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Polymicrobial wound infections: Pathophysiology and current therapeutic approaches

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ABSTRACT

Acute and chronic wounds represent a very common health problem in the entire world. The dermal wounds are colonized by aerobic and anaerobic bacterial and fungal strains, most of them belonging to the resident microbiota of the surrounding skin, oral cavity and gut, or from the external environment, forming polymicrobial communities called biofilms, which are prevalent especially in chronic wounds. A better understanding of the precise mechanisms by which microbial biofilms delay repair processes together with optimizing methods for biofilm detection and prevention may enhance opportunities for chronic wounds healing. The purpose of this minireview is to assess the role of polymicrobial biofilms in the occurrence and evolution of wound infections, as well as the current and future preventive and therapeutic strategies used for the management of polymicrobial wound infections.

1. Introduction

Acute and chronic wounds affect millions of people in the entire world (Demidova-Rice et al., 2012). The acute wound repair process has four phases partially superposed in time and space, *i.e.* (i) coagulation (during this phase, the platelets adhere to the damaged blood vessels and initiate the blood-clotting cascade with hemostatic and protective roles; the platelets are also releasing chemotactic factors for mononuclear and polymorphonuclear phagocytes) (Singer and Clark, 1999; Weyrich and Zimmerman, 2004); (ii) inflammation (this process is mediated by the arrived leukocytes, which are releasing reactive oxygen species with microbicidal activity and proteases that clear the wound of foreign bodies, devitalized tissues and microbial cells; the inflammation is resolved in few days, being accompanied by the apoptosis of inflammatory cells) (Gilroy et al., 2004; Eming et al., 2007); (iii) formation of granulation tissue (this phase is mediated by the proliferation of dermal and epidermal cells and the activation of a strong angiogenic response requiring the activation of endothelial progenitor cells, and having as consequence the synthesis of the extra-cellular matrix forming granulation tissue containing mainly collagen I, irrigated by the newly formed blood vessels) (Humar et al., 2002; Liu and Velazquez, 2008), and iv) remodeling or scar formation phase (this phase is involving wound contraction mediated by differentiated fibroblasts or myofibroblasts, which acquire a smooth muscle actin-containing stress fibers phenotype, matrix remodeling by matrix metalloproteases and scar formation following apoptosis of fibroblastic cells) (Hinz, 2007; Rai et al., 2005).

Chronic wounds, divided in vascular (*e.g.* venous and arterial ulcers), diabetic and pressure ulcers, are characterized by a common sequence of processes impairing the wound healing, such as: (i) prolonged or excessive inflammatory phase (Eming et al., 2007); (ii) persistent infections, with the formation of drug-resistant microbial biofilms (Wolcott et al., 2008) and (iii) the inability of dermal and/or epidermal cells to respond to reparatory stimuli.

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2. Pathophysiology of polymicrobial wound infections

The skin microbiota analysis by advanced molecular methods (pyrosequencing) indicate that a small percent of 1–2% of the skin colonizing microorganisms can be cultivated and also that there is a significantly lower diversity of bacteria found in wounds than in the intact skin, demonstrating the barrier role of the normal microbiota against colonization, proliferation and dissemination of opportunistic and pathogenic microorganisms (Martin et al., 2010; Gontcharova et al., 2010).

The loss of skin integrity favors the exposure of subcutaneous tissue to microbial colonization and proliferation, by providing appropriate moisture, temperature and nutritive conditions. The presence of foreign bodies and of devitalized tissue facilitates microbial proliferation in the absence of early prophylactic antibiotic treatment and surgical debridement (Robson, 1997).

Microorganisms that colonize the damaged tissues often form polymicrobial communities called biofilms, that may be defined as varied consortia of fungi, bacteria, and viruses that exist at a phase or density interface embedded in a self-secreted and/or host-derived, self-hydrated polymer matrix, often consisting of polysaccharides (Brogden et al., 2005), which provides an optimal environment for microbial cells survival, enabling their escape from host immune system and resistance to antibiotic treatment (James et al., 2008; Martin et al., 2010). The presence of one microorganism generates an appropriate environment for other pathogenic microorganisms, which are able to colonize the respective niche, while two or more non-pathogenic microorganisms could synergically interact to cause disease. This is possible because of the fact that the components of the normal microbiota, with a large variety and density, co-evolved for a very long period of time in a relatively small space, favoring the establishment of complex and specific physical and chemical interactions.

The ecological interactions established among the members of microbial associations could be: (i) mutualistic or synergistic, facilitating the adherence to the epithelial surfaces and the efficient utilization of nutrients and metabolic by-products or (ii) competitive/antagonistic (Brian et al., 2012) implicated in many processes, such as: contact-dependent attachment, intercellular communication *via quorum*-sensing cross-talk, colonization enhancement, augmented/changed virulence phenotypes, immunomodulation (Peleg et al., 2010).

Similarly to the virulence concept, that can no longer be associated with a single virulence factor for certain pathogens, some infectious diseases, such as the biofilm-associated ones, can no longer be attributed to a monospecific etiology (Casadevall and Pirofski, 2001). The microbial density, species, associations, as well the host immune response are all predictive factors for the wound healing and infection.

Microbial biofilms are prevalent especially in chronic wounds, such as diabetic foot, pressure, and venous leg ulcers, being often constituted by diverse polymicrobial communities, including aerobic as well as not cultivable, strictly anaerobic bacteria (James et al., 2008; Bowler and Davies, 1999; Bowler, 1998).

The dermal wounds are colonized by aerobic and anaerobic bacterial and fungal strains, most of them belonging to resident microbiota of the surrounding skin, oral cavity and gut, or from the external environment (Bowler et al., 2001). It is considered that aerobic or facultative pathogens such as *Staphylococcus aureus*, coagulase-negative staphylococci, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Enterococcus* spp. and beta-hemolytic streptococci, as well as *Candida* spp. are the primary causes of delayed healing and infection in both acute and chronic wounds, especially the surgical ones (Duerden, 1994; Mangram et al., 1999). The anaerobic *Bacteroides fragilis*, *Clostridium perfringens*, *Porphyromonas* spp., *Peptostreptococcus* spp. and *Prevotella* spp. are the most cited species involved in soft tissues and bite wound infections (Brook, 1996, 1998; Brook and Randolph, 1981; Brook and Frazier, 1990, 1997, 1998a,b; Brook and Finegold, 1981; Bariar et al., 1997).

In diabetes mellitus, the high glucose blood levels lead to osmotic imbalance, dehydration of body tissues, and, if not treated properly, to organ damage (Alberti and Zimmet, 1998), development of peripheral neuropathy and poor blood circulation, especially in limbs, predisposing to an increased risk of infection, with chronic evolution generating ulcerating polymicrobial biofilm-mediated wounds. Due to the inability of diabetic subjects to feel cuts and irritations on visually obscured areas of the feet, these infections often pass unnoticed and progress to more serious illnesses, hard to treat and potentially leading to limb amputation (Wu et al., 2007; Boulton, 2010).

The most abundant aerobic isolates recovered from this kind of infections were *Corynebacterium* spp., *Enterococcus* spp., *E. coli*, *Staphylococcus epidermidis*, and *S. aureus*; among the most commonly isolated anaerobic bacteria were *Fusobacterium* spp., *Porphyromonas* spp., *Prevotella* spp., *Bacteroides* spp., and *Clostridium* spp., often associated in polymicrobial communities with a large diversity (Gardner and Frantz, 2008).

The development of polymicrobial aerobic–anaerobic populations is facilitated by the low oxygen tension (hypoxia or anoxia) and the reduced redox potential of the wound environment (Gerding, 1995).

The wound evolution in diabetic patients is aggravated by the defects in wound healing (*i.e.* the inability of keratinocytes to migrate and differentiate properly, inappropriate levels of angiogenic and growth factors, epidermal barrier function, fibroblast migration, and macrophage function (Falanga, 2005; Galkowska et al., 2006; Maruyama, 2007). It was demonstrated that the presence of biofilm-forming *S. aureus* strains specifically inhibit wound-healing mechanisms and exacerbate disease (Bowling et al., 2009).

Wound infection is the result of virulence factors expression, surpassing the host natural immune system, followed by microbial dissemination and tissue invasion, leading to the occurrence of a local purulent discharge, pain or erythema (Peel, 1992). Live bacteria and their toxins induce excessive inflammatory responses and tissue damage that can lead to abscesses, cellulitis, osteomyelitis or limb loss (Ovington, 2003). The recruited inflammatory cells, as well as bacteria, produce different proteases, which could degrade the ECM and also, growth factors.

A better understanding of the precise mechanisms by which bacterial biofilms delay repair processes together with optimizing methods for biofilm detection and prevention may enhance opportunities for chronic wounds healing. Taking into account that the strictly anaerobic or fastidious microorganisms cannot be recovered by culture methods, combination of molecular and culturing methods is required in order to obtain a more complete characterization of the microbial diversity of chronic wounds, and a deeper understanding of the role of different microbial species in chronic wound pathology and healing (Frank et al., 2009; Gontcharova et al., 2010).

Many studies have been stated that a microbial load of $\geq 10^6$ organisms per quantitative swab sample taken from open burn wounds when bacterial cells were observed in a Gram-stained smear prepared from the same sample or $>5 \times 10^4$–10^5 CFU/g of tissue is predictive for the occurrence of a wound infection (Breidenbach and Trager, 1995; Levine et al., 1976; Raahave et al., 1986).

On the other side, the presence of more bacterial species is associated with a higher probability of wound infection, due to the synergic interactions between species, which could favor, for example, the occurrence of a hypoxic environment appropriate for