IWJ	iwj_12637	B	Dispatch: June 28, 2016	Journal: IWJ	CE:
Journal Name	Manuscript No.		Author Received:	No of pages: 4	TS: suresh

International Wound Journal ISSN 1742-4801

ORIGINAL ARTICLE

Viability of *Lucilia sericata* maggots after exposure to wound antiseptics

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Key words
Lucilia sericata; Maggot therapy; Bio-surgery; Antisepsis; Disinfection; Mobility; Bacterial ingestion; Wound care

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Daeschlein G, Napp M, Assadian O, von Podewils S, Reese K, Hinz P, Matiasek J, Spitzmueller R, Humphreys P, Jünger M, Kramer A. Viability of *Lucilia sericata* maggots after exposure to wound antiseptics. Int Wound J 2016; doi: 10.1111/iwj.12637

Abstract

After debridement and before dressing a wound with maggots of calliphorid flies, one frequently performed step is the application of antiseptics to the prepared wound bed. However, the concomitant application of antiseptic agents during maggot therapy is regarded controversial as antiseptics may interfere with maggots' viability. In this experimental in vitro study, the viability of fly maggots was investigated after exposure to various antiseptics frequently used in wound care. Here, we show that *Lucilia sericata* fly maggots can survive up to an hour's exposure to wound antiseptics such as octenidine, povidone-iodine or polihexanide. Concomitant short-term application of wound antiseptics together with maggots on wound beds is tolerated by larvae and does not impair their viability.

Introduction

Bio-surgical debridement by maggots of calliphorid flies is an accepted method in the therapy of non-healing chronic wounds. Application of fly maggots for wound bed preparation was first introduced in the 1920s (1) and was intermittently abandoned in the 1940s (2,3) after the introduction of improved surgical procedures, together with the discovery of penicillin. In 1988, this method was re-introduced to wound care (4–7) and gained attention among wound care managers because of the favourable non-surgical debridement results and the maggots' ability to eliminate bacteria in an order of > 4 $\log_{10}$ within 48 hours (8). Today, usually, 50–400 free larvae are used for 1–2 days, while larvae sealed in bio-bags may be left on the wound bed for 3–4 days (9,10).

After debridement and before dressing a wound with maggots, one frequently performed step is the application of antiseptics to the prepared wound bed. However, concerns exist that the concomitant application of antiseptic agents during maggot therapy may interfere with maggots' viability. While generally plausible, there is very little published data supporting or rejecting this assumption (11).

As wound antisepsis is also desirable during bio-surgery, the aim of this work was to investigate the viability of fly maggots after exposure to various antiseptic compounds frequently used in wound antisepsis.

Key Message

- Bio-surgical debridement by maggots of calliphorid flies is an accepted method in the therapy of non-healing chronic wounds. However, concerns exist about the concomitant application of antiseptic agents during maggot therapy. While generally plausible, there are no data supporting or rejecting this assumption. Here, it was demonstrated that *Lucilia sericata* fly maggots survived up to an hour's exposure to common wound antiseptics and that concomitant use may be feasible in practice. However, the clinical relevance of this observation requires further studies.

[†]These authors contributed equally to this work.

Table 1 Product, active compound and concentration of tested agents

Product	Active compound	Category	Lot no.	Concentration (diluent)
Octenisept®	0·1% octenidine dihydrochloride, 2% phenoxyethanole	Wound antiseptic	1068542	Undiluted
Lavasept®	0·04% polyhexanide	Wound antiseptic	TFS004N	Undiluted
Betaisodona®	10% povidone-iodine	Wound antiseptic	10023616	Undiluted
Manorapid synergy®	57·6% of ethanol 96%, 10% 1-propanol	Hand rub	03048	Undiluted
Ethanol	70% ethanol	Skin antiseptic	-	Undiluted
Lysoformin 3000®	7.5% glyoxal, 9.5% glutaral, 9.6% didecyldimethyl-ammoniumchloride	Disinfectant	110701	2% (a. bidest.)
Descogen F®	60% potassium peroxymonosulfate	Disinfectant	280704	1.5% (a. bidest.)
Disifin med® (powder)	90% tosylchloramine sodium	Disinfectant	01062001	1% (a. bidest.)
Wofasteril®	35% peracetic acid	Disinfectant	811205	0.25% (a. bidest.)
Wofasept®	5% chlorocresol, 2% chlorofen, 5–15% anionic tensides	Disinfectant	-	2% (a. bidest.)
Control	Sterile saline 0·9%	-	-	0·9%

Material and methods

Sterile maggots of *Lucilia sericata* (Diptera: Calliphoridae) were purchased from Biomonde GmbH (Barsbüttel, Germany). Before being used for the experiments, larvae were first visually examined for their vitality and intactness and thereafter incubated for 48 hours at 37°C on sterile Columbia agar plates with 5% sheep blood (Oxoid, Wesel, Germany) until they reached the third larval stage.

Maggots were tested against three wound antiseptics: octenidine dihydrochloride (Octenisept®; Schülke, Germany), polyhexamethylene biguanide (polyhexanide; Lavasept®; Fresenius Kabi, Germany) and povidone-iodine (Betaisodona®; Mundipharma, ~~Germany~~ Antiseptica GmbG). Furthermore, maggots were also tested against alcohol-based hand rubs containing 58% ethanol and 10% 1-propanol (Manorapid synergy®; Antiseptica, Germany) and 70% ethanol. Finally, five different undiluted surface disinfectants were investigated, containing 7·5% glyoxal, 9·5% glutaral and 9·6% didecyldimethyl-ammoniumchloride (Lysoformin 3000®; Lysoform, Germany), 60% potassium peroxymonosulfate (Descogen F®; Antiseptica, Gemany), 90% tosylchloramide sodium (Disifin med®; Disifin, Germany), 35% peracetic acid (Wolfasteril®; Kesla Pharma Wolfen GmbH, Germany) and 5% chlorocresol with 2% chlorofen (Wofasept®; Kesla Pharma Wolfen GmbH, Germany). Table 1 gives a detailed summary on the tested antimicrobial compounds. All products were used at the concentrations recommended by the manufacturers for use in clinical settings. Sterile 0·9% saline solution was used as a control for all the experiments.

Each experiment was conducted in parallel with 40 viable larvae for every antimicrobial compound or 0·9% NaCl control and exposure time. Initially, maggots were transferred into a 12-ml test tube (Sarstedt, Germany) and incubated at 37°C fully immersed in 10 ml of the test solution, with exposure times of 1, 2, 5, 10, 30 and 60 minutes. After incubation, maggots were removed from the solution and washed three times in sterile saline with gentle shaking for over 1 minute; each maggot was then gently placed into 10-ml sterile 0·9% NaCl with forceps. Finally, maggots were placed onto a pre-warmed (37°C) Columbia agar plates for 2 hours to monitor viability. Maggots that remained initially immobile after exposure to the respective test compound were transferred for three additional days on to Columbia agar and incubated at 37°C to monitor for changes in motility.

Viability was assessed by two methods. First, motility was assessed visually by using a numeric analogue scale (NAS) ranging from 0 (dead) to 1+ (inhibited mobility, only slow movements on agar plate with intermittent pauses of movement), 2+ (slower movements without pauses over 10 s) and 3+ (full motility).

Furthermore, viability was assessed by the maggot's ability to reduce bacteria. Following the same test procedure as above, 40 new maggots were used to investigate their ability to reduce bacterial loads after exposure to antimicrobial compounds using a modified quantitative suspension test as previously described (8). Briefly, exposed maggots were introduced to a freshly prepared *Staphylococcus aureus* suspension containing ten (4) colony-forming units (cfu) suspended in 20 ml of sterile saline for 24 hours at 37°C. After incubation, the reduction factor (rf) was calculated (rf = $\log_{10}$ cfu control minus $\log_{10}$ cfu test) and compared with the rf obtained from 40 maggots of a control experiment without prior exposure to antimicrobial compounds. The *S. aureus* strain used for all experiments was a methicillin-sensitive *S. aureus* strain (MSSA; American Type Culture Collection ATCC 6538 Manassas, VA).

Results

Effects on motility

Exposure to the three wound antiseptics for up to 10 minutes had no observable impact on the motility of the maggots (Table 2). However, from 30 minutes onwards, single maggots started to show obvious, decreased motility with intermitted pauses of movement. After 1 hour of exposure to Octenisept® or Betaisodona®, 33/40 and 32/40 of the tested maggots still showed full motility. Interestingly, 70% ethanol had no impact on the maggots' motility or their ability to reduce the bacterial load in suspension. However, this was not observed when maggots were exposed to an alcohol-based hand rub containing 58% ethanol and 10% 1-propanol (Manorapid synergy®). In this case, all maggots exhibited full motility up to 30 minutes exposure, yet after 60 minutes exposure, eight maggots were dead, and two exhibited severe impairment of movement. This effect may be attributed to the toxicity of the 1-propanol

Table 2 Viability of maggots to antiseptics and disinfectants ($n = 40$ maggots per tested compound)*

Compound	1 minute *n* (NAS)	2 minute *n* (NAS)	5 minute *n* (NAS)	10 minute *n* (NAS)	30 minute *n* (NAS)	60 minute *n* (NAS)
Octenisept®	1 M: 2+; 39 M: 3+	1 M: 2+; 39 M: 3+	40 M: 3+	2 M: 2+; 38 M: 3+	1 M:1+; 4 M: 2+; 35 M: 3+	2 M:1+; 5 M: 2+; 33 M: 3+)
Lavasept®	40 M: 3+	40 M: 3+	2 M: 2+; 38 M: 3+	2 M: 2+; 38 M: 3+	1 M: 1+; 3 M: 2+; 36 M: 3+	2 M: 1+; 3 M: 2+; 35 M: 3+
Betaisodona®	1 M: 2+; 39 M: 3+	40 M: 3+	2 M: 2+; 38 M: 3+	2 M: 2+; 38 M: 3+	2 M: 1+; 4 M: 2+; 34 M: 3+	3 M: 1+; 5 M: 2+; 32 M: 3+
Manorapid synergy®	40 M: 3+	40 M: 3+	40 M: 3+	40 M: 3+	40 M: 3+	8 M: 0; 2 M: 1+; 30 M: 3+
Ethanol 70%	40 M: 3+	40 M: 3+	40 M: 3+	40 M: 3+	40 M: 3+	40 M: 3+
Lysoformin 3000®	40 M: 3+	40 M: 3+	40 M: 3+	40 M: 3+	40 M: 3+	40 M: 3+ †
Descogen F®	40 M: 3+	2 M: 1+; 1 M: 2+; 37 M: 3+	2 M: 1+; 2 M: 2+; 36 M: 3+	2 M: 1+; 4 M: 2+; 34 M: 3+	1 M:1+; 4 M: 2+; 35 M: 3+	5 M:1+; 8 M: 2+; 27 M: 3+
Disifin med®	40 M: 3+	40 M: 3+	40 M: 3+	1 M: 1+; 1 M: 2+; 38 M: 3+	1 M: 1+; 1 M: 2+; 38 M: 3+	1 M: 1+; 2 M: 2+; 37 M: 3+
Wofasteril®	23 M: 0; 9 M: +1; 8 M: 2+	25 M: 0; 11 M: +1; 4 M: 2+	25 M: 0; 12 M: +1; 3 M: 2+	22 M: 0; 13 M: +1; 5 M: 2+	24 M: 0; 14 M: +1; 2 M: 2+	26 M: 0; 14 M: +1
Wofasept®	18 M: 0; 9 M: +1; 13 M: 2+	14 M: 0; 6 M: +1; 20 M: 2+	22 M: 0; 11 M: +1; 7 M: 2+	19 M: 0; 13 M: 1+; 8 M: 2+	21 M: 0; 12 M: 1+; 7 M: 2+	20 M: 0; 15 M: 1+; 5 M: 2+
Control	40 M: 3+	40 M: 3+	40 M: 3+	1 M: 2+; 39 M: 3+	40 M: 3+	40 M: 3+

M, maggot.

*Viability of maggots directly after 1, 2, 5, 10, 30 and 60 minutes exposure to antiseptics and disinfectants following the numerical analogue scale (NAS) ranking from 0 (dead) to 1+ (remarkably inhibited motility, only slow movements on agar with intermittent pauses of movement), 2+ (slower movements without pauses over 10 seconds) and 3+ (full motility).

†3/40 maggots hyperactive for over 20 minutes after 60 minutes exposure.

rather than the ethanol. While not directly comparable, a previous study conducted by McIntosh *et al.* reported similar effects of 2-propanol on fly maggots (11).

The most obvious influence on maggots' motility and bacterial reduction ability was observed for antimicrobial compounds used for surface and/or instrument disinfection. Surprisingly, exposure to Lysoformin 3000® showed no effect on maggots' motility. On the contrary, after 1 hour of exposure, maggots exhibited hyperactive motility for more than 20 minutes, before returning to normal motility. All other surface disinfectants inhibited maggots' motility to a variable degree. For Descogen F® and Disifin med®, a reduction in motility was observed after 2 minutes exposure time, but with no larval death. Wolfasteril® and Wolfasept® exhibited the most adverse impact on the viability of the maggots as both products inhibited motility after 1 minute of exposure, with approximately 50% larval death.

Effects on ability to reduce bacterial load

Exposure to Betaisodona® or Octenisept® reduced the maggots' ability to consume bacteria by 5% and 10%, respectively, when compared to the control maggots incubated in 0·9% sterile saline (Table 3). The control maggots achieved a 2·96 $\log_{10}$ (=100%) reduction in *S. aureus* over a 24-hour incubation period. Exposure to Lavasept® had an even larger effect on the maggots, reducing their impact on *S. aureus* by 19% when compared to the sterile controls.

Descogen F® and Disifin med® not only reduced maggots' motility after 2 minutes exposure but also reduced maggots' ability to reduce *S. aureus* by 74% (Descogen F®) and 61% (Disifin med®).

Table 3 Effect of maggot ($n = 40$) exposure to selected antiseptics and disinfectants on their efficacy to reduce *S. aureus* ($\log_{10}$ RF ± SD following a modified quantitative suspension test). Reduction obtained after exposure to 0·9% saline = 100%

	S. aureus $\log_{10}$ reduction	% $\log_{10}$ reduction compared to control (%)
Control (0·9% sterile saline)	2·96 (±0·6)	100
70% ethanol	2·86 (±0·7)	99·7
Betaisodona®	2·82 (±1·1)	95
Octenisept®	2·67 (±0·4)	90
Lavasept®	2·39 (±0·8)	81
Disifin med®	1·16 (±0·6)	39
Descogen F®	0·77 (±0·5)	26

Discussion

The main beneficial effect of maggots for the treatment of wounds is the debridement of necrotic tissue (i.e. controlled myiasis), the promotion of tissue granulation (12) and the support of wound antisepsis because of the secretion of antibacterial peptides (13–19) together with some degree of bacterial ingestion (8,20). Generally, providers of maggots recommend that the application of wound antiseptics shall be paused during maggot treatment in order not to kill or inactivate the larvae. However, the results of the present study show that particularly antimicrobial compounds that are routinely used for wound antisepsis are well tolerated by *L. sericata* maggots and that a number of other antimicrobial compounds do not interfere significantly with maggots' viability. Therefore, it

may be concluded that both wound antisepsis, particularly with octenidine (Octenisept®) or povidone-iodine (Betaisodona®), and the application of fly maggots on the wound bed may be performed concurrently. However, the clinical relevance of this observation requires further studies.

Our study has a number of limitations. First, the applied method does not allow any assessment on maggots' viability over an exposure period of more than 1 hour. Yet, in clinical practice, an exposure to antiseptics may occur over periods of 24–48 hours and longer. Such long exposure times may have a negative impact on the physiology of maggots. Second, it is difficult to correlate maggots' viability in terms of motility and the ability to reduce *S. aureus* with their clinical performance to debride necrotic tissue from wounds. However, both appear to be good surrogates to assess maggots' ability of secretion and ingestion. Robinson *et al.* (18) described the elimination of bacteria in the digestive tract of viable maggots, with more than two third of the bacteria dying in the hind stomach and significant reduction along the gastrointestinal tract. Using fluorescence-expressing *E. coli*, Mumcuoglu *et al.* (20) demonstrated that during passage of the bacteria through the intestinal tract of maggots, a pronounced colonisation in the crop and anterior midgut occurred, which decreased sharply after passing from the posterior midgut and anterior hindgut, dropping to almost zero near the anal opening. Accordingly, maggot excretions were, in most of the cases, free of labelled *E. coli*. In contrast to this result, the author's recent work (8) showed that strains of MSSA and MRSA remained viable inside maggots for at least 3 days and were excreted to the environment and even remained viable inside the later pupa. Indeed, those antimicrobial compounds with the lowest impact on the maggots' motility did not also influence the ability to reduce *S. aureus*. Contrarily, if motility was influenced within 2–5 minutes of exposure, maggots showed a significant reduction to eliminate *S. aureus* as well.

Finally, it was interesting to observe that none of the tested chemical compounds were able to completely inhibit or kill the tested maggots. Even Wolfasteril®, based on of peracetic acid, and Wolfasept®, based on chlorocresol and chlorofen, which inhibited motility already after 1 minute of exposure, only generated approximately 50% larval death.

In conclusion, *L. sericata* fly maggots can survive up to an hour's exposure to wound antiseptics, particularly octenidine (Octenisept®) or povidone-iodine (Betaisodona®). Concomitant short-term application of wound antiseptics together with maggots on wound beds is well tolerated by larvae and does not impair their viability. However, the ability of maggots to debride wounds under antiseptic conditions needs to be further investigated in clinical trials.

Acknowledgement

The authors declare no competing financial interest or other conflict of interest.

Author contribution

GD, AK and MN formulated the study hypothesis. SvP, RS and OA performed the laboratory experiments and collected the data. All authors were involved in the literature search for the study, drafted the manuscript and were involved in drafting and processing the study results and in interpreting the study data.

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