RESEARCH ARTICLE

Modern possibilities in the treatment of inflammation caused by *Staphylococcus aureus* biofilm with bioactive glass S53P4

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ABSTRACT

Treatment of implant related infections after surgical or dental procedures are challenging complications and may have devastating results for patients. The ability of microorganisms to adhere and form biofilm on implant surface is one of the main reasons for treatment failure in infections. When used in human body implants provide a suitable surface for cell attachment and bacterial colonization. One of the most common bacteria that is isolated in hospital-acquired infections is *Staphylococcus aureus*, methicillin-resistant subtype. When a biofilm is formed, bacteria acquire an arsenal of properties that allow them to survive in an adverse environment, increasing their resistance to antimicrobial agents. Recently, as treatment modalities bioactive glass is used in the treatment of infections with multiresistant bacteria types. Its mechanism of action is to stimulate osteogenesis by releasing biologically active ions and at the same time it has antibacterial function on a number of antibiotic-resistant bacteria due to increasement in osmotic pressure and environmental pH without affecting host tissues. These properties make bioactive glass extremelly suitable for the treatment of infection in bones with destruction and bone loss. The aim of the present study is to review the outcomes reported in the literature on the antimicrobial effectiveness of bioactive glass S53P4 on *Staphyloccocus aureus* (MRSA).

Key words: bioactive glass, inflammation, biofilm, *Staphyloccocus aureus*

Introduction

Misuse and overuse of antimicrobials are the main drivers in the development of drug-resistant pathogens. Antimicrobial resistance (AMR) is a major threat to human and animal life as many of the traditional antimicrobial drugs lose their efficacy. According to a 2019 World Health Organization report. antimicrobial resistance is responsible for about 700,000 deaths per year (WHO, 2019). In addition to being a major cause of morbidity and mortality it also causes huge financial losses to the world economy.

Infection may occur from haematogenous colonization (usually children), post-traumatic (open fractures) or post-operative contamination (Romano et al., 2014). The most common pathogen to induce bone infection (osteomyelitis) is *Staphylococcus aureus* (Lindfors et al., 2017). Intravenous or oral antibiotics fails to eradicate the infection because in devitalized bone there is poor vascularization and drug penetration is limited.

Staphylococcus aureus is a Gram-positive pathogenic bacteria and is a major cause of various infectious diseases in humans and animals (Adams , 2009; Schaumburg et al., 2015). These diseases can range from simple skin and soft tissue infections to more serious and life-threatening conditions as sepsis (Kobayashi et al., 2015). Staphylococcal infections are caused by several different types of staphylococcal microbes including: methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *Staphylococcus aureus* (MSSA), vancomycin-intermediate *Staphylococcus aureus* (VISA), vancomycin-resistant *Staphylococcus aureus* (VRSA). The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection has been growing steadily for more than a decade and in particular among persons undergoing surgical intervention with the placement of implants in the human body, elderly and immunocompromised patients (Segawa et al., 1999).

With the growing threat of antimicrobial resistance more and more attention is being paid to products with antimicrobial effects that can reduce the use of antibiotics. A number of studies have demonstrated the antimicrobial effectiveness of

bioactive glasses and it's osteoinductive potential. Bioactive glass S53P4 does not contain antibiotics and its antimicrobial properties are based on the increasment of pH and higher osmotic pressure caused respectively by the exchange of alkaline ions and the release of salt ions (Zhang et al., 2010).

Staphylococcus aureus biofim

Staphylococcus aureus exists between a planktonic state and a biofilm. As a biofilm, bacteria are attached to a substrate or to each other and embedded in a self-produced extracellular polymeric substance. The biofilm consists of two different components, water (about 97%) and organic matter, which includes EPS/extracellular polymeric substance/and microcolonies (Nazir et al., 2019). EPS constitutes about 50 to 90% of the total organic matter of the biofilm and is a complex of various polymeric substances such as extracellular DNA (eDNA), proteins, and polysaccharides, which provide a protective barrier against both host defense mechanisms and exogenous antibiotic treatments (Donlan, 2002; Idrees et al., 2020). The remainder, 10–25%, consists of microcolonies (Nazir et al., 2019).

In *Staphylococcus aureus* biofilm, the main component of EPS is the polysaccharide intercellular adhesin (PIA) (Reffuveille et al., 2017). The polysaccharide component of EPS is named PIA because of its function, i.e. intercellular adhesion of bacterial cells, and poly-β (1-6)-N-acetylglucosamine (PNAG), due to its chemical composition. PIA are cationic in nature and play significant roles in colonization, biofilm formation and biofilm-associated infections, immune evasion, antimicrobial resistance, and phagocytosis (Nguyen et al., 2020). Placement of implants during surgical operations provide a place for bacteria to adhere and to colonize it. This greatly facilitates the rapid development of staphylococcal bacteria into a resistant biofilm.

Bacteria biofilm exhibit an altered antimicrobial resistance phenotype through a lower metabolic rate and reduced rate of cell division (Hall-Stoodley & Stoodley, 2009). In addition, biofilm can act as a diffusion barrier and also cause inactivation of antimicrobial substances (Hall-Stoodley et al., 2004; Singh et al., 2010). As a result, the minimum inhibitory concentration of antimicrobial compounds on biofilm bacteria can reach 500–1000 times that of their planktonic counterparts (Costerton et al., 1995). A mature biofilm can further shed planktonic bacteria or microcolonies that can travel to other parts of the body causing recurrent infections.

Bioactive glass S53P4

This review focuses on bioactive glass S53P4 (BonAlive Biomaterials Ltd., Turku, Finland), with a composition of 53% SiO_2, 4% P_2O_5, 23% Na_2O, and 20% CaO, which is considered as a biocompatible, osteoconductive bone substitute with the capacity for bone bonding and antibacterial properties (Hench & Paschall, 1973; Andersson & Kangasniemi, 1991; Lindfors NC et al., 2010; Jones, 2013). This composition is increasingly used in clinical practice in the treatment of various inflammations caused by *S. aureus*. Bioactive glass S53P4 has been shown to facilitate and stimulate bone formation and bone defect healing and simultaneously has an antibacterial effect against various pathogens.

The ability of BAG-S53P4 to inhibit bacterial growth is based on a process that occur when the bioactive glass reacts with body fluids. First, sodium is released from the surface of the glass causing an increase in pH that is unfavorable for bacteria. Furthermore, the ions (sodium, calcium, phosphorus and silicon) released from the surface increase the osmotic pressure: a phenomenon that has been shown to kill both planktonic bacteria and biofilm bacteria *in vitro* (Andersson & Kangasniemi, 1991; Hench & Paschall, 1973). This deposition of ions also forms a layer of silica gel near the surface of the bioactive glass to which amorphous calcium phosphate precipitates and subsequently crystallizes into native hydroxyapatite. The osteostimulating properties of this layer activate osteogenic cells and potentially promote angiogenesis (Van Gestel et al., 2015). A proper filling of the defect and adequate soft tissue coverage are necessary prerequisites for successful outcome.

Discussion

We selected the methicillin-resistant *S. aureus* (MRSA) as an observed bacterium due to its increasing frequency as a cause of total bone infections after surgical manipulations and placement of implants in the human organism.

Usually treatment of bone infections is a two-stage procedure. At first stage an aggressive debridement in combination with the usage of antibiotic loaded PMMA pearls is done. Second stage include removal of antibiotic beads and filling the bone defect with different types of bone substitutes. Disadvantages of this technique are that the time of antibiotic release is not always know, bacterial resistance can occur due to prolonged antibiotic release and there is a possibility for pathogens to produce biofilm on the foreign body. The S53P4 bioactive glass can be applied directly to the infected area through a one-step surgical procedure resulting in a less invasive and more cost-effective treatment for patients. This material is especially effective for bone cavity management following debridement. It is also an osteo-conductive biomaterial with bone-bonding, angiogenic and potent antimicrobial properties (Bigoni et al., 2019).

According to literature data, the bioactive glass S53P4 is presented as the most effective of the bioactive glasses for inhibiting the bacterial growth of *Staphylococcus aureus* (MSRA) *in vitro* studied so far. Several different studies have presented the ability of bioactive glass S53P4 to be a stand-alone antibacterial bone substitute (Coraca-Huber et al., 2014;

Drago et al., 2015; Leppa¨ranta et al., 2008). Romano et al. (2014) performed a retrospective cohort study where they compared the treatment of chronic osteomyelitis with debridement combined with systemic and topical antimicrobial therapy in three different groups (Romano et al., 2014; Drago et al., 2013). They compared a group treated with S53P4 bioactive glass with two control groups. Roman et al. (2014) present a retrospective cohort study of treatment of chronic osteomyelitis with debridement. They compared the use of bioactive glass with two local antibiotic therapies applied through a different carrier (antibiotic loaded in hydroxyapatite with calcium sulfate and a combination of tricalcium phosphate and teicoplanin loaded demineralized bone matrix). At a median follow-up of 22 months, no recurrent infections were observed in 92.6% of patients treated with S53P4 bioactive glass. In the group of patients treated with antibiotic hydroxyapatite and calcium sulfate compounds, 88.9% were infection free. In 86.3% of patients treated with a mixture of tricalcium phosphate and antibiotic-loaded demineralized bone, no reinfection occurred. Data on the faster healing of wounds in the group of patients treated with bioactive glass are also presented (Romano et al., 2014; Drago et al., 2013).

McAndrew et al. (2013) also reported successful and complete healing of bone bacterial infections in three patients. Follow-up at 14 to 21 months showed no radiological evidence of osteomyelitis during this period with good integration of the bioactive glass and surrounding bone (McAndrew et al., 2013). A study by Lindfors et al. (2010) described the successful treatment of osteomyelitis with S53P4 bioactive glass beads in eleven patients (Lindfors et al., 2010). There were also no adverse effects for patients resulting from the use of bioactive glass. Clinical outcome was good or excellent in nine patients (median follow-up of 24 months).

Another debatable issue in the treatment of postoperative infections caused by staphylococcal bacteria is the formation of new blood vessels during bone regeneration (Parsons & Strauss, 2004). There are indications that S53P4 bioactive glass has angiogenic potential. However, the evidence is scarce and based only on *in vitro* findings (Detsch et al., 2014). An angiogenic effect may provide a crucial link in the bone healing cascade and remains an important topic for future research.

Eva Steinhausen et al. (2021) compared bioactive glass BAG S53P4 versus autologous bone graft (AB) in 83 patients (bioactive glass n=51, AB n=32) with chronic osteomyelitis and infected nonunion after surgical interventions. Twenty-one patients had re-infection (n=15, 29 %; AB n=6, 19 %). Sixty-four patients had complete bone healing at the end of the follow-up period (BAG n=39, 77 %; AB n=25, 78 %). In the study performed, it was found that patients with multidrug-resistant pathogens had a significantly higher rate of incomplete bone healing (p=0.033) and a 3-fold higher risk of complications in both groups. The authors conclude that biactive glass S53P4 is a suitable bone substitute not only for successful infection control and defect filling, but also for bone healing in cases of infected nonunion (Steinhausen et al., 2021).

A recent study on the use of S53P4 bioactive glass with traditional surgical treatment in septic osteoarthritis of the first MTP joint showed that the bioactive glass was effective. The authors studied adult patients (age > 18 years) with type 1 or 2 diabetes, with a neuropathic plantar or marginal-medial ulcer, and with osteomyelitis involvement of the first MTP joint (diabetic foot). In them, osteomyelitis was confirmed by a positive probe-bone test (with the established presence of *Staphylococcus aureus* methicillin-sensitive and *Staphylococcus aureus* methicillin-sensitive) and an x-ray of the leg. A total of 22 patients were divided into two groups. A first group of 10 patients were treated with segmental resection of the first MTP joint and periarticular bone stabilized with an external fixator and local application of S53P4 BG biomaterial mixed with 5 mL of venous blood. Second group 12 patients who were treated with segmental resection, temporary application of Septopal®, 7.5 mg gentamicin sulfate, and stabilization with external fixator. The authors reported successful healing with complete disappearance of osteomyelitis in all 10 patients of the first group and in 9/12 patients of group B (p = 0.221) (Kastrin et al., 2021).

Coraca-Huber et al. (2014) evaluated the effectiveness of different sizes of S53P4 bioactive glass against *Staphylococcus aureus* biofilms grown on metal discs *in vitro*. Biofilms, they cultured on titanium disks and then contacted with BAG-S53P4 (0.5–0.8 mm and <45 μm). The team found that BAG-S53P4 could suppress *S. aureus* biofilm formation on titanium discs *in vitro*. The suppression rate of biofilm cells by BAG-S53P4 <45 μm was significantly higher than by BAG-S53P4 0.5–0.8 mm. Bioactive glass S53P4 has the potential to be used as a bone substitute to resolve infectious complications in joint replacement surgeries and treat chronic osteomyelitis (Coraca-Huber et al., 2014).

It is of interest to us to follow the behavior of polyresistant bacteria placed on various surfaces previously treated with bioactive glass *in vitro*. If it is found impossible to cultivate them, the results would serve to create an algorithm for surgical manipulations, especially patients placed at a higher risk of developing infections due to a number of chronic diseases.

As a future direction for scientific research work, we propose to investigate the possibility of pre-treatment of the implantable surfaces during surgical manipulations with bioactive glasses *in vitro*.

Conclusion

The antibacterial, osteo-stimulative and angiogenic properties of bioactive glass S53P4 makes it a reliable tool for the treatment of chronic bone infections. Its one-stage surgical application reduces hospitalization time and related complications. However, more randomized, multicenter clinical trials need to be performed in order to determine if bioactive glass could replace the current gold standard two stage revision technique for treatment of chronic bone infections.

The presented brief overview about the current possibilities for the treatment of inflammations caused by *Staphylococcus aureus* in biofilm by applying bioactive glass S53P4 presents a reliable possibility to treat common infections without antibiotic administration.

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