

Review

Antimicrobial Dressing for Diabetic Foot Ulcer Colonized with MRSA

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Abstract: Wound healing in patients with diabetic foot ulcer differs among people. The wound healing process was influenced by factors like nature of the wound, tissue and an immunity of a person. Any measure taken to control bacterial colonies in wound plays a significant role in wound healing. However, recent emergence of Methicillin-resistant *Staphylococcus Aureus* (MRSA) associated with chronic wounds created health concerns worldwide. An MRSA colony present in diabetic wounds vulnerable to prolong the wound healing has reported worldwide. Since MRSA are resistant to a wide range of antibiotics, choosing appropriate dressings to treat MRSA colonized wounds has become a challenge. Either synthetic or natural antimicrobial agents are used to develop dressings that combat against MRSA infections. In today's practice, the incidence of chronic wounds and its associated socioeconomic consequences is rising despite effort and advances in wound management. In this review, an attempt made to summarize various antimicrobial dressings based on its activity against MRSA.

Keywords: Wound, Honey, Silver, Antimicrobial, MRSA

Introduction

The estimated prevalence of diabetes covers 382 million people (Tao *et al.*, 2015). Initially, the diabetic foot was recognized in 19th century, however clinician's attention towards the matter was turned only after last half of the 20th century (Boulton, 2008; Connor, 2008). Any foot ulcer is potentially dangerous in diabetic patients (Boulton *et al.*, 2005). In their lifetime, about 15% of diabetic patients develop Diabetic Foot Ulcer (DFUs) (Kandemir *et al.*, 2007). Worldwide, the prevalence of the diabetic foot accounts for around 20% of hospital admissions (Roberts and Simon, 2012). Diabetes with a complication of DFUs is estimated with high morbidity and mortality rate (Boulton, 2008).

Ischemia, neuropathy and infection are the major pathologies leading to DFUs (Brem and Tomic-Canic, 2007; Boulton, 2008; Connor, 2008). Decreased vascular supply and impaired host immune response promoted bacterial infection in DFU (Roberts and Simon, 2012). Although diabetic foot infections are polymicrobial, the most frequently isolated species is *Staphylococcus aureus* (Lipsky, 2008; Mendes *et al.*, 2012) which is responsible for causing a wide range of illnesses (Murali *et al.*, 2014). Penicillin and its

derivatives, including Methicillin are used for the treatments of infections caused by *Staphylococcus aureus* (Rayner and Munckhof, 2005). The misuse and overuse of antibiotics against *Staphylococcus aureus* aids these microbes become resistant to drugs.

Staphylococcus aureus which is resistant to normal antimicrobial drugs such as chloramphenicol, tetracycline, amino glycosides and macrolides was referred to as Methicillin-resistant *Staphylococcus aureus* (MRSA) (Lee, 2003). It is reported that a *mecA* gene which codes for penicillin-binding protein named PBP 2A is responsible for the resistance against Methicillin (Wielders *et al.*, 2002). Lately, another gene named *mecC* resistance against *Staphylococcus aureus* was described and isolated in humans (Porrero *et al.*, 2014).

Recently, the amount of MRSA infections are increasing worldwide (Dang *et al.*, 2003; Lipsky, 2008). It is estimated about 46% of *Staphylococcus aureus* isolated are MRSA (Tentolouris *et al.*, 2006). In diabetic wounds, presence of MRSA differs from 15-30% (Shankar *et al.*, 2005). Patients with DFUs are extremely at a risk of infections. Once the wound is infected with bacteria, it spreads and ends up in severe destruction of tissues which results in amputation (Kandemir *et al.*, 2007; Mendes *et al.*, 2012; Zubair *et al.*, 2012). In

diabetes, foot ulceration and amputation are closely inter-related (Boulton, 2008). Foot ulceration and infection are the leading risk factors for amputation (Carmona *et al.*, 2005). Approximately 15% of patients with DFUs lead to lower extremity amputation. Additionally, MRSA dwell in surrounding atmosphere and hands of health care workers is a key mode of transmission between patients. Thus, MRSA have a high epidemic potential. Wound infection occurs when the microorganisms present in the wound overwhelm the immune system of the person (Robson, 1997). Prevention and treatment are necessary to prevent morbidity, particularly amputation (Lipsky *et al.*, 2004).

DFU infection followed by amputation not only increases the morbidity (Centers for Disease and Prevention, 2003). Additionally, the psychological impact of an amputation dramatically increases the risk of mortality. Diabetic foot ulcers are the common and costly complications of diabetes (Ramsey *et al.*, 1999). Previous studies have examined the prevalence of diabetic foot wounds (Dang *et al.*, 2003), however this prospective study examined the prevalence of diabetic foot wounds infected with MRSA.

Role of Dressings

An ideal wound care dressings for DFUs should maintain a moist wound healing environment, remove excessive exudate, odor and be effective in treating infection of diabetic foot wounds. The exact level to discriminate bacterial infection from bacterial colonization is not clear. However, it is established that bacteria equal to or greater than 10^5 colony forming units per gram of tissue (cfu/g) is sufficient to confirm infection (Sibbald *et al.*, 2003). Dressing materials incorporated with various antimicrobial compounds such as silver, honey, iodine are in use for millennia. In the early 20th century, the discovery of antibiotics greatly reduced the routine usage of such compounds. More recently, the interest in these compounds has renewed. Manufacturers add these compounds to the dressings, which provide greater antimicrobial action and aid in healing process. Wound dressing products containing these agents have developed to address the needs of chronic wounds with the intent to minimize colonization to prevent local infection, thereby enhancing healing. Some of these products outlined in this review.

Dressing Methods with Natural Antimicrobial Agent

Honey

In traditional medicine, honey had valued place over centuries (Chowdhury, 1999). Honey used for rapid clearance of bacteria in colonized and infected

wounds (Efem, 1988; Vardi *et al.*, 1998). It also removes malodor associated with wounds (Efem, 1988; Subrahmanyam, 1991; Molan, 2002) and promotes autolytic debridement to facilitate the rapid development of a clean, granulating wound bed (Subrahmanyam, 1998). A rapid rate of healing has been reported in chronic wounds treated with honey (Efem, 1988; Wood *et al.*, 1997). Continuous application of honey for 7-10 days enhances sterility in infected wounds as reported earlier (Efem, 1988). Honey exhibits broad-spectrum antimicrobial activity against more than 80 species (Molan, 1992; Blair and Carter, 2005). Honey has a complex chemistry and its activity varies markedly based on its source (Molan, 1992). Depending on the floral source, climate and harvesting conditions the antimicrobial properties of a honey also varies (Molan, 1992; French *et al.*, 2005). However, almost all-natural honey contains flavonoids, phenolic acids, Superoxide Dismutase (SOD), Reduced Glutathione (GSH), Millard reaction products and peptides. Most of antioxidant effect exhibited by these compounds is because of its synergistic properties (Alvarez-Suarez *et al.*, 2010). Many studies illustrating the antimicrobial activity of honey have been published (Al-Waili and Haq, 2004; Blair and Carter, 2005; Molan, 2006).

Mechanism of Action

The precise mode of action of honey has not yet been fully understood, but acidity, osmolarity, generation of hydrogen peroxide and other phytochemical components such as alkaloids, flavonoids are considered important for its antimicrobial activity (Molan, 1992; 1999). Four major properties of honey were responsible for its antimicrobial activity. Initially, honey dehydrates bacteria by withdrawing the moisture out of wound environment. The high sugar content of honey is also hinders the growth of a microbe. But the inhibitory effect of honey on bacteria is independent of its sugar content (Cooper *et al.*, 2002a; 2002b). The high osmolarity of honey protects the skin from maceration and its high viscosity prevents cross-infection of wounds (Bergman *et al.*, 1983; Efem, 1988; Subrahmanyam, 1991; Moore *et al.*, 2001; Molan, 2006). Secondly, the honey is acidic in nature at a pH range between 3.2 and 4.5, which inhibit the growth of bacteria. Thirdly when honey is diluted, it continuously produce hydrogen peroxide by an enzymatic action of glucose oxidize which inhibits bacterial growth (Al-Waili and Haq, 2004; Eddy and Gideonsen, 2005; Molan, 2006). It is reported that the honey is capable of inhibiting common wound infecting bacteria species including *Staphylococcus aureus* even when diluted 10 times or more (Cooper *et al.*, 1999). The minor contribution by several phytochemical factors is also responsible for

antimicrobial activity (Al-Waili and Haq, 2004). The presence of unidentified phytochemical in honey is responsible for its high antimicrobial effect which is also resistant to heat and light (Molan, 1992; Olaitan *et al.*, 2007). All these physical and chemical factors give honey unique property to act as a wound dressing (Basualdo *et al.*, 2007).

Honey is of three types namely hydrogen peroxide honey (Pasture honey), non-peroxide honey (Manuka honey) and artificial honey. Based on its concentration honey can act as either bactericidal or bacteriostatic in nature. Pasture honey (4-8%) and Manuka honey (5-11%) were bacteriostatic whereas bactericidal at 5-10 and 8-15% (v/v) concentrations, respectively. Whereas, artificial honey (sugar solution) was bacteriostatic at a concentration of 20-30% and does not exhibit any bactericidal effect (Mandal and Mandal, 2011). Previously, a number of *in vitro* studies demonstrated the antimicrobial activity of honey against MRSA (Maeda *et al.*, 2008). The MRSA strains were more sensitive to Manuka honey due to the presence of methylglyoxal which is the dominant active antimicrobial component (Adams *et al.*, 2009). The mean minimum inhibitory concentration values of Manuka and Pasture honey against MRSA (6.9 and 7.1% v/v, respectively) (Cooper and Molan, 1999). *In vitro* clinical isolates of Methicillin-susceptible and Methicillin-resistant staphylococci were shown to be equally susceptible to Manuka honey with minimum inhibitory concentration reported as 3% (v/v) (Cooper *et al.*, 1999; 2002).

In staphylococci, *alt* gene 17 encodes for Murein hydrolases enzyme that is highly sensitive to manuka honey. A deficiency in these enzyme leads to failure in cell separation (Rice *et al.*, 2003). It is reported that exposure of *Staphylococcus aureus* with 10% (w/v) manuka Honey for period of 4 h found to interrupt the cell cycle during cytokinesis (Jenkins *et al.*, 2011).

Dressing procedure with honey was generally less painful (Subrahmanyam, 1991). This is due to the ability of the honey to maintain the moisture of the wounds without adhesion to the granulating surface. The clinical use of honey has been its success in eradicating MRSA from colonized chronic wounds (Eddy and Gideonsen, 2005; Visavadia *et al.*, 2008). It has used successfully on DFU patients who was threatened with amputation (Eddy and Gideonsen, 2005).

DFUs usually take a longer time to heal. For this reason, the affordability for the treatment was an issue. Therefore, the overall cost for wound dressing with honey is relatively cheaper and hence it does provide an economical and practical option for the management of wound ulcers in diabetic patients.

Chitosan

Chitosan is a polymer obtained from the shells of crustaceans which is highly biocompatible and

biodegradable (Foda *et al.*, 2007; Lee *et al.*, 2009; Jayakumar *et al.*, 2011). The bacteriostatic and fungistatic properties of chitosan are particularly useful in the wound care management (Sun and Li, 2011). Chitosan incorporated dressings used in the wound management system because of their unique properties like biocompatibility, biodegradability, non-toxic nature etc.

Mechanism of Action

It works by creating a positive charge, hence interacting with negatively charged molecules such as gram-positive bacteria, blood cells, proteins and lipids (Lee *et al.*, 2009). Chitosan also has an accelerating effect on wound healing (Jayakumar *et al.*, 2011) by activating immune cells through its cytokine production, giant cell migration and stimulating type IV collagen synthesis (Mezzana, 2007).

KytoCel is an absorbent dressing composed of natural, biodegradable acylated chitosan fibers. These fibers bond with wound exudate to form a clear gel that locks-in fluid and absorbs pathogens. The absorbent properties of KytoCel enable it to bind and lock away commonly encountered wound pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and MRSA, thereby reducing wound bioburden and the risk of cross-contamination at dressing change (Li *et al.*, 1992; Khor and Lim, 2003; Foda *et al.*, 2007).

Maggots

Several ancient tribes and Mayan healers use maggots (*Lucilia sericata*) to treat chronic wounds (Parnés and Lagan, 2007). Maggot therapy removes dead tissues in order to promote healing (Mumcuoglu, 2012). It is also known as larval therapy, biosurgery, therapeutic myiasis, Maggot Debridement Therapy (MDT), bio debridement (Singh *et al.*, 2014). However, its use was declined in late 1900s due to introduction of antibiotics and surgical procedures in the treatment of wound (Chan *et al.*, 2007).

Mechanism of Action

Use of maggots is helpful in achieving wound disinfection (Sherman *et al.*, 2007; Tantawi *et al.*, 2007), wound healing (Horobin *et al.*, 2006) and biofilm inhibition. Maggot feeds on dead tissues, exudates and cell debris in the wound (Parnés and Lagan, 2007). Disinfection of wound achieved by ingestion of bacteria from infected tissues. Maggots secrete proteolytic enzymes that include collagenase (Ziffren *et al.*, 1953) and trypsin and chymotrypsin like enzymes which aid in lysis of dead tissue and bacteria (Steenvoorde *et al.*, 2005). Other alkaline medium containing calcium, urea and ammonium

bicarbonate also secreted by maggots that further inhibit bacteria growth (Chan *et al.*, 2007). A number of studies demonstrated that all microorganisms were eradicated within 10-15 min after ingestion by the maggot (Simmons, 1935).

The primary purpose of maggot therapy is the breakdown of necrotic tissue and transform into an acute wound (Beasley and Hirst, 2004). A study by Courtenay suggested that maggot therapy is an extremely efficient alternative option for fast and efficient wound management (Courtenay *et al.*, 2000). Although, maggot therapy promoted wound healing in few studies, its effectiveness compared to other standard therapy is still under argument (Beasley and Hirst, 2004). There is limited literature on patient experiences on maggot therapy.

Dressing Methods with Synthetic Antimicrobial Agent

Iodine

In 1839, Davies first used aqueous potassium iodine. Later, Iodine used in aqueous and alcoholic preparations for hand washing and skin preparation prior to surgery (Leaper and Durani, 2008). However, earlier use of iodine resulted in undesired side effects such as skin irritation and staining. It is toxic, but dose-dependent is not harmful to humans but will be detrimental to bacteria (Leaper and Durani, 2008).

Mechanism of Action

Molecular iodine is active against bacteria, fungi and viruses. Iodine's exact antimicrobial mode of action is not fully understood, but it is believed to be associated with its ability to rapidly penetrate the cell wall of microorganisms and denature proteins, nucleotides and fatty acids leading to cell death (Chang, 1971; McDonnell and Russell, 1999). Iodine denatures proteins and enzymes by binding to thiol and sulphhydryl groups and alters phospholipid membrane structures by blocking hydrogen bonding (Leaper and Durani, 2008).

Iodine released from the dressing transported by blood, which is readily absorbed in thyroid. Therefore, use of iodine-based dressings has declined because of its toxic effects (Simon *et al.*, 2009). However, it is believed that the development of products such as cadexomer iodine has reduced the number of iodine-associated toxicity cases (Michaels *et al.*, 2009). In addition, it has suggested that the overall risk of toxicity to normal patients is minimal, but caution should be taken with children, pregnant or lactating women, patients with previously existing thyroid dysfunction or extensive burns (Leaper and Durani, 2008).

Iodine dressings based on its physical characteristics exists in two distinct forms cadexomer iodine and Povidone iodine. Cadexomer iodine is a starch lattice that contains 0.9% iodine micro beads (Sundberg and Meller, 1997). A sustained release of iodine from cadexomer iodine exhibit antimicrobial activity by absorbing excess exudates in the wound bed (Fleck, 2006). Povidone iodine is an iodophor which is referred as polyvinylpyrrolidone iodine (PVP-I). PVP-I is composed of hydrophilic elemental iodine and a synthetic polymer (Mayer and Tsapogas, 1993). PVP-I on microbial cells affects the structure and functions of cellular proteins by blocking hydrogen bonding and altering the membrane structure (Cooper, 2004). Both cadexomer iodine and PVP-I are highly effective against MRSA. Lacey and Catto (1992) determined that more than 99% of MRSA cells were killed within 10 sec of exposure to Povidone-iodine. Mertz *et al.* (1999) found that Cadexomer iodine significantly reduced MRSA. Effectiveness of Cadexomer iodine, *in vivo* against *Staphylococcus aureus* and MRSA was also established (Percival *et al.*, 2005).

Silver

In medical field, the usage of silver was documented for the past thousands of years (Sinclair and Ryan, 1993; Thomas and McCubbin, 2003a). The use of Silver Nitrate (AgNO₃) to treat ulcers was demonstrated in 18th century (Klasen, 2000). In 1920s, the use of colloidal silver in wound management was accepted by the US Food and Drug Administration (FDA) (Demling and Desanti, 2001). However, after the introduction of penicillin in the 1940s, antibiotics became the standard treatment for bacterial infections and the use of silver diminished. More recently, clinicians have turned back to silver for wound dressings because of the emergence and increase of antibiotic-resistant bacteria like MRSA (Gemmell *et al.*, 2006).

Silver contained dressings are available in a variety of forms such as foams and hydrocolloids constituting free silver ions as the active ingredient (Ovington, 2001). The potency of silver as an antimicrobial agent was found to be depends on the amount and rate of free silver released onto the wound bed (Lansdown, 2002). Silver dressings may contain different forms of silver such as; silver nitrate, silver ions or silver-based crystalline nanoparticles (Bradshaw, 2011). The exact composition of these dressings was difficult to determine because many of the manufacturers would not disclose their product ingredients. It has suggested that elemental silver has little or no antimicrobial activity. However, its cationic form is highly active. In the presence of wound exudate, silver readily ionized to form cations (Lansdown, 2004). But the amount of silver released from the dressing differs

between manufactures (Lansdown, 2004). The amount and rate of free silver released onto the wound surface will influence its antimicrobial activity and Lansdown has reported that levels in excess of 20 mg/L demonstrate the best results (Lansdown, 2002).

Silver is more universally effective against the growth of fungi, viruses, yeast, gram negative and gram-positive bacteria including MRSA. At particular concentrations, ionic silver kills detrimental microbes without affecting the proliferating granulation tissue (Forrest, 1982). Research has shown silver dressings are effective against MRSA (Maple *et al.*, 1992; Russell *et al.*, 1994; Wright *et al.*, 1998; Fleck, 2006). Loh *et al.* (2009) tested MRSA isolated from wounds for known silver resistance genes and although these were identified in some of the isolates, all strains were found to be susceptible to the silver dressing used (Bradshaw, 2011). Both nanocrystalline silver, as well as ionic silver showed similar antimicrobial efficacy on in vitro testing. There has been a lot of variation in the test methods used to determine silver concentration in numerous publications. There appears to be no standard technique (Maple *et al.*, 1992).

Mechanism of Action

In acidic environment of wound, silver ions converted to positive charged ionic salts and attracted towards negatively charged bacterial cell membrane, mediating the passage of silver into the bacterial cell (Lansdown, 2002; Brett, 2006; Michaels *et al.*, 2009). The interaction of silver salt with bacterial thiol (2SH) groups leads to inactivation, the blocking of key pathways such as cellular respiration and structural changes in the bacterial membrane and the blocking of enzyme and transport systems (Bradshaw, 2011). Silver bind to tissue proteins and disturb the cell membrane leading to the bacterial death. Silver also bind and denature the bacterial DNA and RNA, thus inhibiting cell replication (Tredget *et al.*, 1998; Wright *et al.*, 1998; Yin *et al.*, 1999; Lansdown, 2002; Fong and Wood, 2006).

In market a wide range of silver-based dressings are available with different therapeutic options. Some studies have demonstrated that silver is significantly cytotoxic towards fibroblasts and keratinocytes an essential components involved in wound repair (Burd *et al.*, 2007; Du Toit and Page, 2009). Sustained release of silver is essential to achieve wound healing. Acticoat, one of the silver dressing when moistened with sterile water and placed on the wound releases clusters of highly reactive silver cations up to 100 parts per million, causing block in electron transport leading to inactivation of bacterial cell DNA and cell membrane damage (Dritch *et al.*, 1987; Ovington, 2001; Lansdown,

2002). Acticoat 7 active against MRSA was demonstrated (Thomas and McCubbin, 2003b).

PHMB

Polyhexamethylenebiguanide (PHMB) has used as an antimicrobial agent for about 60 years including contact lens solution and pool cleaning. Recently, PHMB dressings introduced into wound management successfully. A wide range of PHMB dressings are available in market including gauze, drains and intravenous sponges and hydrogels (Motta and Trigilia, 2005; Moore and Gray, 2007). The PHMB molecule either chemically bound to dressing material or delivered freely into the wound tissues.

Mechanism of Action

The chemical structure of PHMB molecule is similar to the structure of Antimicrobial Peptides (AMPs). AMPs are produced by keratinocytes and inflammatory neutrophils to protect the wound infection (Sørensen *et al.*, 2003). AMPs works by interfering with the cell metabolism of microorganisms, preventing them from absorbing nutrients and disposing of waste products, which ultimately results in microorganism death while the host cells remain unaffected. It is believed that PHMB molecule and AMPs possess the same mechanism of action because of its structural similarity (Moore and Gray, 2007). The PHMB component of the dressing has a proven broad-spectrum action with efficacy against bacteria, fungi and yeasts (Mueller and Krebsbach, 2008; Müller and Kramer, 2008), but it does not have an adverse effect on healthy host cells (Larkin *et al.*, 1992; Moore and Gray, 2007; Gilliver, 2009).

A test of PHMB for biocompatibility resulted in less damage to healthy wound cells compared to other antimicrobial agents such as chlorhexidine, Povidone-iodine, triclosan, silver and sulfadiazine (Müller and Kramer, 2008). Suprasorb X+PHMB also successfully used to manage a MRSA-infected foot ulcer patient (Easy, 2015).

Conclusion

Diabetes is a metabolic disorder affecting function of a tissue. The individual with such metabolic disorder develop diabetic foot ulcers at certain part of their life, which is chronic in nature. The chronicity of diabetic foot ulcer increased with the presence of infectious microbes. Worldwide, an increase in antibiotic resistant bacteria such as MRSA had put the diabetic foot ulcer patient at high risk. To overcome these issues, selecting an appropriate dressing material is essential to reduce the morbidity and mortality of the patients. Compounds such as honey, iodine, PHMB and silver incorporated into dressings to provide antimicrobial

action and aid the healing process. Selecting an appropriate dressing material for diabetic foot ulcer colonized with MRSA is somewhat challenging because each product has its own benefits and drawbacks. Dressing choice depends on relative size and nature of the wound. Much of the published literature on the antimicrobial properties of silver, honey, PHMB, chitosan and iodine-containing dressings is contradictory, with varying degrees of efficacy reported. The mechanisms of action of these compounds are yet poorly understood. Further studies are required to determine the mode of action of these compounds, which is promising in the development of new antimicrobial dressings.

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Parthasarathy Ravichandran: Contributed to the acquisition, analysis and interpretation of the data. Drafted final approval of the version to publish.

Sai Prasad Chitti: Contributed sustainably to the design of study. Provided critical revision of the manuscript.

Ethics

All authors have read and approved the manuscript and declare that there is no conflict of interest.

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