Effect of medical grade indian honey on biofilms: a case series


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The presence of biofilms in a wound impairs healing. It is now thought that most chronic wounds contain biofilms, which are resistant to most antibiotics. A number of agents have been recently proposed to be effective in removing biofilms, one such agent being honey. Honey has been used for centuries in wound healing. Different types of honey have different properties, and although manuka honey is well known for its antimicrobial properties, there are other honeys which are also effective in wound management. One type of honey from India is obtained from *Apis dorsata* bee which is found in Western Ghats. Here we report our experience of three cases wherein the medical grade Indian rock bee honey was found to be effective in management of wounds in with biofilms.

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Diabetic foot ulcers (DFU), pressure ulcers (PU) and leg ulcers are common chronic wounds seen in clinical practice worldwide. (Clinton and Carter, 2015). A wound is said to be chronic when wound healing fails to progress normally (Clinton and Carter, 2015; Han and Ceilley, 2017).

Biofilms are common in both acute and chronic wounds, less than 10% of acute wounds and greater than 60% of chronic wounds have biofilms (Clinton and Carter, 2015, Malone et al, 2017).

Biofilms are complex, dynamic (Mahoney, 2015) dimensional mosaic consortia of microorganisms that are encased in a extracellular polymeric substances (EPS) made of sugars and proteins (Clinton and Carter, 2015; Mahoney, 2015; Philips et al, 2010) These biofilms are polymicrobial in nature and attaches themselves to living and non-living surfaces (Clinton and Carter, 2015; Philips et al, 2010; Omar, 2017). The EPS could be consider like a house that the bacteria live in, offering protection from external environment (Clinton and Carter, 2015).

The structure of biofilms shields the microorganisms against the action of host immune system as well as antimicrobial agents, both topically as well as systemic ones (Bowen et al, 2016). This protection increases the virulence of infection and complicates wound management (Clinton and Carter, 2015). The EPS matrix of the biofilms also hinders permeation of antibiotics (Banu et al, 2015). There are *in vitro* experiments which shows antibiotic resistance of bacteria in biofilms is up to 1000 times (Neut et al, 2012). *Staphylococcus aureus* and *Pseudomonas aeruginosa* readily forms biofilm, in diabetic foot wounds (Banu et al, 2015).

There are various proposed strategies to treat biofilms include repeated debridement, use of antiseptics like polyhexamethylene biguanide (PHMB) and octenidine and use of topical agents like silver, cadexomer iodine and honey, (Mahoney, 2015; Bowen et al, 2016), which has been shown to be effective in treating biofilms (Mahoney, 2015).

Here we report cases where the wound biofilms were treated effectively with Indian medical grade honey.

Case 1

A 48-year-old male with a history of diabetes mellitus and ischaemic heart disease presented to with a history of swelling of the left foot and leg for five days along with pain and pus discharge. A diagnosis of abscess was made (Wagner's grade 3) and patient underwent debridement twice. Postoperatively patient was put on three cycles of negative pressure wound therapy (NPWT), which resulted in a good granulation tissue. The patient then underwent regular dressing changes at his hometown

Case 1

- A 48-year-old male with diabetes mellitus and ischaemic heart disease presented to with a swollen left foot and leg with pain and pus discharge
- Following other unsuccessful treatments the Indian medical grade honey on the wounds
- Once good granulation tissue and no visible signs of biofilms were observed after three weeks of honey application treatment returned to standard dressing

Biofilm on the wound	Biofilms being easily removed	Honey applied to the wound
		
Wound at 3rd application of honey (day 5). *Note the decrease in biofilm*	**Healthy red granulation after three weeks of honey before skin grafting**	**Split-thickness skin graft on the wound**
		

and presented to us after few weeks where we noticed the wound to be covered entirely with biofilms (visual indicators). The patient was undergoing regular removal of biofilms and poly(hexamethylenebiguabide)hydrochloride solution (PHMB) were used as an antiseptic, however, biofilms kept forming/reforming. Biofilms culture of wound showed *P. aeruginosa*.

At this point, we decided to use Indian medical grade honey on the wounds. The wound was irrigated with normal saline. Around 5–6ml of sterilised honey was applied to the sterile guage and this was then applied to the wound, over which sterile pads were placed and roller guage was used to secure the dressing. Honey dressings was applied every two days for two weeks. We noticed the gradual disappearance of biofilms and wound appearing healthy. Once the wound had good granualtion and no visual indicators of biofilm, regular dressings were used. The wound was cleaned with normal saline, then hydrogel was applied to the wound, over which we placed gauge and pads and roller bandage was used to secure the dressing. At week four the patient underwent split-thickness skin grafting *(Case 1)*.

Case 2

A 46-year-old male patient with diabetes and hypertension presented to us with abscess over left upper leg on lateral aspect for which he underwent debridement. Patient was undergoing regular dressings with PHMB and hydrogel. The patient presented to us after

Case 2

- A 46-year-old male patient with diabetes and hypertension presented with abscess over left upper leg on lateral aspect
- Bacterial culture grew *Staphylococcus aureus*
- He was treated with medical grade honey over 8 days. After which normal saline and hydrogel were used
- At end of week three the wound was granulating with no obvious signs of biofilm

Wound with biofilm and unhealthy granulation	Honey dressing applied	Wound after two weeks	Wound at end of three weeks
			

three weeks, when biofilms were noticed on his wound with unhealthy granulation tissue. Bacterial culture grew *Staphylococcus aureus*. He was treated with medical grade Indian honey over 8 days and 4 sittings were used after which treatment was changed to normal saline and hydrogel. At end of week three the wound was granulating excellently with disappearance of biofilms *(Case 2)*. A repeat culture did not show any growth. However, the COVID-19 pandemic lead to an emergency lockdown and the patient could not undergo skin grafting, leaving the wound to heal by secondary intention.

Case 3

A 65-year-old male with diabetes presented to us with abscess over right foot near great toe (Wagners grade 3) for which he underwent debridement. Patient was undergoing regular dressing with PMHB and hydrogel. However we noticed presence of biofilm *(Case 3)* over the wound when he presented, so we used honey dressings on the wound. After 2.5 weeks the wound had healthy red granulation with no biofilm visually. It was allowed to heal with secondary intention.

Discussion

Although, one of the successful strategy in treating biofilms is physical removal, along with proper cleansing of wound, it is seen known that biofilms can reformed quickly (Neut et al, 2012). It has been reported that a mature biofilm can reform on the wound within 24 hours (Bowen et al, 2016). Bioflims delays wound healing and ensures that the inflammatory phase is maintained, rendering the wound chronic (Bowen et al, 2016).

Case study 3

- A 65-year-old male with diabetes with abscess over right foot (Wagners grade 3)
- After 2.5 weeks the wound had healthy red granulation, a reduction in wound size with no visual signs of biofilms

Visual signs of biofilms on the wound	After 2.5 weeks honey treatment
	

Although biofilms are microscopic structures, there are several visual indicators of wound biofilm (Mahoney, 2015; Metcalf et al, 2014). Presence of excessive exudates, presence of pale green or yellow slimy layer, an easily removable gelatinous material which reforms quickly and a suspected biofilm layer than can be removed with forceps automatically revealing underlying granulation tissue are some of the visual indicators (Mahoney, 2015; Metcalf et al, 2014).

Honey has been used in wound for more than 2000 years (Stephen-Haynes and Callaghan, 2011). Its anti-inflammatory, anti-oxidant and antibacterial properties are well known among wound care experts (Yaghobi, 2013). Low PH, osmotic effect and production of hydrogen peroxide in honey is responsible for its antimicrobial activity (Alam, 2014).

Different medical grade honey used worldwide includes manuka, tulang and gelans (Jain and Apoorva, 2020). The honey we used in this case is obtained from giant comb of Apis Dorsata, which is a Indian rock bee (Jain and Apoorva, 2020). It is commonly found in South Asia. More than 90% of honey in India is from *Apis dorsata* colonies (Basavarajappa, 2017).

A study by Qameer et al (2008) on physicochemical properties of *Apis dorsata* honey showed that this honey has PH of 3.8 to 4.7 with 5-hydroxymethylfurfural (HMF)content of 50–56mg/Kg and moisture content of 20.5–26%. A study by Phadke et al (1967) in India, showed moisture content of *Apis dorsata* honey to be around 20.9% (Joshi et al, 2000; Phadke, 1967). Invertase and proline content are also high in *Apis dorsata* honey compared with *Apis mellifera* and *Apis cerena* honeys. The glucose oxidaxe content are higher in *Apis dorsata* honey (8.51µg) compared with other Apis honey (Joshi et al. 2000).

We obtained raw fresh honey, sterilised it with 15KGy gamma irradiation. Post-irradiation, the honey was cultured and no growth was seen. We then used it on wounds.

Honey of species is known to have antimicrobial properties against various microorganisms including *P. aeruginosa*, *Escherichia coli*, *S. aureus*, *Candida Albicans*, *Klebsiella pneumoniae* (Fahim, 2014). A study by Bhushanam et al, in India, showed that honey from different Apis including *Apis dorsata* exhibited broad spectrum antibacterial activity *in vitro*. (Bhushanam, 2019).

A study by Shenoy et al (2012) showed Indian origin honey have good activity against *P. aeruginosa* wound isolates and it was comparable with manuka and tulang honey.

Another study, from South India, reported that natural unprocessed honey was effective in eradicating biofilms from chronic wound and reduced healing time (Vallabha, 2019). Honey in general has both bacteriostatic and bactericidal action (Alam, 2014). The acidity of honey is known to restrict growth of microorganism (Stephen-Haynes and Callaghan, 2011). The antibiofilm action of honey is due to its sugar component fructose (Lu et al, 2019; Cooper, 2014). Honey is also effective in disrupting the established biofilms (Cooper, 2014). The acidity of honey is likely to reduce the biofilm (Al-Kafaween, 2020). It is known that honey can disrupt biofilm formation by inhibiting down-regulating binding proteins normally used by microorganisms to attach to human proteins, (Cooper, 2017).

Conclusion

Honey has been used in wounds because of its antimicrobial activity. Like many other antimicrobial agent, honey is effective against biofilms. It can prevent as well as disrupt established biofilms. This case series shows that Indian honey obtained from *Apis dorsata* is effective in treating wounds with biofilms. Larger studies are needed to establish its efficacy.

Conflict of Interest: The authors have no conflict of interest to declare

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