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ORIGINAL ARTICLE

Is Honey an Answer for Eradication of Biofilms?

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Abstract
Biofilm formation in chronic ulcers is an important cause for delayed healing of wounds. Eradication of biofilms is a challenge as this is not amenable to standard methods of ulcer management. Currently, there are very limited options for its eradication except manual debridement which is painful, needs repeated anesthesia, and cannot be done on daily basis. Unprocessed naturally extracted honey has multiple unique properties as a local applicant which aid in faster healing. This article presents the incidence of biofilms in chronic ulcers and use of honey as a local applicant in enhancing healing. Sixty-four patients with ulcers were studied for presence of biofilm. Among them, 43 (67%) had biofilms which took significantly long time to heal in comparison with ulcers without biofilms ($p < 0.002$). Later, efficacy of honey in eradication of biofilms was studied in 56 patients with chronic ulcers having biofilms. There was a significant reduction in time for eradication, ($p < 0.0175$) healing of ulcers, and total duration of hospital stay in comparison with ulcers managed by standard methods. Results suggest that natural unprocessed honey has a great potential in eradication of biofilms.

Keywords Biofilm · Honey · Eradication of biofilms · Delayed healing of ulcers · Chronic wounds

Introduction

Wounds or ulcers which do not heal in 30 days are loosely termed as chronic wounds. Ischemia, poor sensation, and poor nutrition are known to delay healing. Persistent infection due to biofilms is being recognized as one of the important cause [1]. Research on wound healing and infections has traditionally focused on planktonic or free-floating bacteria which could be cultured, antibiotic resistance or sensitivity established. This led to narrow the focus on long-term/short-term antibiotic therapy which often failed to eradicate infections [2]. This was due to persistence of bacteria as biofilms [3, 4] which are recalcitrant to antimicrobial therapy and immune response of the host [5]. Biofilm-related infections are known to lurk in catheters and cardiac valves until these are removed [6–9]. The incidence of biofilms is 60% in chronic ulcers and 6% in acute ulcers [1]. Biofilms are associated with 65% of hospital-acquired infections with financial burden of 1 billion dollars annually in the USA. Management of biofilms is a challenge to surgeons as they do not respond to standard local or systemic antibiotic therapy.

At present, mechanical debridement is the accepted method for biofilm management which is painful and needs repeated anesthesia [10]. The other methods mentioned like enzymatic debridement [10], silver-based hydrogel, and iodine-based hydrogels [11] are under trial.

Raw unprocessed honey is one of the local applications with known antimicrobial activity, [12, 13] hygroscopic properties [6, 9, 14], anti-inflammatory [15–17], and autolytic activity [18], and acts as barrier for cross contamination preventing biofilm formation. Even though there are reports of the use of honey for management of wounds [19], its utility in eradication of biofilms is acknowledged mainly in vitro studies [20–22]. Few clinical studies encourage its use but are still nascent. Though there was a clinical study (http://onlinelibrary.wiley.com/doi/10.1111/j.13652702.2008.02558.x/abstract;jsessionid=5F90C2DB605DA86B4DDE8180B640BFD7.f02t01), it was retracted due to errors in analysis.

Looking at the incidence of biofilms in the study, time taken for healing, nonvisibility of biofilms, and difficulty and pain during repeated mechanical debridement's, desperate search for feasible remedy in the form of local application of honey was considered. This formed another study.

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This article aims to assess the use of unprocessed honey as a topical agent in eradicating biofilms in wounds and compare with the observational study done earlier in the same unit in terms of time taken for appearance of granulation tissue, eradication of biofilms, definitive therapy, and duration of hospital stay.

Methodology

This article is a combined report of two studies conducted at our institute. The first study [control group] was an observational study to detect biofilms in chronic wounds. The second study [study group] aimed at eradicating biofilms using unprocessed honey by local application. Both studies were conducted prospectively after obtaining institutional ethical committee clearance and written informed consent of all subjects.

Table 1 shows details of the methodology adopted and work done.

The ulcer was inspected for transparent membrane and pus. Pus/tissue was collected and submitted immediately for further process. The specimens were cultured and tested for bacterial isolates as per standard methods. Isolates were subjected to antimicrobial susceptibility test by disc diffusion technique as per CL51 guidelines. These were further tested for biofilms by tube adherence method [23] which is a qualitative method for biofilm detection.

Biofilm was considered to be formed when a film lining the wall of the tube was visible (Fig. 1). The test was repeated three times and confirmed. Ulcers were managed by regular dressings after cleaning with 10% povidone-iodine solution [Betadine] as local application. They were observed for appearance of granulation tissue, reduction in size, discharge, and time taken for definitive treatment (Figs. 2 and 3).

Based on this observation, a prospective interventional study was taken up to study the efficacy of honey on biofilm eradication from Oct 13 to Jun 2015.

Patients with acute/chronic ulcers with biofilms were included ($n = 60$) [1].

Once presence of biofilm was confirmed by tube adherence test, honey dressing was done regularly after recording parameters like size, discharge, foul smell, and presence or absence of granulation tissue on day 1. These ulcers were cleaned with normal saline, and 10–30 ml of honey (Dabur) was applied on sterile gauze, spread over ulcer bed, and the ulcer was dressed daily. Assessment of ulcer was done every fifth day using same parameters, and swab/tissue was taken for culture and sent for detection of biofilm. The procedure was continued until the ulcer was biofilm-free and subjected to definitive therapy by split-

Table 1 Details of the methodology adopted and work done

	Control group (observational study)	Study group (interventional study)
Duration	1 and 1/2 years	
Period	Oct 2011 to June 2013	Oct 2013 to June 2015
Sample size	64 [1]	60 [1]
Inclusion criteria	All chronic ulcers of 30 days or > 30 days duration	
Exclusion criteria	Ulcers < 30 days, malignant ulcers, and immune-compromised patients	Chronic ulcers without biofilms, malignant ulcers, and immune-compromised patients
Age, gender, comorbidities	Noted and recorded	
Site of ulcer	Predominantly lower limbs	
Culture sensitivity of pus/tissue	At admission as per CL5 guidelines Repeated every 5th day if the ulcer showed biofilm Continued until culture reported sterile and − ve for biofilm	At admission as per CL5 guidelines, confirmed presence of biofilm Repeated every 5th day until culture reported sterile and − ve for biofilm
Methodology adopted to detect biofilms	Tube adherence method [23]	
Management of ulcers	Initial debridement and regular dressings using 10% povidone-iodine as local application	Initial debridement and regular dressings using unprocessed honey [Dabur] as local application
Systemic therapy	Oral/I v antibiotics as per culture sensitivity report, correction of nutrition and control of diabetes	
Appearance of healthy granulation tissue	Noted and recorded every 5th day	
Tube test − ve indicating clearance of biofilm	Noted and taken up for definitive therapy, mainly split-thickness skin grafts [STSG] or delayed suturing whenever feasible. Number of days taken recorded	
Time taken for final definitive therapy STSG or healing	Noted from day of initial debridement to day of STSG/ healing of ulcer	
Total duration of hospital stay	Noted	

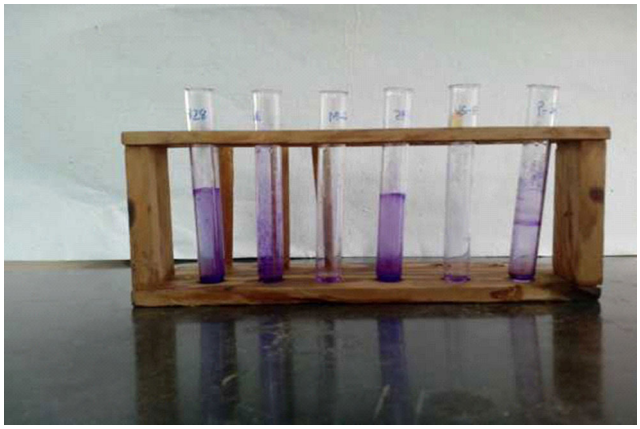

Fig. 1 Biofilm coating the walls of the test tube (violet color)

thickness skin grafts (STSG). Results were analyzed by applying mean, Student's *t* test, chi-square, and *p* value.

Results

Observational Study

Age of patients ranged from 18 to 80 and the mean was 60. Maximum patients were in the range of 41–70. M:F ratio was 3:1. The causes of ulcers were postcellulitic ulcers, diabetic foot ulcers, and others. Among 64 patients, 43 had biofilms in their ulcers (67.2%). The overall duration of healing was 52.31 days in individuals who had biofilms and 42.56 days in ulcers who did not have biofilms which is statistically significant ($p < 0.002$).

An interesting observation in this study was correlation of diabetes mellitus and biofilms. Among 64 patients, 47 were diabetics and 31/41 had biofilms in their ulcers. The mean healing time in this group was delayed in comparison to the total sample (54.38 days versus 52.31). Diabetics who did not have biofilms in their ulcers had a mean healing time of 43.68 days which is statistically significant ($p < 0.0175$).

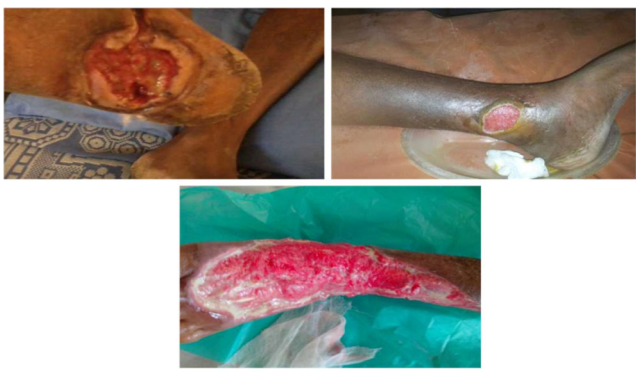

Fig. 2 Ulcers with biofilms in the observational group

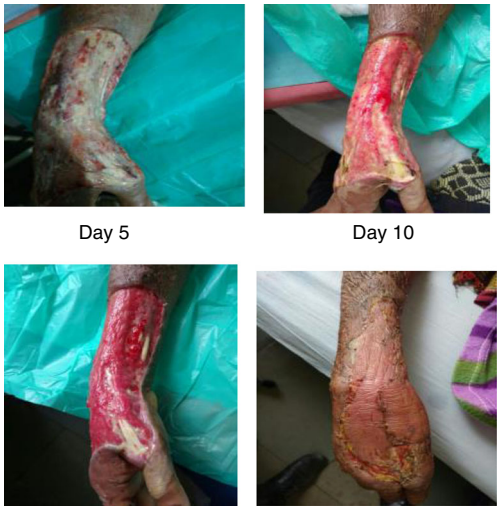

Fig. 3 Progressive stages of healing after honey application

Interventional Group [Ulcers Treated with Honey Application]

Patients with acute/chronic ulcers with biofilms were included. The sample size was 60 [1].

The demographic profile and causes were similar to the observational study. The age ranged from 21 to 70. Male to female ratio was 5:1. Among 60 patients, 4 were acute ulcers, and hence not included in the discussion. Fifty-four had chronic ulcers and 15 had diabetes among them. Irrespective of the diabetic status, there was no significant increase in the time taken for healing (Table 2).

The average duration for ulcers to form healthy granulation tissue was 10–30 days with a mean of 18.1 ± 5.5 days. Forty-three percent showed healthy granulation in 10–15 days. This was irrespective of the patient being diabetic or otherwise. Fifty-five percent of patients underwent skin grafting by 15 days, 26.7% by 20 days, and 18.3% took more than 20 days for definitive therapy. Biofilms were eradicated maximum by 25 days with an average of 17.5 days and minimum 10 days. The duration of hospital stay was minimum 20 days to maximum 30 days with a mean of 26.4 ± 3.1 days.

There were no significant local irritation or reaction seen in both groups. Patients complained of more pain in honey group, but pain scores were not included in the study (Table 3).

Discussion

Traditional focus on free-floating bacteria led to poor recognition of biofilm as a cause for persistence of infections which are resistant to standard methods of management [5] due to different behavior from those found on the surface of the

Table 2 Common organisms cultured

Sl. no	Organisms	Number in control group	Percent	Number in study group	Percent
1	Staph aureus	22	34	35	58.3
2	Pseudomonas A	11	17	9	15
3	*E. coli*	08	12.5	8	13.3
4	Klebsiella P	0	0	2	3.3
5	Klebsiella oxytoca	03	4.5	1	1.7
6	Citrobacter	01	1.5	1	1.7
7	Proteus V	01	1.5	0	0
8	KP and EC	0	0	1	1.7
9	SA and EC	0	0	2	3.3
10	SA and PA	0	0	1	1.7
11	Sterile	18	28	NA	NA

wounds. It is estimated that around 80% of infections are biofilm-related [4]. These biofilms are mainly composed of extracellular polymeric substances (EPS) or slime (80%), and 20% are microbes residing as communities encased in EPS matrix [24, 25]. These microbes are heterogeneous with different geno/phenotypes resistant to antimicrobial therapy and immune response of host due to poor penetration, metabolic inhibition, and protected quiescent bacteria [26–28].

In early stages, they can be controlled by immune-mediated defense of the host. But once the ulcers become chronic, microbes attach to the wound surface and proliferate. These microbes secrete different proteins form an extracellular matrix and get embedded deeply. Here, these are well-protected, less active, and resistant to therapy. Only the microbes situated at the margin of the wound are metabolically active and vulnerable to antibiotics, antiseptics, and host defenses.

Management of biofilms is a challenge to surgeons, as they do not respond to standard local and systemic therapies. The accepted method till date is mechanical debridement [10], is painful and needs repeated sessions which in

Table 3 Comparison of findings in the observational study and intervention with application of honey

Sample size	Observational study = 64	Interventional study using honey = 64 [chronic ulcers = 60]
Age range	30–90	21–88
Highest number of patients were in age group of	41–50	46–65
Mean age	60	54.5
M:F ratio	M = 48, F = 16 3:1	M = 50, F = 10 5:1
Chronic ulcers	64	56/60
Ulcers +ve for biofilms	43 (67.2%)	60 (100%)
Considered for study	43/64	56/64
Comorbidities	DM:47	DM:15
Wound measurements	2 × 2 cm to 16 × 12 cm	3 × 2 cm to 16 × 12 cm
Time taken for eradication of biofilm and definitive therapy	Range 20–86 days Mean of 52.31 ± 5.8 days in ulcers with biofilms 42.56 ± 28 days in ulcers without biofilms - *p* value 0.002	Range 10–26 days Mean 16.9 ± 4 days - *p* value 0.0001
Appearance of granulation tissue in days	Min − 20 max − 40 Mean 32 ± 7.5	Min 10 max 30 Mean 18.1 ± 5.5
Time taken for STSG/complete healing of ulcers	Min 20 max 72 Mean 52.31 ± 10.2 days in biofilm + ve group (control group) 42.56 ± 4.2 days in biofilm − ve group *P* value: <0.002	Min 10 max 26 days Mean 16.9 ± 4.4 *p* value 0.0001
Duration of hospital stay in days	Biofilm +ve min 21 max 85 Mean 56.05 ± 5.8 Biofilm − ve min 21 max 59 Mean 43 ± 4.32	Min 20 max 30 Mean 26.4 ± 3.1

turn increase duration of healing, hospital stay, and subsequently, cost of treatment.

There are few other methods suggested for eradication of biofilms like enzymatic debridement [10], silver-based hydrogels, and iodine-based hydrogels [11] which have been tried and are under evaluation. Unlike these, raw unprocessed honey is known to have antimicrobial activity [12, 13], hygroscopic properties [6–9, 14], and anti-inflammatory [15–17] and autolytic activity [18]. Honey is easily available, easy to apply, and reduces the need of multiple debridement, and there is no evidence of resistance. Though experimental studies [20–22] have shown good action of honey on biofilms, clinical studies in chronic wounds with biofilms where honey was used as local applicant for eradication of biofilms are few [24, 27–29]. One of the published studies was retracted later (http://onlinelibrary.wiley.com/doi/10.1111/j.13652702.2008.02558.x/abstract;jsessionid=5F90C2DB605DA86B4DDE8180B640BFD7.f02t01). Utility of honey in burns and wounds [19] is known; its utility in attempting to eradicate chronic wounds with biofilms in natural clinical settings as a part of routine wound care is the focus of this work. Our results clearly demonstrate the benefits of application of honey in terms of eradication of biofilms in both diabetic and nondiabetic individuals, reduction in time taken for development of healthy granulation tissue, and definitive treatment. This reduced overall duration of hospital stay significantly and improved the final outcome.

Conclusion

Raw unprocessed honey as a local application eradicates biofilms in chronic wounds and reduces healing time with better final outcome.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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