vitro, maggot secretions were found to adequately kill Gram-positive bacteria but Gram-negative bacteria were killed less effectively[10]. Gram-negative bacteria appeared to grow faster in the presence of maggots, possibly as a result of an increase in the pH of the wound.

This retrospective study showed that the chance of culturing a Gram-positive bacteria is higher before than after treatment with maggot therapy (p=0.07), and found the opposite effect for Gram-negative cultures (p=0.001). Looking at a subgroup of these 16 patients, namely the four patients in which the chance of culturing a Gram-negative bacteria after treatment with maggots increases (patient 1, 4, 9 and 12), shows an interesting effect. The only difference between this subgroup and the other 12 patients is that fewer maggots were applied (645 in the subgroup *vs* 1020 in the other group). Looking at another subgroup, namely the patients who were treated with a minimum of 1000 maggots in total (patients 2, 3, 11, 14, 15 and 16), the chance of culturing a Gram-negative bacteria decreased after treatment with maggots.

The number of maggots needed to debride a wound is estimated at 10 larvae per cm^2 of wound, but there seems to be no maximum number of larvae per cm^2 of wound[17]. Special calculators have been developed to calculate the number of maggots needed to debride a wound, based on size and percentage of wound area covered with slough[18]. In accordance with in-vitro findings[10,14–16], maggot therapy appears to be more effective against Gram-positive bacteria. Reasons for faster growing of Gram-negative bacteria during maggot treatment could be because of a result of an increase in the pH of the growth medium. Another reason could be that endotoxins produced by Gram-negative bacteria are capable of destroying secretions produced by maggots.

Although the methodological limitations of the present open label, non-comparative cohort study precludes a definite conclusion concerning the effectiveness of maggots against Gram-positive and Gram-negative infected wounds, the authors believe that, for these patients, Gram-positive bacteria are digested and killed more easily than Gram-negative bacteria. The authors believe that a higher number of maggots is not only needed for a larger wound, or for a wound covered with a higher percentage of slough, but also for a Gram-negative infected wound. A limitation of the present study was that all patients who were septic or had a severe wound infection were treated with antibiotics directed at the causative agent which would probably have influenced the subsequent cultures.

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