Microbiology and malodourous wounds

KEY WORDS
- Leg ulcers
- Malodour
- Odourants
- Volatiles

People with chronic wounds often have malodour problems because of their heavily colonised wounds. This causes social and psychological problems for the patient and their family. Most microorganisms produce a wide array of volatile chemical molecules and the common types of chronic wound contain a mixture of aerobic and anaerobic organisms which produce pungent odours. Reduction in bioburden or absorption of volatile odours can help reduce the smell associated with chronic wounds but ultimately closure of the wound is the key. Patients with fungating wounds need to be managed with the correct topical treatments to reduce bioburden and have good dressings made available to them to help reduce odours.

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Chronic wounds are colonised with a mixture of aerobic and anaerobic bacteria and occasionally fungi. Recent studies show that in the wound up to 80% exist as a biofilm (Wolcott et al, 2013; Malone et al, 2017). Chronic wounds can be difficult to heal due to a number of issues including poor vascularity and the microorganisms proliferate in these conditions, accounting for the malodour and profuse exudate. The organisms often do not cause active infection, but the resultant biofilm makes wound healing difficult. This has consequences for the patient in terms of a non-healing exuding wound and associated malodour and these can lead to a range of problems such as depression, lack of self-respect and self-esteem to name a few (Bale et al 2004). The negative impact of these wounds on an individual's quality of life (QoL) have been reported, citing odour as being distressing, leading to social isolation, depression, feelings of guilt and repulsion (Gethin et al, 2014; Ousey and Roberts, 2016).

Biofilms are difficult to remove unless active wound management procedures including aggressive wound cleansing and debridement and application of topical antimicrobial agents are used (Phillips et al, 2010). In a study on fungating malignant breast cancer wounds where no infection was present, the microbiology showed that biofilms were present, and they were colonised with mixed species of bacteria, with an average number per patient of 3.6 aerobic species and 1.7 anaerobic species. The presence of anaerobic bacterial strains was evidenced in 70% of the wounds; biofilm was observed in 35% of the cases. Odour was a reliable indicator of colonisation by anaerobes (Fromantin et al, 2013). Other microbiologically based studies of chronic wounds and malodour have demonstrated the presence of mixed microorganisms and demonstrated the presence of anaerobes as a major contributor to malodour in wounds (Bowler et al, 1999).

Many bacteria possess a wide range of enzymes that allow them to break down a variety of substrates including lipid, protein, carbohydrates, and other hydrocarbons, and by-products of any these biochemical processes may produce volatile end products with associated odour. Many of the common aerobic bacteria isolated from chronic wounds are members of the family Enterobacteriacae (Gram negative aerobic bacilli) which are commonly found in human faeces. In addition, many chronic wounds are also colonised by anaerobic bacteria which are also found in the gastrointestinal tract (Dowd et al, 2008). These bacterial species are known to produce odourous volatiles such as indole, skatole, and thiols (sulfur-containing compounds), as well as the inorganic gas hydrogen sulfide. These are the same compounds that are responsible for the pungent odour associated with faeces and flatulence. In addition, anaerobic bacteria are well known for producing unpleasant odours as the result of the anaerobic digestion of a range of substrates. In particular, the breakdown of a variety of amino acids produce strong smelling short chain fatty acids such as *n-butyric*, *n-valeric*, *n-caproic*, *n-propanoic*, and iso valeric acids (Holdeman et al, 1977). Other odours such as cadaverine and putrescine are released from necrotic tissue and are foul-smelling organic chemical compounds produced by the breakdown of amino acids in dead tissue (O Brien, 2012).

Anaerobic bacteria found in wounds with malodour and known to produce odourous volatile metabolites

Table 1. Odour assessment tool (Haughton and Young 1995)	
Strong odour	Evident on entering the room with dressing intact
Moderate odour	Evident on entering room with the dressing removed
Slight odour	Evident close to the patient with the dressing removed
No odour	No odour evident with dressing removed

include *Bacteroides sp*, *Clostridium sp*, *Prevotella sp*, *Porphyromonas sp*, and *Fusobacterium nucleatum* (Bowler et al, 1999). Aerobic bacteria associated with malodour have been identified as *Proteus sp*, *Klebsiella sp*, *Pseudomonas sp*, and methicillin-resistant *staphylococci* (Thomas et al, 2010).

Common wound pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* produce an array of volatile compounds and these odours are often the first identifying feature of the bacteria. *S. aureus* smell (in my personal view) cheesy and *P. aeruginosa* smell fishy. Several studies using head space analysis of the volatiles produced have been used to identify these common pathogens and two studies (Filipiak et al, 2012; Zhu et al, 2010) assessed the suitability for diagnostic purposes using gas chromatography mass spectrometry and electrospray mass spectrometry. They were able to identify the organisms on the basis of the volatile mixtures they produced.

HOW DO WE IDENTIFY ODOURS?

There can be difficulties quantifying and describing malodour in the wound as individuals differ in their ability to smell and describe odours. Haughton and Young (1995) devised a method for quantifying odour with an odour assessment scoring tool (*Table 1*).

The human nose has over 5 million scent receptors and can detect very low concentrations of volatile chemical compounds measured in parts per billion (ppb), or less in air. These receptors have an affinity and can distinguish between a range of odours that activate neurons in the olfactory nerve and ultimately the central nervous system. Small changes in the chemical composition of the volatiles can change the smell that is perceived by the receptor and some odours perceived as pleasant by one individual may be perceived as unpleasant by another. In addition, many individuals attribute a smell or an odour with something they have come into contact with in their environment. An interesting example of this from a microbiology perspective is that the bacterium *Clostridium difficile* produces an odour supposedly similar to that of elephant dung! Odours can also be a trigger for a range of reactions, particularly appetite (most people love the smell of freshly baked bread). A more detailed analysis of each aspect of the human olfactory system and comparison with electronic noses can be found in Gardner and Bartlett (1999).

Individuals are acutely aware of body odours and as such hygiene has become an important aspect of everyday lives. Halitosis (bad breath), smelly feet, flatulence and body odour are the major problems that can cause embarrassment for individuals and the volatile chemical compounds that cause these smells have been identified. For example, in the majority of cases of bad breath, it is the production of volatile sulfur compounds that is the problem. *Hydrogen sulfide* has a smell of rotting eggs, *methanethiol* and *dimethyl sulfide* have the smell of rotting cabbage, or garlic. At low levels, these compounds cannot be detected by the human nose, but when there are high levels of bacteria and as such high levels of the by-products, then these volatile compounds become detectable and bad breath becomes a problem. The threshold to detect *hydrogen sulphide* is 0.00047 parts per million (Franklin, 2013). In order to reduce bad breath, good oral hygiene is recommended using a tooth brush to loosen bacteria attached to the teeth where they grow as a biofilm and a toothpaste containing the antiseptic, fluoride. In extreme cases, an individual may be referred to a dental hygienist to remove the buildup of plaque (mature biofilm) in the tooth crevices and in between the teeth by physically scraping this away. Reducing bacterial numbers reduces the levels of the volatiles that cause bad breath.

In the moist areas of the human body, for example, axillae, groin and feet, bacteria can proliferate on the skin, some utilising fatty acids and other molecules. When they do so, a consequence of their growth is the production of by-products, some of which are volatile molecules resulting in body odour. The smell of body odour is characteristic and constitutes a number of chemical volatile molecules including two organic compounds, *3-methyl-2-hexenoic acid* (which smells goat-like), *3-hydroxy-3-methylhexanoic acid* (which smells cumin like) and sulphur containing compounds (Troccaz et al, 2004).

Foot odour is often described as cheesy and two main compounds contributing to the cheesy smell are propanoic and isovaleric acid. Propanoic acid has a pungent, sour and rancid odour, whilst isovaleric acid is described as cheesy, rancid and fermented (Katsutoshi et al, 2006). Interestingly, isovaleric acid is actually the result of a bacterium used in the production of some strongly pungent cheeses. Also, propanoic and isovaleric acid are produced in high amounts by anaerobic bacteria

Table 2. Compounds associated with specific odour description

Compound	Odour description
Acetic acid	Sour
Isobutyric acid, Isovaleric	Cheesy
Butyric acid	Cheesy and vomit
3-methyl-2-hexenoic acid	Goat like
dimethyl sulfide, methaniol	Rotting cabbage
Hydrogen sulphide	Rotting eggs

Table 3. Products used to manage odour

Products	Description
Charcoal	Absorbs odours
Silver	Reduces bioburden
Honey	Reduces bioburden and reduces odours
Iodine	Reduces bioburden
Metronidazole	Reduces bioburden
Essential Oils*	Masks odours

Adapted from Akhmetova et al 2016. *Essential oils (fragrant plant oils) should only be used under the supervision of a clinically trained complementary therapist

following the breakdown of amino acids. Anaerobic bacteria are frequently found in heavily colonised chronic wounds such as leg ulcers and contribute to the rancid smells sometimes described by practitioners (O Brien, 2012) *(Table 2)*.

In order to minimise our bodily smells, we bathe or shower frequently to reduce the numbers of bacteria and associated odours. In addition, most individuals will apply deodorants that contain pleasant smelling molecules that help to mask the unpleasant smell of body odour. Some individuals use antiperspirants that reduce moisture production in these areas, which the bacteria need to grow and produce odour. Currently similar principles are used to reduce the smell of malodourous wounds.

HOW TO MANAGE MALODOUR

From a microbiologist's perspective the most appropriate way to manage malodour in wounds is a very similar approach to reducing odours in other areas of the body *(Table 3)*. That is, reducing bioburden and the bacteria responsible for producing the odours and masking the smells with deodorants. Biofilm based wound care for chronic wounds is being promoted for all types of wounds including venous leg ulcers, pressure ulcers and diabetic foot ulcers (Malone and Swanson, 2017, Wolcott et al 2009; Wolcott et al 2010). Studies have shown that in diabetic foot ulcers, economic savings to this approach can be as high as 68% (Wolcott, 2015).

Biofilms are also a problem in malignant fungating wounds, where cancerous cells invade the epithelium and infiltrate supporting blood and lymph vessels resulting in a loss of vascularity and leading to tissue death and necrosis. For these and other wound types where an aggressive approach of debridement, cleaning and application of a topical antimicrobial may not be possible, the best way to minimise odour is to reduce the numbers of bacteria in the wound using topical antimicrobials, absorb the volatiles or mask the odours (O' Brien, 2012). This can be difficult for these wound types and a different approach may have to be taken depending upon the individual wound and treatment, especially in malignant wounds. The approach often shifts from healing the wound to maintaining quality of life; falling into 2 categories: physical and psychological management (O'Brien, 2012). Therefore, the following approaches should be used:

1. Cleaning and gentle debriding the wound to reduce organism numbers
2. Application of a topical antimicrobial agent to reduce bacterial numbers
3. Application of odour absorbing dressings to reduce odours (Grocott, 2000)

Typical treatment used by practitioners in these cases is administration of systemic metronidazole (targeting the anaerobic bacteria in the wound), topical metronidazole gel and/or antimicrobials (antiseptics which are broad spectrum) and odour absorbing dressings (Thomas et al, 1998, Williams 2000).

COULD WE USE MALODOUR AS A DIAGNOSTIC MICROBIOLOGICAL METHOD?

Most wound care practitioners would say that the unpleasant smell from a leg ulcer colonised with certain bacteria emits a characteristic smell, for example *Pseudomonas aeruginosa*. This concept has not gone unnoticed and some researchers developed the electronic nose for biomedical applications in the 1980s and 1990s (Persaud and Dodd 1982, Persaud, 1992) which mimics the olfactory system. The electronic noses include three major parts: a sample system, a detection system and a computing system. The sample system generates the volatile compounds and these are injected into the detection system of the electronic nose, which consists of a sensor set (a sensor array) that reacts with the specific volatile molecules and creates a change of electrical properties. The specific response is transformed into digital values and the data then computed based on statistical models often using principal component analysis (Persuad and Dodd 1982).

Other researchers have developed biosensors which look for specific molecules from different bacterial species (Dargaville et al 2013; Trill 2007; Pavlou et al 2002; Wilson and Baietto 2011) and hopefully these will

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be developed into a diagnostic point of care test in the future. In addition, changes in odour could possibly be used as an early detector of infection or wound changes and an e-nose could possibly be another tool to be used to benefit patients in order to start antibiotic therapy specific to the causative bacteria (Ousey et al, 2017).

CONCLUSION

The future of wound care is very exciting because over the last decade microbiologists working with wound care practitioners have come to realise that bacterial colonisation and formation of biofilms markedly influence the chronicity of wounds. Knowing this, there is a huge potential to work together to formulate new wound management procedures and new products to either prevent wound biofilm formation or to disrupt the biofilm. If we can conquer these problems then associated problems such as high levels of exudate and malodour may become a thing of the past. Whilst we wait for these, we should be mindful of some simple strategies. Assume in a non-healing malodourous wound that there are high numbers of bacteria, probably in a biofilm and reduction of these bacterial numbers will help reduce odours. Use appropriate cleansing and debridement and keep applying a topical antimicrobial dressing until the problem subsides. The frequency of the suggested regular debridement procedure still needs to be fully determined but recommendations are proposed by Phillips et al (2010) in their publication *Biofilms Made Easy.* WUK

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