Diabetic wound infection: A review on microbial population and infection control

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Abstract. A diabetic foot ulcer is one of the major complications of diabetes and it leads to lower extremity amputation in patients. This review explores the current research on microbial populations on diabetic wounds, and also treatment alternatives to combat the infection on chronic diabetic wounds. The microbial communities exist in diabetic wound infection are diverse. Microbes rarely survived in single species of planktonic cells. They usually exist in a complex polymicrobial biofilm population which consists of different types of microorganisms. Furthermore, the development of bacterial biofilm on the wound that usually consists of multidrug-resistant pathogens also delayed the wound healing. To overcome this problem, many types of modern wound dressing were developed including hydrocolloid, hydrogel, alginate and collagen wound dressing. Besides, modern biotechnological advancements such as cell therapy, bioengineered skin, dermal scaffolds, tissue-engineered artificial skin and growth factors were also employed to promote the recovery of the wound. In conclusion, diabetes mellitus is a major health care challenge worldwide. Diabetic patients are at risk for developing foot ulcer which ultimately leads to amputation; hence a safe and effective alternative treatment is required to improve diabetic patients' quality life.

Keywords: Diabetic wound, infection control, microbial population

INTRODUCTION

Diabetic foot ulcer is described as ulceration, infection and destruction of deep tissues, in a foot of person associated with neurological abnormalities and peripheral vascular disease in the lower limb (Van Netten *et al.*, 2019). Diabetic foot ulcer is one of the major complications of diabetes and it possibly leads to lower extremity amputation in patients. Furthermore, a study conducted from 2007 to 2008 in China reported that the prevalence of foot ulcer was 92.4 million for diabetes and 148.2 million for pre-diabetes

(Yang *et al.*, 2010). About 24% of diabetes patients underwent amputation within the first year after being affected with foot ulcers. This can be accessed via physical inspection such as the skin of vascular, neurological, and musculoskeletal systems (Alexiadou and Doupis, 2012). It is important to identify the early clinical signs of vascular damage in the limbs before ulcer formation such as atrophic foot, lack of hair growth on the dorsum, cool temperature of the limb, thin shining and atrophic skin (Ahmad,

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2016). Microbial infections become a major issue that may affect deep tissue, joints and bones (Noor *et al.*, 2017). The early stage of diabetic foot ulcer formation is the loss of sensation due to peripheral neuropathy factor (Pallela and Narahari, 2017). Due to the loss of sensation, the repetitive minor injuries may eventually lead to foot ulcer (Alexiadou and Doupis, 2012). Furthermore, the ulcers are prone to the colonization of various pathogenic microorganisms (Weledji and Fokam, 2014).

One of the issues associated with a diabetic ulcer is the expensive medical treatment cost. The total expenses on wound management products have increased by 4.6% annually to 28.7 billion USD in 2013. Diabetic patients with foot ulcer showed a 150-fold increased risk of amputation, which is always associated with poor wound management (Pednekar *et al.*, 2015). On the other hand, diabetic foot ulcer requires an intensive multidisciplinary assessment that usually handled by experts like nurses, doctors and specialists. Proper management of the ulcer may contribute to the avoidance of wound infection. A good wound management system significantly lowered the risk of amputation by simple practice on daily basis such as good footwear, chiropody, cleanliness, aggressive surgical radical debridement, off-loading, and ulcer management (Ahmad, 2016).

Diabetic wound infection

High bioburden delays the healing of infected wounds. The development of wound infection is an ongoing problem among diabetic patients. Usually, diabetic patients are more susceptible to develop infections as the immune system was diminished. Furthermore, severe wound infections also increase the risk of lower extremity amputations. Thus, wound infection causes prolonged hospitalization, thereby increasing the cost of the treatment (Tipton *et al.*, 2019). There are several classic clinical signs of infections including erythema, localized warmth around the site of wound, edema, purulence exudate and fever (Green, 2012). Proper care practices are necessary to prevent morbidity, especially amputations.

Generally, the wounds are easily infected once they are exposed to the colonization of pathogenic microorganism. Wound colonization

refers to the multiplying of various microbial species on a wound (Tipton *et al.*, 2019). This leads to wound infection due to the apparent microbial growth and colonization. *Staphylococcus aureus* is the most common bacterium found in a diabetic wound that leads to the alteration of wound healing (Gardner *et al.*, 2013). Hirsch *et al.* (2008) performed a study of wound healing on animal model inoculated with *S. aureus*. The results showed that *S. aureus* significantly impaired the wound healing of diabetic wound and the infection also promotes invasive co-infection of other bacteria.

Microbial species on diabetic wound

The microbial communities exist in diabetic wound infection are diverse. Microbes rarely survived in single species of planktonic cells. They usually exist in a complex polymicrobial biofilm population which is consist of different types of microorganisms (Peters *et al.*, 2012). The common bacteria in wound are *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter* spp. (Hirsch *et al.*, 2008). *S. aureus* and *P. aeruginosa* are the most common bacteria isolated from chronic wound (Fazli *et al.*, 2009; Serra *et al.*, 2015). Numerous studies have been conducted to study the role of multi-species pathogenic biofilms related to chronic diabetic wound before determining an effective method for wound infection treatment (Dalton *et al.*, 2011; Tipton *et al.*, 2020; Wu *et al.*, 2019). Various methods have been employed to identify the bacterial population in the chronic wound such as qualitative measures, quantitative measures, polymerase chain reaction, and metagenomics methods (Martin *et al.*, 2010; Wu *et al.*, 2019). However, these techniques require a substantial amount of repetition to obtain accurate results. To overcome this problem, newly developed techniques such as pyrosequencing, microarrays and next generation sequencing were used (Wu *et al.*, 2019). In such, a study was performed by Dowd *et al.* (2008) to determine the bacterial diversity in the chronic wound by utilizing pyrosequencing, denaturing gradient gel electrophoresis, and full ribosome shotgun sequencing. The study revealed that different bacterial populations were identified in

different types of wound which majored by 62% of an obligate anaerobic microorganism.

The obligate anaerobic bacteria found in diabetic foot ulcer are *Peptostreptococcus*, *Prevotella*, *Finnegoldia* and *Peptoniphilus* (Howell-Jones *et al.*, 2005). Granov *et al.* (2019) reported that about 74 to 95 % anaerobic bacteria found in the site of the infected wound were *Peptostreptococcus* and *Bacteroides*. Although the prevalence of anaerobic bacteria is less, it is still the cause of infections (Van Netten *et al.*, 2019). In contrast, Senneville *et al.* (2006) reported that anaerobic bacteria play a minor role in diabetic wound infection. Dezfulian *et al.* (2011) reported that *S. aureus* was the most frequently isolated bacteria from a patient with diabetic foot infections in Iranian Hospital. Furthermore, the finding is supported by Gonzalez *et al.* (2003) where they reported *S. aureus* was the predominant bacteria found in the infection site followed by *Enterococcus*, *Streptococcus*, and *Enterobacteriaceae* (Gonzalez *et al.*, 2003).

In fact, *S. aureus* has been found as an opportunistic pathogen that able to adapt easily in different environmental conditions (Nair *et al.*, 2014). The most commonly isolated bacteria from diabetic foot infection were *P. aeruginosa* contributing about (35%) followed by *S. aureus* (19%) and MRSA (6%) (Parsa and Samani, 2015). *P. aeruginosa* develops acquired antibiotic resistance due to its ability to secrete virulence factors such as pili, type III secretion systems, quorum-sensing molecules, extracellular products like pyocyanin, and polysaccharides (Sadikot *et al.*, 2005). Most *P. aeruginosa* strains secrete pyocyanin (N-methyl-1-hydroxyphenazine), a blue redox-active secondary metabolite with ability to induced production of hydrogen peroxide in both eukaryotic and prokaryotic cells (Lau *et al.*, 2004). This process causes cell death due to oxygen reduction. Colvin *et al.* (2012) stated that *P. aeruginosa* produced at least three types of polysaccharides to form biofilm. These polysaccharides form strong biofilm structure that protect them from the antimicrobial agents. Biofilm of *S. aureus* and *P. aeruginosa* have been well studied. The interaction of polymicrobial infections between these two microorganisms have been derived from both *in vitro* and *in vivo* studies. Besides, the conflict of *S. aureus* and *P. aeruginosa* also resulted in a chronic infection that can impair the wound healing process and

promote antibiotic resistance (Colvin *et al.*, 2012; Short *et al.*, 2014). In agreement, Pastar *et al.* (2013) demonstrated the interactive effect of MRSA and *P. aeruginosa* by utilizing porcine wound healing model. The co-infection works well in slowing down the healing process and limits the antibiotic response. This implication significantly indicates the synergistic effect of both pathogenic species. In the following years, Serra *et al.* (2015) published a review on the role of *S. aureus* and *P. aeruginosa* in a chronic wound. They observed that these most common bacteria isolated from chronic wound exhibit a few virulence factors which make them acquired antibiotic resistance.

Biofilm in diabetic wound

Biofilm is developed when any group of microorganisms embed to each other and form a matrix of extracellular polymeric substance (Wei *et al.*, 2019). The development of bacterial biofilm usually consists of multidrug-resistant pathogens that cause hospital-acquired infection. It has been reported that different microbial species were found on wound biofilm (Elias and Banin, 2012). Besides, according to the National Institute of Health, 65 to 80% of chronic infections are associated with biofilm formation including oral soft tissues, dental implants, urinary tract prostheses, and lung tissues. Since then, various strategies have been employed to prevent and eradicate the biofilm formation. Majorities of microbial biofilm studies in the last decade were based on *in vitro* models such as microtiter plate and continuous flow chamber/reactor (Fabrega *et al.*, 2009; Durmus *et al.*, 2012). Although limited evidence was collected to comprehend the biofilm formation using these techniques, the outcomes of the studies provide important information on biofilm survival, eradication and mechanisms of microbial cell in biofilm development.

Extracellular polysaccharides are the main components in the biofilm matrix. These polysaccharides help the bacteria to adhere to a host cell and protect them from environmental stress (Limoli *et al.*, 2015). Bacterial biofilms delay the diabetic wound healing and it is also crucial to demonstrate the role of biofilms in delaying the healing of the wound (Percival *et al.*, 2015). These polysaccharides block complement activation, depress the lymphoproliferative response and prevent opsonin detection on bacterial cell wall by

phagocytes, hence the wound recovery is delayed (Bunyan *et al.*, 2019). Besides, biofilm also limits the leukocytes to the wound and attenuates their ability to produce antimicrobial reactive oxygen species (Campoccia *et al.*, 2019). More importantly, the exopolysaccharides in the biofilm serves as a physical barrier, which is a cause of antibiotic resistance. The polysaccharides slow down the diffusion of antibiotic to the wound (Bunyan *et al.*, 2019). Various studies were conducted to investigate the wound associated with diabetic patients. Several *in vivo* models were used for biofilm study. The formation of biofilm delayed the wound healing of diabetic rats infected with *P. aeruginosa* (Zhao *et al.*, 2010).

Biofilm is a unique community where different types of microorganisms work synergistically in forming a structure. Numerous stages of biofilm development were reported, including initial attachment, irreversible attachment, maturation I, maturation II and dispersion (Monroe, 2007). Initially, the development of biofilm begins with the free-floating of microbial cells at the wound surface. At this stage, the movement of the bacterial communities was driven by flagella and type IV pili that promote the colonization of micro-colony against subtracting (Conrad, 2012). The planktonic cells swim along the surface in Brown motion. Colonization of bacteria communities is formed *via* Van der Waals interaction between the outer cell wall of bacterial cell with the surface (Renner and Weibel, 2011). Then, this situation leads to the development of the matured three-dimensional structure of biofilm followed by dispersion (Renner and Weibel, 2011). During colonization, the communities of the cells can communicate with each other using a chemical signal called quorum sensing which also coordinates the gene expression (Geske *et al.*, 2005). Quorum sensing is a mechanism where the cells produce and deliver their signals called auto-inducers to increase the cell density (Hense and Schuster, 2015). Thus, quorum sensing is another factor contributing to antimicrobial-resistant and it accelerates the growth of biofilm (Flickinger *et al.*, 2011).

Furthermore, biofilms can grow virtually in any surfaces with several requirements. Environmental factors including pH, temperature, rheological as well as adhesive

properties, need to be considered in biofilm formation (Garrett *et al.*, 2008). Basically, the biofilm is actually an adaption of the microbial cells with the environmental conditions that vary on several stresses (Zhang *et al.*, 2013). O'toole *et al.* (2000) discovered that genes and regulatory circuits are important mechanisms for initial cell-surface interaction, biofilm maturation and the return of biofilm microorganisms to a planktonic mode of growth. Antibiotic treatment of diabetic wound with biofilm formation has always been challenging. The efficacy of systemic antibiotic therapy was deduced by 25 to 20% with the biofilm formation (Wei *et al.*, 2019). Besides, the drug resistance of bacteria can be increased to 1000 to 1500 times after biofilm formation, compared to the planktonic cells (Mendoza *et al.*, 2019). Antibiotic resistance of wound bacteria has a significant influence on wound healing among diabetic patients.

Infection control

Wound dressing is one of the primary treatments against foot ulcer. The dressing is designed to maintain moisture, promote wound healing, lower the risk of scar formation and protect the wound from any other impairments (Moura *et al.*, 2013). Traditionally, bandages, plaster, lint and gauze were used in wound management. However, traditional wound dressing failed to provide a moist environment and maintain the pH of wound sites (Boateng *et al.*, 2008). To overcome this problem, many types of modern wound dressing were developed include hydrocolloid, hydrogel, alginate and collagen wound dressing (Dhivya *et al.*, 2015; Weller *et al.*, 2020). Hydrocolloid dressings are composed of hydrocolloid materials such as gelatin, pectin, and carboxymethyl cellulose which provide a moist environment for wound healing and barrier to external microorganisms (Dorner *et al.*, 2009). In addition, hydrocolloid dressings are able to absorb a large amount of wound fluid and exudates for a sustained period. Thus, the frequency of dressing changes can be reduced (Hilton *et al.*, 2004). Due to its oxygen impermeable nature, the dressing increases the rate of collagen synthesis during wound recovery (Oliveira *et al.*, 2020).

Hydrogel dressing is commonly used for necrotic, sloughy, granulating, epithelializing wounds (Moody, 2006). It is designed in a

hydrophilic polymer matrix which made up from synthetic polymer like poly (methacrylates) and polyvinyl pyrrolidone with high water content (Dhivya *et al.*, 2015). It is suitable for all four stages of wound. Dumville *et al.* (2013) conducted a study by using hydrogel wound dressing on a diabetic foot ulcer. The study showed that hydrogel dressing was more effective than basic wound dressing on diabetic foot ulcer and there is no statistical difference in a number of heal ulcer with larval therapy, platelet-derived growth factor, and a different brand of hydrogel dressing. In order to meet a high-quality wound dressing, researchers have also exploited smart polymers to cooperate with hydrogel. For instance, N-isopropylacrylamide (NIPAM), a thermo-responsive drug carrier is grafted within the hydrogel layer in dermal patches. The substance becomes hydrophilic at a temperature of 32°C and below, but hydrophobic at the higher temperature. This condition can be activated through copolymerization for optimal drug release (Wei *et al.*, 2009). The charge of the hydrogel materials can be also modified to control the release of the drug to the wound, thus can frequency of dressing changing can be reduced (Venault *et al.*, 2019).

Alginate dressing is another type of modern dressing used in direct contact with a wound. Alginate is a natural polymer derived from brown seaweed (Hilton *et al.*, 2004). Alginate has a higher absorbency compared to normal dressing where it can absorb 20 times of their weights which make them effective to be used for mild to heavy exudate (Baker, 2005). A controlled random trial performed by Lalau *et al.* (2002) showed that alginate wound dressing improved the wound healing of diabetic patients compared to normal dressing. However, alginate dressing displays a few disadvantages that limit the usage; it may be painful for a patient with small amount of exudate since it requires a large moisture content to function effectively and it always require a secondary dressing which will increase the cost of dressing changes (Dabiri *et al.*, 2016). Furthermore, there is no evidence on the effectiveness of alginate dressing in healing diabetic foot ulcer (Dumville *et al.*, 2013). Latest advanced wound dressing was made up of collagen. Due to its chemotactic properties, it stimulates new tissue growth by migrating cells

like fibroblasts and keratinocytes (Fleck and Simman, 2010). However, collagen dressing triggers an allergic response in some patients (Chattopadhyay and Raines, 2014). Recently, a clinical study by Djavid *et al.* (2020) also revealed that the usage of collagen matrix dressing significantly accelerated the healing to hard-to-heal diabetic foot ulcers.

Recently, various types of antimicrobial agents, nanoparticles and natural products have been incorporated into the wound dressing. Unfortunately, the choice of antimicrobial agent showed no clear evidence if one better than others in treating diabetic food ulcer because the data from previous reports were heterogeneous (Selva *et al.*, 2015). The inappropriate and indiscriminate use of antibiotics is a key reason contributing to antibiotic resistance in microorganisms. Silver finished cottons are widely used in wound care products. In addition, an *in vivo* study conducted by Kostenko *et al.* (2010) on animals mimic the condition found in a bed wound showed that silver-based wound dressing exhibited a significant reduction number of biofilm viable cells in 7 days treatment. Kalishwaralal *et al.* (2010) reported that silver nanoparticles eradicate the biofilm production in *P. aeruginosa* and *S. epidermis* by 98%. Despite of this, silver-finished product could lead to increased morbidity with chronic ingestions. Moreover, inhalation of heavy metal preparations also leads to deposition of heavy metal particles in the skin, eyes, kidneys and livers (Rovira *et al.*, 2015). In other words, due to the exposure or ingestion of excessive silver element, the skin discoloration in the wound area or argyria appeared (Baker *et al.*, 2007). This skin staining usually remained visible after treatment (Zweiker *et al.*, 2014). For the silver ion to function as an effective bactericidal agent, it needs to reach a concentration of 30-40 ppm (Murphy and Evans, 2012). However, as recorded by Poon and Burd (2004), the silver ion happens to be toxic to keratinocytes and fibroblasts at 33 ppm and above, especially. There are a thousand types of wound dressing available in last decades in different shapes, size and color. However, it is a challenging task for clinicians to find the most appropriate wound dressing for different types of wound as no single wound dressing is suitable for all wound treatment.

Many advanced technologies were developed to help the recovery of wound, these modern therapies including cell therapy, bioengineered skin, dermal scaffolds, tissue-engineered artificial skin and growth factors (Debels *et al.*, 2015; Falanga, 2020; Frueh *et al.*, 2017). However, they are expensive and not easy to use. Negative pressure wound is another therapy that may represent a treatment that uses vacuum to remove access wound fluid that usually enhances and promotes wound healing (Bovill *et al.*, 2008). However, the results were inconsistent. A comparative study by Ravari *et al.* (2013) demonstrated that vacuum-assisted closure displayed a significant improvement of the wound after 2 weeks with no single amputation case was reported. However, moist wound dressing did not show significant improvement in the recovery of diabetic foot ulcer. Besides, tissue engineering can be applied to substitute the damaged skin and to improve new tissues regeneration. The artificial skin is highly useful for treating chronic wounds; however, this method is highly expensive (Langer and Rogowski, 2009).

CONCLUSION

In conclusion, diabetes mellitus is a major health care challenge worldwide. Clinically, diabetic patients are at risk for developing foot ulcer which ultimately leads to amputation. Besides, wound healing in diabetic patients is frequently impaired where the delay in healing time is commonly associated with microbial infection. Due to this, a safe and effective alternative treatment is required to improve diabetic patients' quality life.

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