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BIOCOMPATIBILITY STUDIES

Original Research

Effect of S53P4 bone substitute on staphylococcal adhesion and biofilm formation on other implant materials in normal and hypoxic conditions

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Abstract To study the effect of bioactive glass bone substitute granules (S53P4) on bacterial adhesion and biofilm formation on other simultaneously used implant materials and the role of the hypoxic conditions to the adhesion. Bacterial and biofilm formation were studied on materials used both in middle ear prostheses and in fracture fixtures (titanium, polytetrafluoroethylene, polydimethylsiloxane and bioactive glass plates) in the presence or absence of S53P4 granules. The experiments were done either in normal atmosphere or in hypoxia simulating atmospheric conditions of middle ear, mastoid cavity and sinuses. We used two collection strains of *Staphylococcus aureus* and *Staphylococcus epidermidis*. In the presence of bioglass and hypoxic conditions the adhesion of the planktonic bacterial cells was decreased for most of the materials. The biofilm formation was decreased for *S. epidermidis* on titanium and polydimethylsiloxane in both atmospheric conditions and on bioglass plates in normoxia. For *S. aureus* the biofilm formation was decreased on bioglass plates and polytetrafluoroethylene in normoxia. Hypoxia produces a decrease in the biofilm formation only for *S. aureus* on polytetrafluoroethylene and for *S. epidermidis* on bioglass plates. However, in none of the cases bioactive glass increased the bacterial or biofilm adhesion. The presence of bioglass in normoxic and hypoxic conditions prevents the bacterial and biofilm adhesion on surfaces of several typical prosthesis materials in vitro. This may lead to diminishing postoperative infections, however, further in vivo studies are needed.

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1 Introduction

Highest possible sterility is the keystone of surgery when implant materials are used. The presence of an implanted device results in an increased susceptibility to postoperative infection [1, 2] because the number of bacteria required to produce an implant infection is reduced [3, 4]. This is due to the fact that bacteria adhere easier to an inactive substratum, which is not incorporated to the host defense system. Implant infection is one of the most scared and devastating complications in surgery due to its recalcitrant nature and implant removal is often the only way to cure the infection [5–7]. The risk of an implant infection is naturally higher in operations of a contaminated or infected bone defect such as in chronic mastoiditis or sinusitis. Furthermore, the infection risk is increased by the fact that

the human bone cavities tend to be hypoxic even after the successful revision surgery [8–10].

In obliteration of an infected bone sequester, the contaminated bone is revised and the resulting cavity is filled using autologous material or bone substitutes such as bioactive glass (BAG) [8, 11, 12]. Similar operation is often performed in dislocated frontal sinus fractures. Usually rigid fixation materials are also needed to restore the weight bearing properties of the bone, the facial contour or conductive hearing in case of middle-ear surgery [11].

In general, bioactive materials are defined as materials eliciting a specific biological response at the interface of material and tissue, resulting in the formation of a bond between them. The term refers to the ability of these materials to form a bone mineral-like calcium phosphate layer on their surfaces [13–15].

BAGsof different compositions have been studied for decades for clinical use and there are several applications in dentistry, orthopedic surgery and otorhinolaryngology at present [13, 15]. First BAGwas introduced in the early 1970s by Hench and his co-workers, who discovered BAG 45S5, capable of forming direct chemical bonds with both hard and soft tissue [16]. In vivo studies have shown that BAGs bond with bone more rapidly than other bioceramics, and in vitro studies indicate that their osteogenic properties are due to their dissolution products stimulating osteoprogenitor cells at the genetic level [14].

The BAG S53P4 has been used as a substitute for the reconstruction of defected bones. It provides bone conductive properties and serves as excellent reconstruction material in osteomyelitis revision surgery, in craniofacial defects and in sinus or mastoid cavity obliteration [10, 12, 17]. It has been shown that S53P4 has antimicrobial properties [18–20] probably based on several factors, including high pH and osmotic effects caused by the non physiological concentration of the dissolved ions [9, 15] and that the presence of S53P4 granules interfere with bacterial biofilm production and decrease its adherence on titanium surface [18, 21].

This study was conducted to investigate the effect of the presence of S53P4 on the bacterial adhesion and biofilm formation on implant materials such as titanium, polytetrafluoroethylene (PTFE) and polydimethylsiloxane (PDMS). Furthermore, the test was conducted both in normal atmospheric oxygen concentration and in hypoxic conditions to mimic the effect of low oxygen level of human bone cavities.

2 Materials and methods

2.1 Materials

2.1.1 S53P4 bioactive glass

The standard sized S53P4 bioactive glass (BonAlive Biomaterials Ltd., Turku, Finland) samples in form of granules and bulk plates were used. The composition of the bioactive glass in weight percentages is SiO_2 53 %, Na_2O 23 %, CaO 20 % and P_2O_5 4 %. S53P4 plates were cut using a low speed diamond saw by the Process Chemistry Centre of Åbo Akademi University in Turku, Finland. All glass plates were manufactured in the same way and finally the plates were tailored to average surface roughness (Ra) of 500–630 nm by using the same SiC abrasive papers as with other materials (120 grit). The size of glass granules was 0.5–0.8 mm and the plate size was $5 \times 5 \times 1.5$ mm^3.

2.1.2 Titanium samples

Magnetron sputtering technique was used for deposition of titanium samples. Titanium samples were coated on 2 mm thick (8×10 cm^2 surface area) glass substrate. Ra of 300–400 nm was applied on glass surface by using SiC abrasive paper (120 grit). Before the deposition, sample surfaces were cleaned with argon sputter (SAM-7 kV, Minsk, Belarus) in vacuum. The initial vacuum chamber pressure was 8×10^{-4} Pa. The sputtering time was 2 times 5 min and the voltage and current used were 4 kV and 20 mA, respectively. During the argon sputtering the vacuum chamber pressure was 10^{-2} Pa. The purity of argon was 99.999 % (Instrument Argon 5.0, Oy AGA Ab, Espoo, Finland). Deposition process was continued directly after the argon sputtering without breaking the vacuum. Magnetron sputtering system (Stiletto Series ST20, AJA International Inc., North Scituate, MA, USA) was used to produce the titanium samples where a negative target potential of 530 V with 1 A current was used to accelerate the positively charged Ar ions to the high purity (+99.7 %) elemental titanium target (Goodfellow Cambridge Ltd, Huntingdon, England). In the deposition process the vacuum chamber pressure was 4×10^{-2} Pa, current 1 A and the deposition time was 4 times 1 min (with 2 min intervals). After the coating process the titanium-coated glass was cut to 9×9 mm^2 sample size by using EXAKT-cutting grinding system (EXAKT-Apparatebau, Hamburg, Germany).

2.1.3 PDMS and PTFE samples

The bulk PTFE and PDMS polymers were obtained from a commercial supplier of industrial polymers (ETRA, Helsinki, Finland). PTFE samples were cut from 1 mm thick polymer sheet and PDMS samples from 2 mm thick sheet to size of 9 × 9 mm. Average surface roughness Ra was approx. 300–400 nm for both materials.

2.2 pH study

S53P4 granules were added to a solution of phosphate buffered saline (PBS) pH 7.4 in ratio 1/10 (100 mg/ml). The pH of the solution was measured after 1, 2, and 24 h in normoxia (0.035 % CO_2 and 20.9 % O_2) and hypoxia at 37°C resembling the situation in the normal middle ear (7 % CO_2 and 6 % O_2) [22, 23]. The Invivo 2 Hypoxia Workstation (Ruskinn Technologies, Ltd., Sanford, Maine, USA) was used to produce the hypoxia. The pH studies were done in triplicate.

2.3 Staphylococcal adhesion experiments

Bacterial adhesion was studied in four different conditions: (1) In normoxia at the presence of PBS, (2) in normoxia at the presence of S53P4 granules using PBS pretreated 2 h with S53P4, (3) in hypoxia at the presence of PBS and 4) in hypoxia at the presence of S53P4 granules using PBS pretreated 2 h with S53P4.

Staphylococcal adhesion experiments were performed in well culture plates (Thermo Scientific Nunc, Roskilde, Denmark) as described by Kinnari et al. [24]. The biofilm-forming collection strains of *S. aureus* 15981 [25] and *S. epidermidis* ATCC 35984 were suspended and diluted to 10^8 colony forming units (CFU/ml) using normal PBS or PBS pretreated 2 h with S53P4 in ratio 1/10 (100 mg/ml). The biomaterial discs were placed in the wells in polycarbonate membrane (Thermo Scientific Nunc, Goteborg, Sweden) inserts to keep the sample out of direct contact to the S53P4 granules. The bacterial suspension was added to the well and incubated for 90 min at 37 °C. Afterwards, the biomaterial plates were rinsed three times with sterile PBS to remove any no adherent bacteria. The dried plates were stained for 15 min with a rapid fluorescence staining method using the Live/Dead BacLight™ Bacterial Viability Kit (Molecular Probes Inc., Eugene, OR, USA) [26]. On each plate, 10 fields were viewed and photographed with Nikon Coolpix 8400 (Nikon, Melville, NY, USA) under a fluorescence microscope at ×40 magnification. The photographs were taken from identical locations according to a premade plan. All experiments were performed in triplicate. The surface area covered with adhered bacteria was calculated using the ImageJ software (National Institute of Health, Bethesda, MD, USA).

2.4 Static biofilm method

The experiment was performed in the same media, atmospheric and temperature conditions as the bacterial adhesion experiment. Biofilm was grown with no fluid shear conditions according to static biofilm method described by Buckingham-Meyer et al. [27]. Sterile filter paper (Whatman Qualitative Grade 2, GE Healthcare Life Sciences, Little Chalfont, UK) was placed on top of a tryptic soy agar (TSA) plate (Becton–Dickinson, Helsinki, Finland) and inoculated with 1.5 ml of a bacterial solution of 10^8 CFU/ml prepared in PBS or PBS pretreated 2 h with S53P4 granules. A sample of each four material was placed symmetrically on top of the filter paper. The S53P4 granules recovered from the PBS solution were added at the same distance from each material in a symmetric disposition on top of the inoculated filter paper as shown in Fig. 1. Plates were incubated for 48 h at 37 °C. After 24 h, the filter paper was remoistened with 1.5 ml of 3 g/l tryptic soy broth (TSB). After 48 h incubation time, samples were washed softly in order to remove the planktonic bacteria and sonicated with an ultrasonic cleaning bath USC100T (VWR, Leuven, Belgium) at 45 kHz with a power output of 300 W for 5 min in 2.5 ml of PBS using the protocol of Esteban et al. [28]. The 5 min sonication time was chosen as Kobayashi et al. recommend that a sonication time of 1–5 min is ideal for dislodging biofilm bacteria without

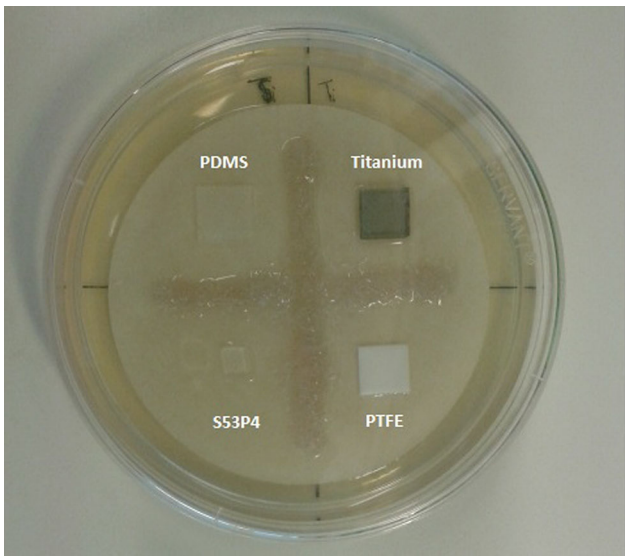

Fig. 1 Static biofilm method: The S53P4 granules were distributed in a cross shape in the middle of the inoculated filter paper at the same distance from samples of titanium, polytetrafluoroethylene (PTFE), polydimethylsiloxane (PDMS) and S53P4 plate. The diameter of the petri dish is 10 mm

affecting bacterial viability [29]. The number of bacteria in each sonication product was quantified by drop plate method [30]. The experiments were carried out in triplicate for each strain and for each atmospheric condition.

2.5 Statistical analysis

Statistical analysis was performed using the EPI-INFO software version 3.5.1 (CDC, Atlanta, GA, USA). Kruskal–Wallis analysis was performed for 3 or more samples. In case of statistical difference, Mann–Whitney test was performed. Non-parametric test was realized, in case data followed non-normal distribution. Normality was tested by Shapiro–Wilk test.

3 Results

3.1 pH study

The effect of S53P4 granules to the pH of PBS in normoxia and hypoxia at different time points is shown in Fig. 2. The pH increased during the first 2 h and there after remained stable. The pH was lower in hypoxia compared to normoxia.

3.2 Bacterial adhesion

The bacterial adhesion test with both strains indicate significantly less bacterial adhesion ($P < 0.05$) in presence of S53P4 granules in both atmospheric conditions except for *S. aureus* on titanium in normoxia ($P = 0.061$) and for *S. epidermidis* on S53P4 plates in both atmospheric conditions ($P = 0.4918$ and $P = 0.0712$). Also the hypoxic conditions decreased bacterial adhesion except for *S. epidermidis* on S53P4 plates ($P = 0.0992$) (Fig. 3). In none of the cases bacterial adhesion increased in presence of S53P4 granules. Image of the adhesion of *S. epidermidis* on titanium is shown in Fig. 4.

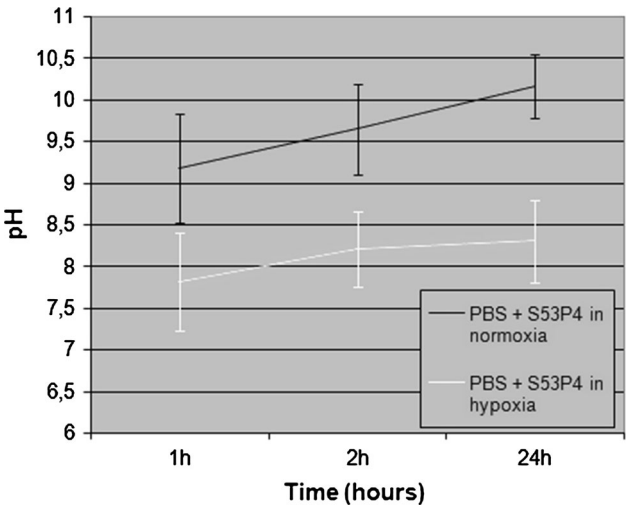

Fig. 2 The pH of the PBS with S53P4 granules was measured in 1, 2 and 24 h both in normoxia and hypoxia. The *error bars* represent the standard deviation

3.3 Percentage of dead bacteria on each surface

The proportion of dead bacteria on each biomaterial in different conditions is shown in Fig. 5. The presence of S53P4 granules causes mainly increase of the proportion of the dead bacteria, however exceptions occur. For *S. aureus* in normoxia the granules cause decrease of the proportion of dead bacteria on PTFE and no difference on S53P4 plates and for *S. epidermidis* no difference was found in titanium and in PTFE in hypoxia and in S53P4 plates in normoxia. Furthermore, hypoxia induced an increase in the percentage of dead bacteria in case of *S. aureus* with PDMS ($P < 0.0001$) and *S. epidermidis* with PTFE ($P = 0.018$).

3.4 Biofilm formation

In seven out of sixteen conditions the presence of S53P4 granules produced a statistically significant decrease in the biofilm formation (Figs. 6, 7). In normoxic conditions the decrease of biofilm formation was significant in all situations except for *S. aureus* on titanium and PDMS and for *S. epidermidis* on PTFE ($P = 0.5127$ and $P = 0.1266$ for *S. aureus* respectively and $P = 0.1266$ for *S. epidermidis*). In hypoxic conditions the S53P4 granules had less significant effect on biofilm formation. Decrease occurred in case of *S. epidermidis* on titanium and PDMS ($P = 0.0495$ for both materials), otherwise there was no statistically significant effect ($P > 0.05$).

The biofilm reduction effect of S53P4 is presented in Fig. 7. The tendency for reduction of biofilm formation by S53P4 was seen in all materials in both atmospheric conditions. The reduction was higher in hypoxia than in normoxia for titanium with both bacteria and for PDMS with *S. epidermidis*. For PTFE and S53P4 plates with *S. aureus* the reduction was higher in normoxia.

The effect of hypoxic conditions on biofilm formation produced a decrease only for *S. aureus* with PTFE ($P = 0.0495$) and for *S. epidermidis* with S53P4 plates ($P = 0.0495$). Hypoxia caused an increase of biofilm formation on titanium for both bacteria ($P = 0.0495$) and on PDMS for *S. epidermidis* ($P = 0.0495$) (Fig. 6).

4 Discussion

With infected and revised bone cavities there is an evident hazard of implant infection that often impedes the use of a prosthetic or filling materials in the first place and several

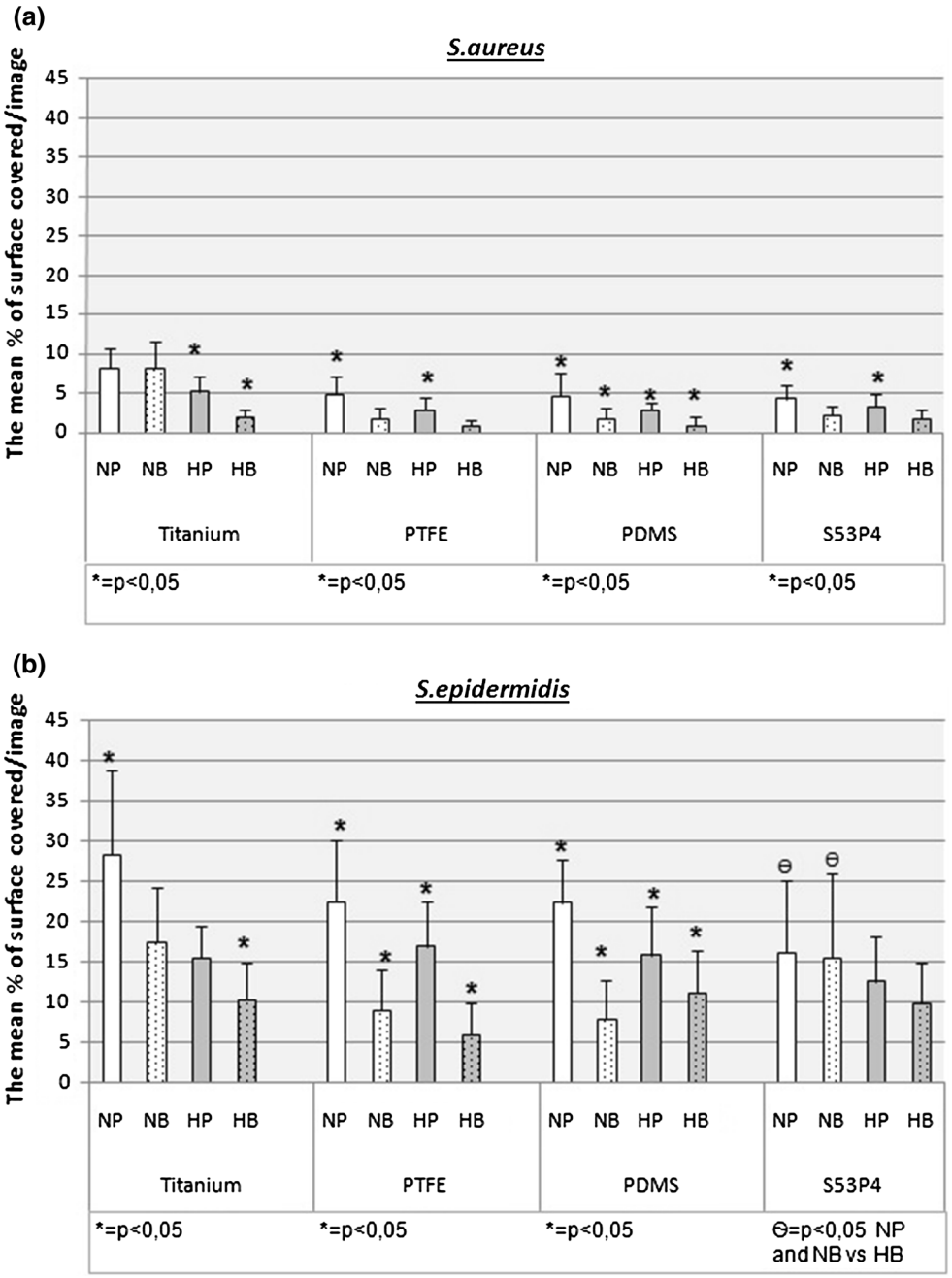

Fig. 3 Bacterial adhesion: The mean percentage of each biomaterial surface covered with **a** *S. aureus* and **b** *S. epidermidis*. *NP* normoxic conditions, *NB* normoxic conditions with S53P4 granules, *HP* hypoxic conditions, *HB* hypoxic conditions with S53P4 granules. The *error bars* represent the standard deviation. *Asterisk* statistically significant difference. *Theta* statistically significant difference compared to hypoxic conditions with S53P4 granules

operations may be needed. The impact of hypoxia on bacterial adhesion and biofilm formation on biomaterials is poorly understood. Hypoxia can influence the bacterial adhesion in many ways such as by modifying the expression of virulence and antibiotic resistance genes [31].

High alkaline environment is not well tolerated by micro-organisms [32]. The optimum pH for the staphylococci is between 7.0 and 7.5 [33]. The increase in the pH during the time of incubation caused by release of alkali ions from S53P4 particles is certainly a critical factor for the antibacterial effects of bioactive glass as shown in other studies [33]. Other factors causing antibacterial properties may be related to the increased osmotic pressure caused by non-physiological concentration of ions of calcium and alkalis released from the S53P4. This could cause perturbations of the membrane potential of bacteria [10, 13, 15]. Nevertheless, S53P4 is well tolerated and seems to be a good implant material. The advantage is that the constituent chemicals are all found in the body, which may decrease the possibility that bacteria develop resistance to these materials [15]. A typical roughness of commercial middle ear implants was chosen for this study. The

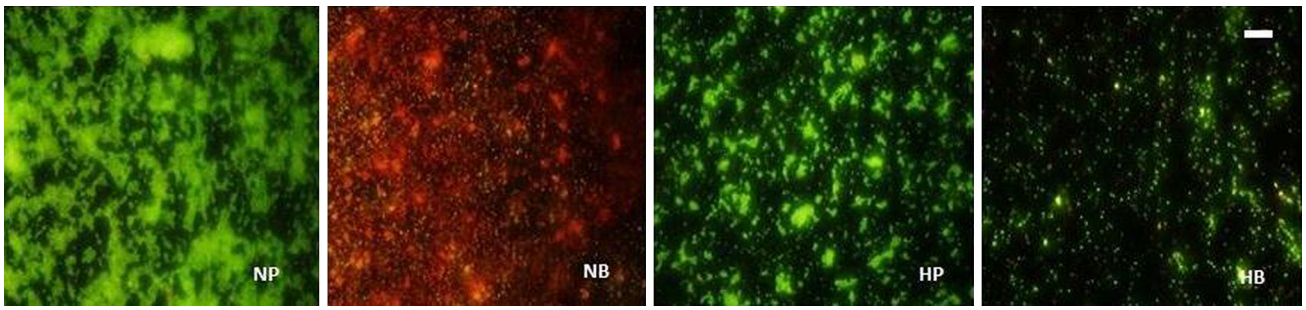

Fig. 4 Fluorescent microscope images of the adhesion of *S. epidermidis* on titanium surface in different conditions (×40 magnification): *NP* normoxic conditions, *NB* normoxic conditions with S53P4 granules, *HP* hypoxic conditions, *HB* hypoxic conditions with S53P4 granules. The samples were stained with BacLight LiveDead stain (Molecular Probes), a combination of propidium iodide and SYTO9 dyes. The *white bar* represents 10 μm length

implants used with bone contact are preferably slightly rough to provide a good attachment between the prosthesis and the surrounding tissue [34].

The medium was pretreated with S53P4 granules for 2 h due to the fact that the pH and alkaline effect of the bioactive glass to the medium becomes quite slowly and no real effect would be available during the two phases of the bacterial adhesion and the biofilm maturation process. We used PBS pretreated with 100 mg/ml of S53P4 because previous studies [32] showed that the antibacterial effect is dose-dependent at concentration of 50 mg/ml and below. In clinical settings proteins of serum or extracellular fluid rapidly coat the implant material surfaces and thus the microbials encounter not a bulk biomaterial but a substratum pretreated with a mixture of host proteins. This should also be taken in consideration in in vitro experiments. We, however, decided not to pretreat the samples for these experiments as one of our main focuses was the mastoid surgery where the titanium and other implants are used to restore the conductive hearing in a dry middle ear meanwhile the infected mastoid cavity is revised and filled with bioglass granules. In these circumstances perioperative bacterial adhesion mainly happens on a bulk biomaterial substratum.

In hypoxic conditions, the pH of the solution containing S53P4 granules is lower compared to normoxic conditions. This is possibly due to the fact that the high CO_2 level of the hypoxia chamber (7 %) moves the pH of the solution towards acidic via the formation of weak carbonic acid from the diffused CO_2. Carbonic acid will dissolve into two carbonate forms HCO_3^- and CO_3^{2-} which also contributes to the pH change [15]. Furthermore, the adaptation of staphylococci to hypoxic conditions may influence to their adhesion characteristics. Cramton et al. have suggested a mechanism whereby ica gene expression and increased production of polysaccharides, the main component of biofilm glycocalyx, may act as a virulence factor for staphylococci in an anaerobic environment in vivo [35].

However, the reduction in bacterial adhesion and percentage of dead bacteria by the effect of S53P4 was visible also in lower pH value in hypoxic conditions. The mechanism implicated is a combination of antimicrobial activity against planktonic cells and interference with bacterial adhesion dynamics and consequent biofilm formation. Because in the majority of cases by the presence of S53P4 there is a decrease of attached bacteria and increase in number of dead bacteria we believe it is a consequence of continuous antibacterial effect that influence also the bacteria adhered to the substrata. The percentage of dead *Staphylococcus aureus* on PTFE was surprisingly decreased by the presence of S53P4. There is no clear explanation to this, however, it is possible that those weak *S. aureus* cells, that die soon after they adhere on other biomaterials, are not able to adhere on the repellent PTFE surface in the first place. This phenomenon must be specific to the species because similar effect was not visible in *S. epidermidis.*

We conclude that the mechanisms of the antibacterial effect of S53P4 may depend on several other aspects such as:

- An increment of the ionic strength as the leaching of ions occurs from the S53P4 [20].
- Antibacterial effect of the inert SiO_2 which is present in the S53P4 composition and may produce a coating on surrounding substrata [36].
- Physical damage to cell wall coming from S53P4 debris that could be related to its antibacterial effect [31].
- The S53P4 could generate oxygen radicals that would react with all biological molecules and cause an antimicrobial effect [13].

The results show that the bioglass effect was more evident on bacterial adhesion than on biofilm formation and that the effect on adhesion was not always correlated to the biofilm formation, same as found by Hu et al. [32]. The

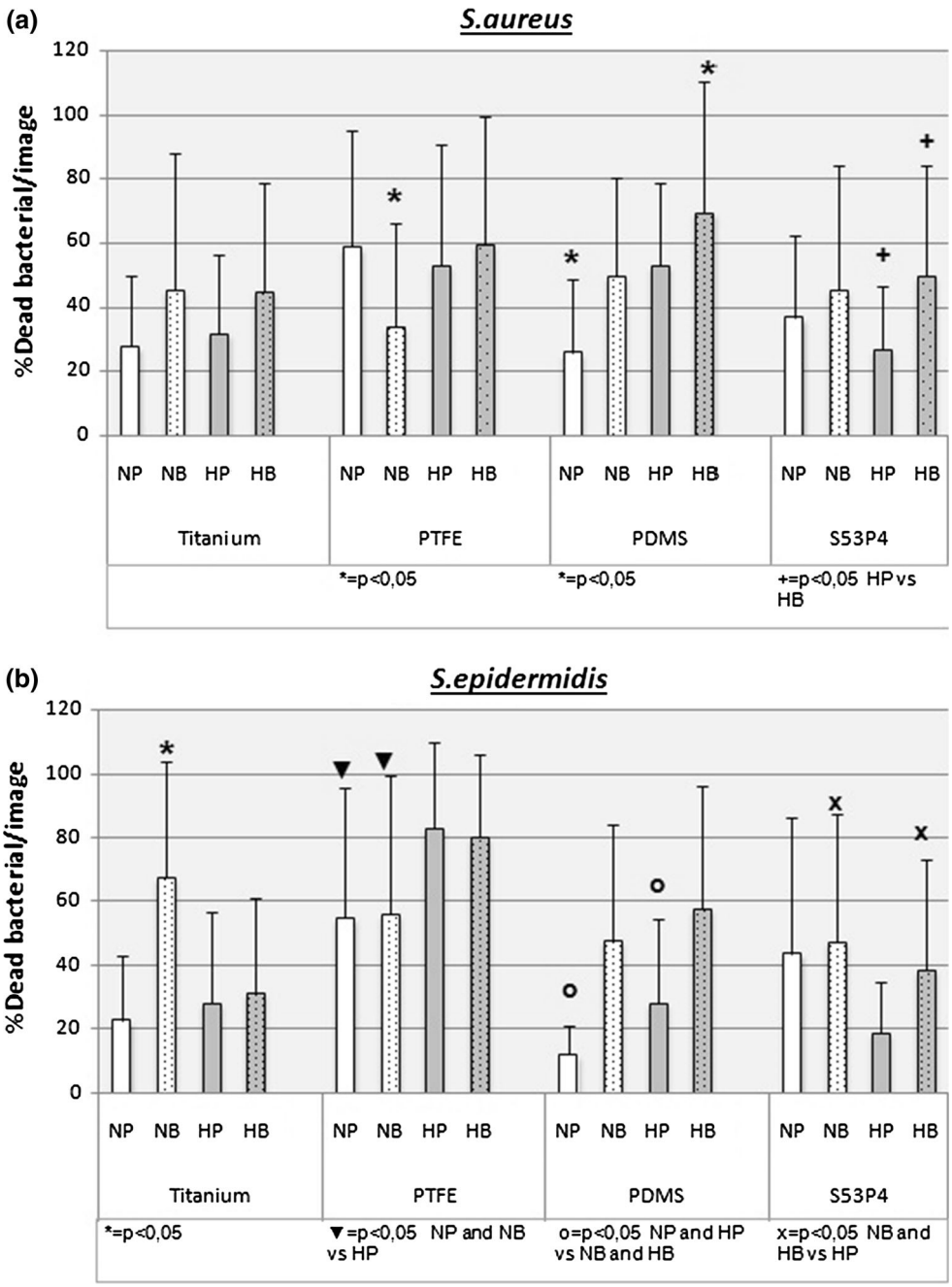

Fig. 5 The mean percentage of dead bacteria adhered on each biomaterial in the different conditions. The *error bars* represent the standard deviation. *Asterisk* statistically significant difference. *Plus sign* the presence of S53P4 in hypoxic conditions produced statistically significant difference. *Black down pointing triangle* statistically significant difference compared to hypoxic conditions without S53P4 granules. *White circle* statistically significant difference compared to both atmospheric conditions withS53P4 granules. *Times symbol* statistically significant difference compared to hypoxic conditions without S53P4

effect of S53P4 on the viability of the planktonic inoculum could be the reason for the fact that less planktonic bacteria attached on some materials. However, in some cases few bacteria are enough to proliferate and develop a mature biofilm due to the biofilm promoting effect of this substratum. The antibacterial effect of S53P4 was also studied at 90 min and 2 days without presence of the biomaterial substrata. A statistically significant reduction of CFU was found in all the conditions except in hypoxic conditions for *S. epidermidis* at 90 min and for *S. aureus* at 2 days. This corroborates the findings that S53P4 has antibacterial properties on clinically important bacteria [11, 14, 16, 19]. Further experiments should be performed to evaluate whether S53P4 has a straight effect on the bacterial adhesion mechanisms or only on the bacterial viability. Compared to the case of normal surgical procedure the bacterial concentrations used in this study were high.

We evaluated the adhesion of reference strains. Biofilm formation should be studied also using clinical strains isolated from patients with a diagnosis of prosthetic infection, which can show different properties than strains adapted to laboratory. These wild type strains, due to their

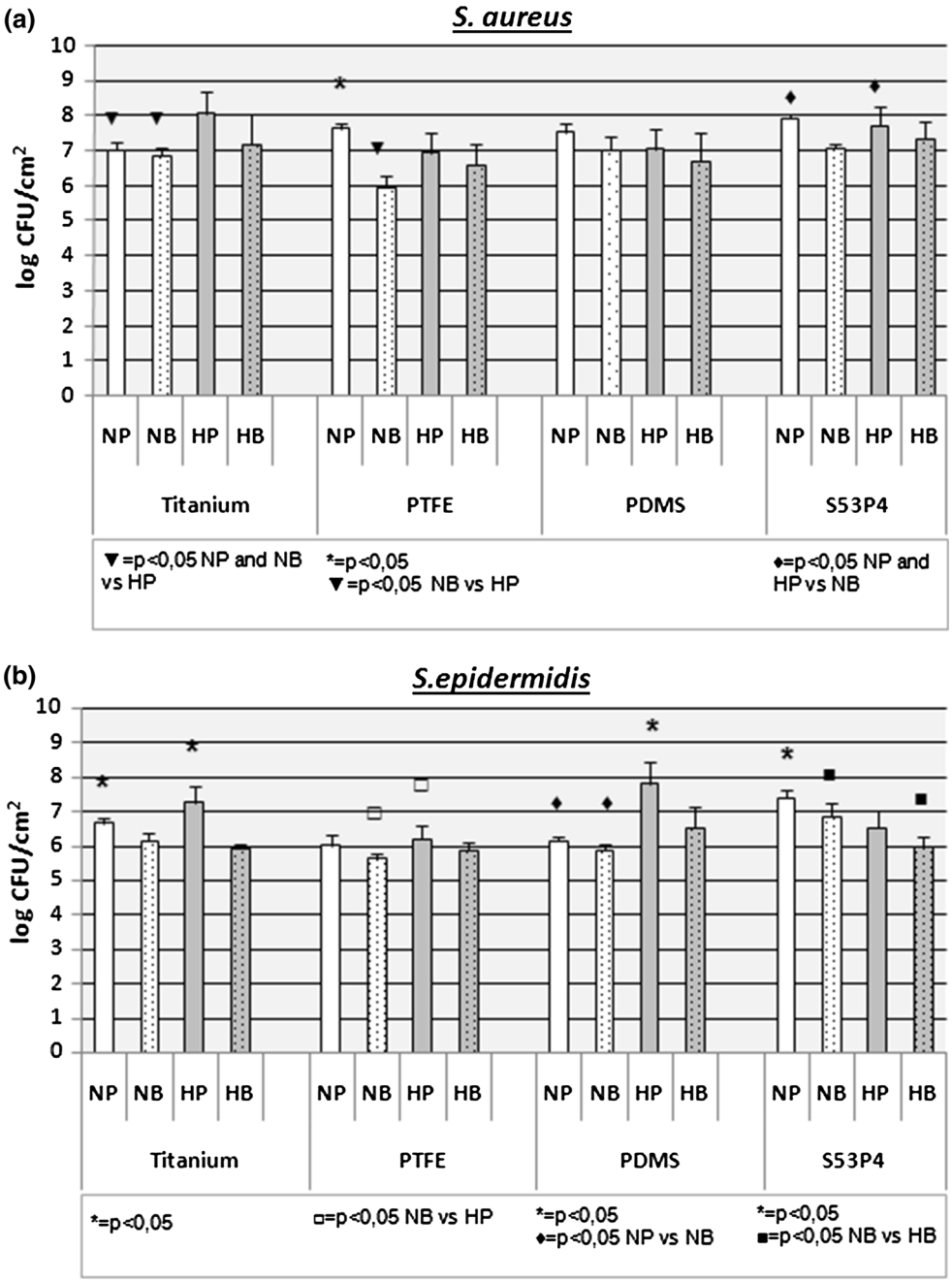

Fig. 6 Biofilm formation in colony forming units with respect to cm^2 ($logCFU/cm^2$) of surface of different biomaterials in each condition in logarithmic scale. The *error bars* represent the standard deviation. *Asterisk* statistically significant difference. *Black down pointing triangle* statistically significant difference compared to hypoxic conditions without S53P4 granules. *Black diamond* statistically significant difference compared to normoxic conditions with S53P4 granules. *White square* statistically significant difference between normoxic conditions with S53P4 and hypoxic conditions without S53P4 granules. *Black square* statistically significant difference between normoxic conditions with S53P4 granules and hypoxic conditions with S53P4 granules

different pathogenic properties might show more reliably the effect of the presence of S53P4 and hypoxic conditions in vivo.

Moreover, in vivo studies should be performed in the future primarily to assess the effect of the host's immune system on the infection itself and the efficiency with which the S53P4 produces a change in pH and osmolarity in an animal model.

Finally, it may be discussed if this growth inhibitory effect in bacterial cells could also be seen in eukaryotic cells. However, the clinical experience and our first experimental results using human cells are contrary to this.

Bioactive glass S53P4 has been used successfully as a bone substitute. The aim of this study was to find out whether simultaneous use of bioglass with other implant materials would decrease the risk of implant infection in vitro. It was revealed that the S53P4 has an inhibitory effect on staphylococcal adhesion and biofilm formation. The mechanism is probably based on a combination of several factors, including high pH and osmotic effects

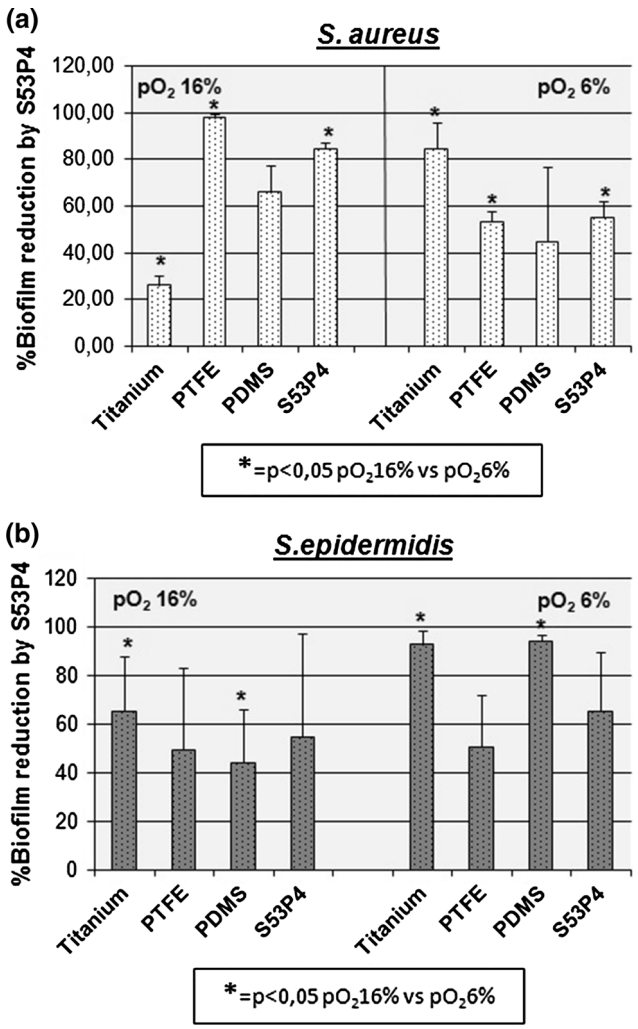

Fig. 7 Biofilm reduction effect of S53P4 on each material and atmospheric condition in percents. **a** Using *S. aureus* or **b** *S. epidermidis*. The *error bars* represent the standard deviation. *Asterisk* statistically significant difference between both atmospheric condition

caused by dissolution of the bioglass. Further in vivo studies are needed to show if S53P4 is suitable filling material in cases of contaminated cavities.

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Compliance with ethical standards

Conflict of interest None to declare.

References

1. Andriole VT, Nagel DA, Southwick WO. A paradigm for human chronic osteomyelitis. J Bone Joint Surg Am. 1973;55(7):1511–5.
2. Rochford ET, Richards RG, Moriarty TF. Influence of material on the development of device-associated infections. Clin Microbiol Infect. 2012;18(12):1162–7.
3. Guggenbichler JP, Assadian O, Boeswald M, Kramer A. Incidence and clinical implication of nosocomial infections associated with implantable biomaterials—catheters, ventilator-associated pneumonia, urinary tract infections. GMS Krankenhhyg Interdiszip. 2011;6(1):Doc18.
4. Elek SD, Conen PE. The virulence of *Staphylococcus pyogenes* for man; a study of the problems of wound infection. Br J Exp Pathol. 1957;38(6):573–86.
5. Deysine M. Infections associated with surgical implants. N Engl J Med. 2004;351(2):193–5.
6. Katsikogianni M, Missirlis YF. Concise review of mechanisms of bacterial adhesion to biomaterials and of techniques used in estimating bacteria-material interactions. Eur Cell Mater. 2004;8:37–57.
7. Perez-Tanoira R, Perez-Jorge C, Endrino JL, Gomez-Barrena E, Horwat D, Pierson JF, et al. Bacterial adhesion on biomedical surfaces covered by micrometric silver Islands. J Biomed Mater Res Part A. 2012;100(6):1521–8.
8. Stoor P, Pulkkinen J, Grenman R. Bioactive glass S53P4 in the filling of cavities in the mastoid cell area in surgery for chronic otitis media. Ann Otol Rhinol Laryngol. 2010;119(6):377–82.
9. Stoor P, Soderling E, Grenman R. Bioactive glass S53P4 in repair of septal perforations and its interactions with the respiratory infection-associated microorganisms *Haemophilus influenzae* and *Streptococcus pneumoniae*. J Biomed Mater Res. 2001;58(1):113–20.
10. Stoor P, Soderling E, Grenman R. Interactions between the bioactive glass S53P4 and the atrophic rhinitis-associated microorganism *Klebsiella ozaenae*. J Biomed Mater Res. 1999;48(6):869–74.
11. Yung M, Tassone P, Moumoulidis I, Vivekanandan S. Surgical management of troublesome mastoid cavities. J Laryngol Otol. 2011;125(3):221–6.
12. Peltola M, Aitasalo K, Suonpaa J, Varpula M, Yli-Urpo A. Bioactive glass S53P4 in frontal sinus obliteration: a long-term clinical experience. Head Neck. 2006;28(9):834–41.
13. Munukka E, Leppäranta O, Korkeamäki M, Vaahtio M, Peltola T, Zhang D, et al. Bactericidal effects of bioactive glasses on clinically important aerobic bacteria. J Mater Sci Mater Med. 2008;19:27–32.
14. Jones JR. Review of bioactive glass: from Hench to hybrids. Acta Biomater. 2013;9(1):4457–86.
15. Leppäranta O, Vaahtio M, Peltola T, Zhang D, Hupa L, Hupa M, et al. Antibacterial effect of bioactive glasses on clinically important anaerobic bacteria in vitro. J Mater Sci Mater Med. 2008;19:547–51.
16. Hench LLPH. Direct chemical bond of bioactive glass-ceramic materials to bone and muscle. J Biomed Mater Res. 1973;7(3):25–42.
17. Cui X, Zhao C, Gu Y, Li L, Wang H, Huang W, et al. A novel injectable borate bioactive glass cement for local delivery of vancomycin to cure osteomyelitis and regenerate bone. J Mater Sci Mater Med. 2014;25(3):733–45.
18. Coraca-Huber DC, Fille M, Hausdorfer J, Putzer D, Nogler M. Efficacy of antibacterial bioactive glass S53P4 against *S. aureus* biofilms grown on titanium discs in vitro. J Orthop Res. 2014;32(1):175–7.
19. Drago L, Romano D, De Vecchi E, Vassena C, Logoluso N, Mattina R, et al. Bioactive glass BAG-S53P4 for the adjunctive treatment of chronic osteomyelitis of the long bones: an in vitro and prospective clinical study. BMC Infect Dis. 2013;13:584.

20. Stoor P, Soderling E, Salonen JI. Antibacterial effects of a bioactive glass paste on oral microorganisms. Acta Odontol Scand. 1998;56(3):161–5.
21. Drago L, Vassena C, Fenu S, De Vecchi E, Signori V, De Francesco R, et al. In vitro antibiofilm activity of bioactive glass S53P4. Future Microbiol. 2014;9(5):593–601.
22. Luntz M, Levi D, Sade J, Herman M. Relationship between the gas composition of the middle ear and the venous blood at steady state. Laryngoscope. 1995;105(5 Pt 1):510–2.
23. Hergils L, Magnuson B. Human middle ear gas composition studied by mass spectrometry. Acta Otolaryngol. 1990;110(1–2):92–9.
24. Kinnari TJ, Soininen A, Esteban J, Zamora N, Alakoski E, Kouri VP, et al. Adhesion of staphylococcal and Caco-2 cells on diamond-like carbon polymer hybrid coating. J Biomed Mater Res Part A. 2008;86(3):760–8.
25. Valle J, Toledo-Arana A, Berasain C, Ghigo JM, Amorena B, Penades JR, et al. SarA and not sigmaB is essential for biofilm development by *Staphylococcus aureus*. Mol Microbiol. 2003; 48(4):1075–87.
26. Boulos L, Prevost M, Barbeau B, Coallier J, Desjardins R. LIVE/DEAD BacLight : application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. J Microbiol Methods. 1999;37(1):77–86.
27. Buckingham-Meyer K, Goeres DM, Hamilton MA. Comparative evaluation of biofilm disinfectant efficacy tests. J Microbiol Methods. 2007;70(2):236–44.
28. Esteban J, Gomez-Barrena E, Cordero J, Martin-de-Hijas NZ, Kinnari TJ, Fernandez-Roblas R. Evaluation of quantitative analysis of cultures from sonicated retrieved orthopedic implants in diagnosis of orthopedic infection. J Clin Microbiol. 2008; 46(2):488–92.
29. Kobayashi N, Bauer TW, Tuohy MJ, Fujishiro T, Procop GW. Brief ultrasonication improves detection of biofilm-formative bacteria around a metal implant. Clin Orthop Relat Res. 2007; 457:210–3.
30. Herigstad B, Hamilton M, Heersink J. How to optimize the drop plate method for enumerating bacteria. J Microbiol Methods. 2001;44(2):121–9.
31. Schaible B, Taylor CT, Schaffer K. Hypoxia increases antibiotic resistance in *Pseudomonas aeruginosa* through altering the composition of multidrug efflux pumps. Antimicrob Agents Chemother. 2012;56(4):2114–8.
32. Hu S, Chang J, Liu M, Ning C. Study on antibacterial effect of 45S5 Bioglass. J Mater Sci Mater Med. 2009;20(1):281–6.
33. Rivadeneira J, Carina Audisio M, Boccaccini AR, Gorustovich AA. In vitro antistaphylococcal effects of a novel 45S5 bioglass/agar-gelatin biocomposite films. J Appl Microbiol. 2013;115(2): 604–12.
34. Kwok P, Fisch U, Strutz J, Jacob P. Comparative electron microscopic study of the surface structure of gold, Teflon, and titanium stapes prostheses. Otol Neurotol. 2001;22(5):608–13.
35. Cramton SE, Ulrich M, Gotz F, Doring G. Anaerobic conditions induce expression of polysaccharide intercellular adhesin in *Staphylococcus aureus* and *Staphylococcus epidermidis*. Infect Immun. 2001;69(6):4079–85.
36. Borzabadi-Farahani A, Borzabadi E, Lynch E. Nanoparticles in orthodontics, a review of antimicrobial and anti-caries applications. Acta Odontol Scand. 2014;72(6):413–7.