sciendo

PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B,
Vol. 73 (2019), No. 4 (721), pp. 325–331.

DOI: 10.2478/prolas-2019-0051

ADHESION AND COLONISATION OF MICROORGANISMS ON POROUS TIO_2 AND TIO_2-SILVER BIOMATERIALS

Ingus Skadiņš[1,#], Lana Micko[2], Liene Zvaigzne[2], Inga Narkevica[3,4], and Juta Kroiča[1]

[1] Department of Biology and Microbiology, Rīga Stradiņš University, 16 Dzirciema Str., Rīga, LV-1007, LATVIA

[2] Faculty of Medicine, Rīga Stradiņš University, 16 Dzirciema Str., Rīga, LV-1007, LATVIA

[3] Rūdolfs Cimdiņš Rīga Biomaterial Innovations and Development Centre, Rīga Technical University, 3 Pulka Str., Rīga, LV-1007, LATVIA

[4] Faculty of Material Science and Applied Chemistry, Rīga Technical University, 3/7 Paula Valdena Str., Rīga, LV-1048, LATVIA

[#] Corresponding author, ingus.skadins@rsu.lv

Communicated by Māra Pilmane

Bone graft transplantation is one of the most common transplants in the world and there has been a significant increase in the use of biomaterials in this sector. Bone substitutes are widely used in traumatology, orthopaedics, maxillofacial surgery and dentistry. The culturing method was used to determine microorganism ability to attach and form biofilms on originally synthesised porous TiO_2 and TiO_2Ag ceramics. The aim of this study was to determine and compare the intensity of adhesion and colonisation of Staphylococcus epidermidis*,* Pseudomonas aeruginosa *and* Candida albicans *on TiO_2 and TiO_2Ag ceramics. The lowest adhesion and colonisation were on TiO_2Ag samples for* S. epidermidis *and* P. aeruginosa*. No* C. albicans *adhesion and colonisation differences were found on TiO_2 and TiO_2Ag ceramic samples.*

Key words: *biomaterial-associated infections,* C. albicans, S. epidermidis, P. aeruginosa.

INTRODUCTION

Biomaterials are used in almost all medical sectors, including craniofacial reconstructive surgery, where bone substitutes are used for the elimination of large bone defects. Major bone defects can develop as a result of trauma, non-acute fractures, tumours, cysts or tumours, infections, and congenital skeletal abnormalities, for example, palate fractures (Huebsch and Mooney, 2009).

In tissue engineering, the cooperation of engineers and surgeons has led to the development of original scaffolds. These are mechanical structures that serve to fill the material space and make tissue matrix (Eldesoqi *et al*., 2013). Nowadays, artificial bone substitutes are used not only to replace missing bone tissue but also with the aim to create an osteo-computational environment for the recovery of bone tissue based on bone formation on the surface of biomaterial (Albrektsson and Johansson, 2002; Hyun *et al*., 2013).

The increased use of biomaterials gives rise to two problems: biomaterial-associated infections (BAI) and inadequate tissue integration. Biomaterial properties that make them suitable for use in living organisms also create favourable conditions for bacteria. Organisms and proteins of the immune system can easily bind bacteria that continue to develop with biofilm and bacterial colonies, which is the cause of one of the most common implant complications or the development of associated infections in biomaterials (Lewis, 2001; Hall-Stoodley *et al*., 2004).

Biomaterial interactions with bacteria and tissue cells depend not only on the specific receptors of the cellular and bacterial outer membranes, but also on the surface structure of the material on the atomic level and the influence of electric forces (O'Gara and Humphryes, 2001).

Biomaterials do not prevent the formation of biofilms. However there are ongoing attempts to modify the existing biomaterials, or synthesise new biomaterials, with the aim to obtain a bone graft that reduces bacterial adhesion on its surface, preventing phenotypic changes in microbial adhesion, including extracellular polymer interactions that produce biofilms (Flemming, 2010).

Bacterial biofilm is made up of polysaccharides and is considered to serve as a medium for the development of infection of the implanted material (Post, 2001).

The sources of biomaterial-associated infections may be different, as microorganisms can gain access to the transplant in several ways and a technically successful operation does not protect from the development of a biomaterial-associated infection. If there is no penetrating trauma of the skin, then the causative agent of biomaterial infections is likely to attach to the implant during surgery (perioperative contusion) or during hospitalisation, before wound suturing (early postoperative contamination) (Zimmerli *et al*., 2004).

The main aim of this study was to compare the intensity of adhesion and colonisation of *S. epidermidis*, *P. aeruginosa* and *C. albicans* on porous TiO_2 and TiO_2Ag biomaterials, and to determine effect of weight of the biomaterial on intensity of adhesion and colonisation.

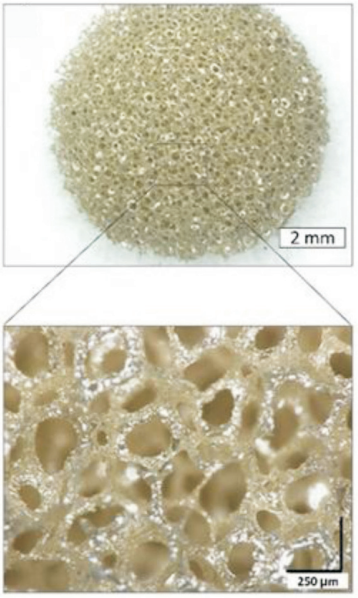

Fig. 1. Digital microscopy image of porous TiO_2 ceramics.

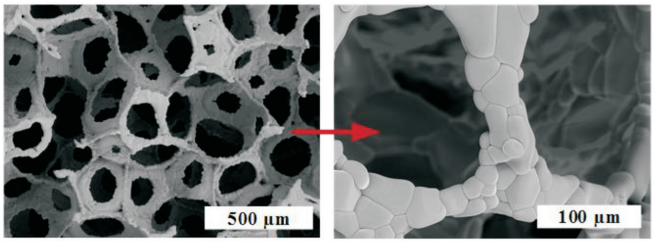

Fig. 2. SEM microphotography of porous TiO_2 ceramics.

MATERIALS AND METHODS

Synthesis and characteristics of TiO_2 ceramic biomaterials. Commercially available TiO_2 anatase nanopowder (puriss., Nanostructured & Amorphous Materials, Inc.) with an average particle size of 15 nm was used to prepare disk-shaped compacts of 10 mm in diameter by uniaxial pressing applying 64 MPa pressure. The compacted green bodies were thermally treated in air and high vacuum conditions. Samples were sintered in air at 700 °C and 1000 °C with heating rate 5 °C /min, held for 10 h and then cooled with cooling rate 5 °C /min. Samples sintered in air at 1000 °C were additionally thermally treated in high vacuum conditions at 1000 °C for 5 h. TiO_2 scaffolds were coated with silver for obtaining TiO_2Ag ceramic samples.

The obtained TiO_2 ceramic biomaterial (Fig. 1 and Fig. 2) had open porosity and permeable pore structure. TiO_2 scaffolds had a porosity of 96 ± 1% with a characteristic pore size of 70–350 µm and a ceramic wall thickness varying from 34 ± 10 µm. The compressive strength of the samples was 0.38 ± 0.13 MPa.

Determining microorganism adhesion *in vitro*. Suspensions of 10^2 and 10^3 CFU/ml *S. epidermidis* and *P. aeruginosa* pure cultures and 10^2 CFU/ml *C. albicans* pure culture were made under sterile conditions. Before *in vitro* tests, the biomaterial samples were weighed.

The samples were incubated in the microorganism suspension at 37 °C for two hours to determine the adhesion intensity on the surface of the biomaterial. After incubation, non-attached microorganisms were rinsed off. To remove microorganisms attached to the surface of the biomaterial, samples were treated with a Vortex centrifuge for 1 minute and an ultrasound bath (45 kHz frequency) for 1 minute (Trampuz *et al*., 2007).

Each sample was placed on Sabouraud media (Oxoid, UK) to determine adhesion of *C. albicans*, and on Trypticase soy agar (TSA) (Oxoid, UK) to determine adhesion of *S. epidermidis* and *P. aeruginosa*. Samples were cultured for 24 h at 37 °C to determine the number of colony forming units.

Determining microorganism colonisation *in vitro*. Suspensions of 10^2 and 10^3 CFU/ml of *S. epidermidis* and *P. aeruginosa* pure cultures and a suspension of 10^2 CFU/ml *C. albicans* pure culture were made under sterile conditions. Before *in vitro* tests, the biomaterial samples were weighed.

The samples were incubated in the microorganism suspension at 37 °C for 24 hours to determine the colonisation intensity on the surface of the biomaterial. After incubation, microorganisms that were not attached to the biomaterials were rinsed off. To remove the microorganisms attached to the surface of the biomaterial, samples were treated with a Vortex centrifuge for 1 minute and an ultrasound bath (45 kHz frequency) for 1 minute (Trampuz *et al*., 2007).

Each sample was then placed on Sabouraud media (Oxoid, UK) to determine colonisation of *C. albicans*, and on Trypticase soy agar (TSA) (Oxoid, UK) to determine adhesion of *S. epidermidis* and *P. aeruginosa*. Samples were cultured for 24 h at 37 °C to determine the number of colony forming units.

Bacterial cultures. *S. epidermidis* (ATCC 12228), *P. aeruginosa* (ATCC 27853) and *C. albicans* (ATCC 10231) reference cultures were used for testing of intensity of adhesion and colonisation.

Statistical analysis. The results were analysed by non-parametric statistics. The Spearman ranking correlation coefficient and Mann–Whitney test were used to assess whether there were statistically significant differences between duration of antibacterial effect of different biomaterial samples. Statistical significance was assumed if a p value was less or equal to 0.05. Statistical analysis was performed with SPSS 22.0.

RESULTS

Bacterial adhesion on porous TiO_2 and TiO_2Ag ceramic samples. The intensity of adhesion of the microorganisms at different bacterial suspension concentrations was determined on the porous TiO_2 ceramics. The mean adhesion intensity of *S. epidermidis* at concentration of 10^2 CFU/ml was 3.3 ± SD 0.47 CFU/0.01 g and at 10^3 CFU/ml adhesion intensity was 15.0 ± SD 0.82 CFU/0.01g. The mean adhesion of *P. aeruginosa* on TiO_2 ceramics at concentration of 10^2 CFU/ml was 5.0 ± SD 0.55 CFU/0.01g, and at concentration of 10^3 CFU/ml was 48.0 ± SD 1.52 CFU/0.01g (Table 1).

The mean *S. epidermidis* and *P. aeruginosa* adhesion intensity on TiO_2Ag samples was lower than on TiO_2 samples. At a suspension concentration of 10^2 CFU/ml, *S. epidermidis* adhesion was 1.1 ± SD 0.29 CFU/0.01g, but *P. aeruginosa* adhesion was 3.2 ± SD 0.41 CFU/0.01 g. Also, at higher concentrations of bacterial suspensions adhesion of bacteria on the silver-based samples was lower than on samples without silver. *S. epidermidis* adhesion intensity reached 10 ± SD 0.72 CFU/0.01 g, and *P. aeruginosa* 32.5 ± SD 1.31CFU/0.01g (Table 2) on TiO_2Ag samples.

Although the adhesion intensity of *S. epidermidis* at 10^2 CFU/ml was lower than that of *P. aeruginosa*, this difference was not statistically significant, $p > 0.05$ ($p = 0.105$) — Mann–Whitney test. At a suspension concentration of 10^3 CFU/ml, *P. aeruginosa* adhesion intensity was significantly higher than the adhesion intensity of *S. epidermidis* $p < 0.05$ ($p = 0.015$).

There was no significant difference between *S. epidermidis* and *P. aeruginosa* adhesion intensity on TiO_2 and TiO_2Ag at a concentration of 10^2 CFU/ml, $p > 0.05$ ($p = 0.105$) — Mann–Whitney test. A significant difference was detected in adhesion intensity of *S. epidermidis* and *P. aeruginosa* at a suspension concentration of 10^3 CFU/ml on TiO_2 (Fig. 3) and TiO_2Ag samples, $p < 0.05$ ($p = 0.015$).

Bacterial colonisation on porous TiO_2 and TiO_2Ag ceramic samples. *S. epidermidis* mean colonisation intensity at a concentration of 10^2 CFU/ml, was 21587± SD 1892 CFU/0.01g on TiO_2 samples and at a concentration of 10^3 CFU/ml - 42193.75 ± SD 3654 CFU/0.01g. *P. aeruginosa* mean colonisation intensity at a concentration of 10^2 CFU/ml was 39250 ± SD 3254 CFU/0.01 g on TiO_2 samples and at a concentration of 10^3 CFU/ml — 46118 ± SD 3996 CFU/0.01 g (Table 3).

Table 1

BACTERIAL ADHESION INTENSITY ON TIO_2

	Adhesion intensity on TiO_2 (CFU/0.01g)	
Suspension concentration	10^2 CFU/ml	10^3 CFU/ml
S. epidermidis	3.3 (± 0.47) (n = 10)	15.0 (± 0.82) (n = 10)
P. aeruginosa	5.0 (± 0.55) (n = 10)	48.0 (± 1.52) (n = 10)

Data presented as mean ± SD

Table 2

BACTERIAL ADHESION INTENSITY ON TIO_2AG

	Adhesion intensity on TiO_2Ag (CFU/0.01g)	
Suspension concentration	10^2 CFU/ml	10^3 CFU/ml
S. epidermidis	1.1 (± 0.29) (n = 10)	10.0 (± 0.72) (n = 10)
P. aeruginosa	3.2 (± 0.41) (n = 10)	32.5 (± 1.31) (n = 10)

Data presented as mean ± SD

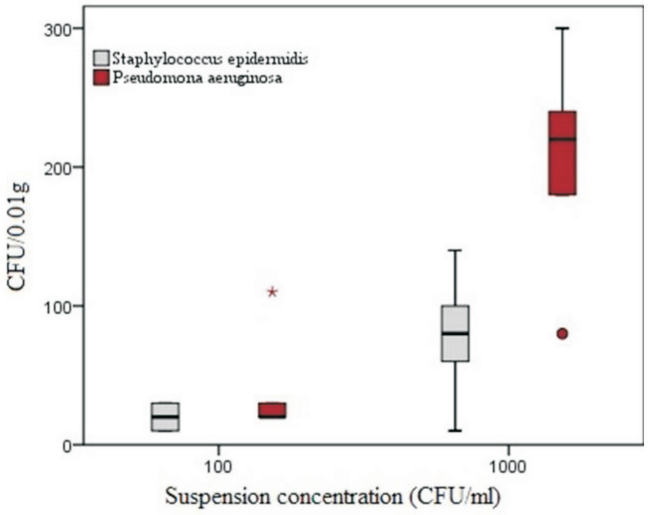

Fig. 3. The intensity of adhesion of *S. epidermidis* and *P. aeruginosa* to the various suspension concentrations of the TiO2 ceramics.

Table 3

BACTERIAL COLONISATION INTENSITY ON TIO_2

	Colonisation intensity on TiO_2 (CFU/0.01g)	
Suspension concentration	10^2 CFU/ml	10^3 CFU/ml
S. epidermidis	21587 (± 1892) (n = 10)	42193 (± 3654) (n = 10)
P. aeruginosa	39250 (± 3254) (n = 10)	46118 (± 3996) (n = 10)

Data presented as mean ± SD

There was no statistically significant difference in colonisation intensity on porous TiO_2 ceramics between *S. epidermidis* and *P. aeruginosa* at a suspension concentration of 10^2 CFU/ml. Similarly, a statistically significant difference in colonisation intensity between the two bacteria was also not found at a concentration of 10^3 CFU/ml (Fig. 4) $p > 0.05$ ($p = 1.04$). A statistically significant difference in colonisation intensity was found between colonisation intensity

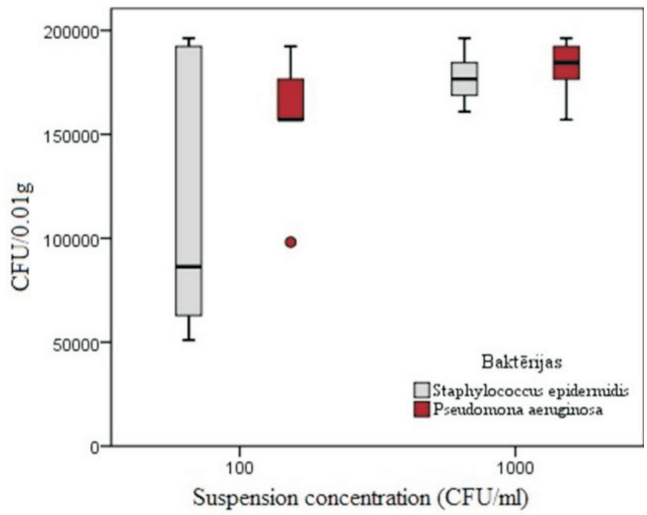

Fig. 4. The intensity of *S. epidermidis* and *P. aeruginosa* colonisation on the TiO_2 ceramics.

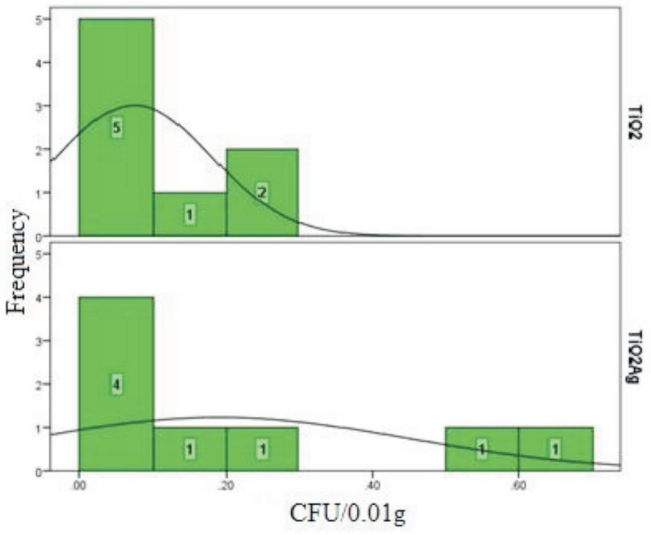

Fig. 5. Adhesion intensity on TiO_2 and TiO_2Ag ceramic samples.

Table 4

BACTERIAL COLONISATION INTENSITY ON TiO_2Ag

	Colonisation intensity on TiO_2Ag (CFU/0.01g)	
Suspension concentration	10^2 CFU/ml	10^3 CFU/ml
S. epidermidis	9585 (± 1892) (n = 10)	19852 (± 3654) (n = 10)
P. aeruginosa	21000 (± 3254) (n = 10)	24852 (± 3996) (n = 10)

Data presented as mean ± SD

on TiO_2 and TiO_2Ag for both bacterial cultures, $p < 0.05$ ($p = 0.01$) — Mann–Whitney test.

The mean *S. epidermidis* colonisation intensity on TiO_2Ag samples at a concentration of 10^2 CFU/ml was 9585 ± SD 1892 CFU/0.01g, and at a concentration of 10^3 CFU/ml — 19852 ± SD 3654 CFU/0.01 g. This difference was statistically significant; $p < 0.05$ ($p = 0.001$). *P. aeruginosa* colonisation intensity on TiO_2Ag samples in both concentrations was higher than the colonization intensity of *S. epidermidis* (Table 4).

***Candida albicans* adhesion on TiO_2 and TiO_2Ag ceramic samples.** The mean weight of TiO_2 samples with *C. albicans* adhesion was 0.045 ± SD 0.02 g, with maximum sample weight 0.09 g and minimum sample weight was 0.02 g. The mean weight of TiO_2Ag samples was 0.084 ± SD 0.02 g, with maximum sample weight 0.13 g and minimum sample weight 0.06 g.

The adhesion of *C. albicans* on the two biomaterials used in the study (TiO_2 and TiO_2Ag) was minor. The mean adhesion of *C. albicans* to TiO_2 surface biomaterials was 0.074 ± SD 0.11 CFU/0.01g, maximum — 0.25 CFU/0.01 g. Adhesion intensity was slightly higher is on TiO_2Ag biomaterials, but the difference between the two groups of biomaterials is not statistically significant (Mann–Whitney, $p = 0.345$) (Fig. 5). The mean adhesion intensity of *C. albicans* to the TiO_2Ag surface was 0.19 ± SD 0.26 CFU/0.01 g.

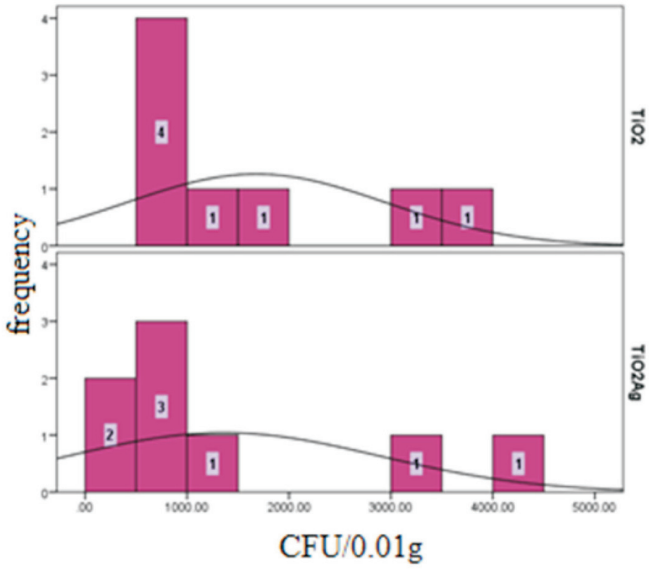

Fig. 6. Colonisation intensity on TiO2 and TiO2Ag ceramic samples.

***Candida albicans* colonisation on TiO_2 and TiO_2Ag ceramic samples.** The mean weight of TiO_2 samples with *C. albicans* colonisation intensity was 0.05 ± SD 0.01 g, with maximum sample weight 0.06 g and minimum sample weight 0.03 g. The mean weight of the TiO_2Ag samples was 0.075 ± SD 0.009 g, with maximum sample weight 0.09 g and minimum sample weight 0.06 g.

Colonisation of *C. albicans* on both biomaterials used in the study (TiO_2 and TiO_2Ag) was relatively high (Fig. 6). A statistically significant difference between colonisation intensity on the biomaterials was not found (Mann–Whitney test $p = 0.29$). The mean colonisation of *C. albicans* on TiO_2 was 1677.46 ± SD 1265.55 CFU/0.01 g with maximum 3924.0 CFU/0.01 g and minimum 586.0 CFU/0.01 g, while the mean colonisation intensity on TiO_2Ag samples was very similar — 1359.37 ± SD 1530.85 CFU/0.01 g.

Effect of biomaterial weight on bacterial adhesion and colonisation ability on TiO_2. Using the Spearman rank correlation coefficient, it was determined whether the weight of biomaterials affects bacterial adhesion and colonisation ability on porous TiO_2 ceramics. A statistically significant correlation between biomaterial weight and bacterial adhesion intensity was not found: Rs = 0.41, $p > 0.05$ ($p = 0.86$) (Fig. 7). However, a statistically significant negative correlation was observed (Fig. 8) between the biomaterial weight and the intensity of colonisation of bacteria: Rs = –0.75, $p < 0.001$. No effect of biomaterial weight on bacterial adhesion and colonisation ability on TiO_2Ag ceramics was found.

Effect of biomaterial weight on *C. albicans* adhesion and colonisation ability. There was no significant correlation between sample weight on adhesion intensity (Figs. 9 and 10)

DISCUSSION

Although the use of biomaterials is largely associated with a successful outcome, improving the quality of life and survival of the patient, complications occur often. One of the most common complications is a BAI (Oliveira *et al.*, 2018). Nosocomial infections associated with implantable medical devices are relatively common. It is estimated that they account for about 50% of all hospital-acquired infections (Sampedro and Patel, 2007).

In order to reduce the risk of infection, it is very important to choose a suitable biomaterial, since biomaterials are known to have different levels of bacterial adhesion and colonisation. Differences in bacterial adhesion are determined by a multitude of factors. One of these is the surface texture of the biomaterial, such as roughness, which contributes to bacterial adhesion in comparison to smooth surfaces. Yet, a biomaterial with a rough surface is preferred from an osseointegration perspective, because it facilitates the attachment of bone-forming cells to the biomaterial. This, in turn, increases bacterial adhesion and the risk of BAI. Therefore, it is necessary to find a biomaterial that has good osseointegration properties and yields a low risk of infection. Other important factors contributing to bacterial adhesion are the morphology of bacteria and the variability in virulence factors. Increased virulence and a propensity to

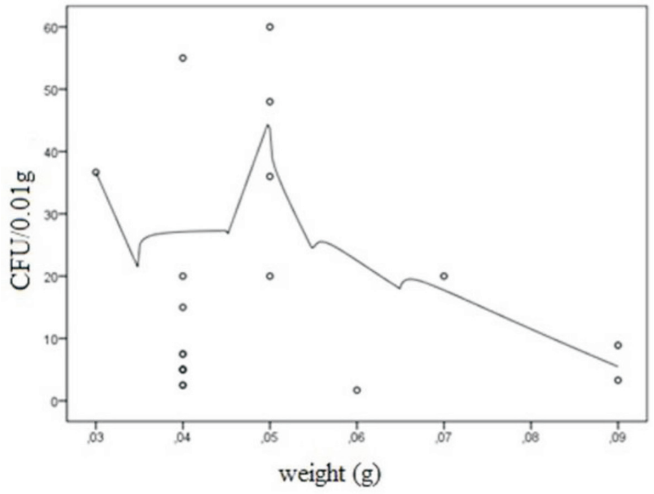

Fig. 7. Impact of biomaterial weight on bacterial adhesion intensity.

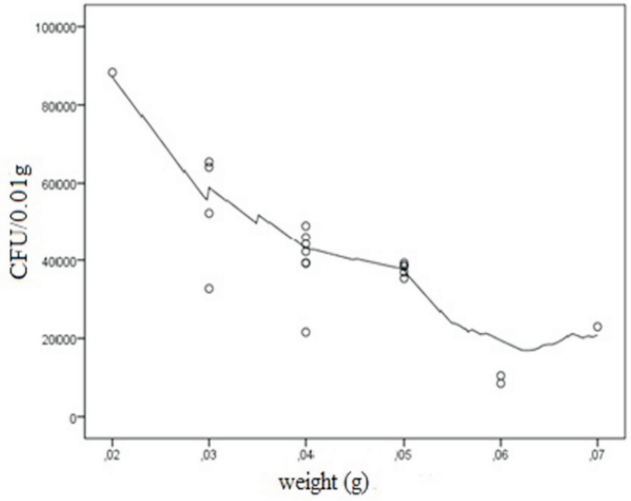

Fig. 8. Impact of biomaterial weight on bacterial colonisation intensity.

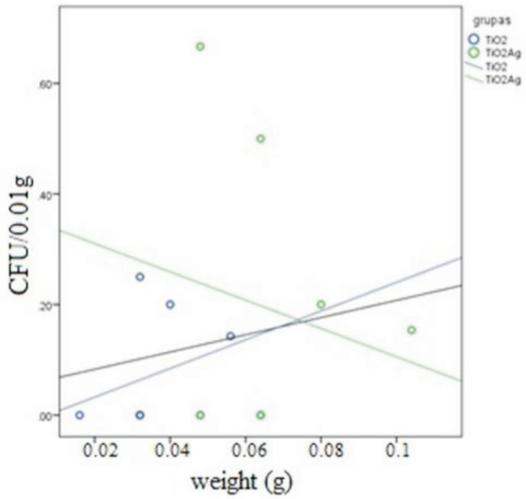

Fig. 9. Impact of biomaterial weight on *C. albicans* adhesion intensity.

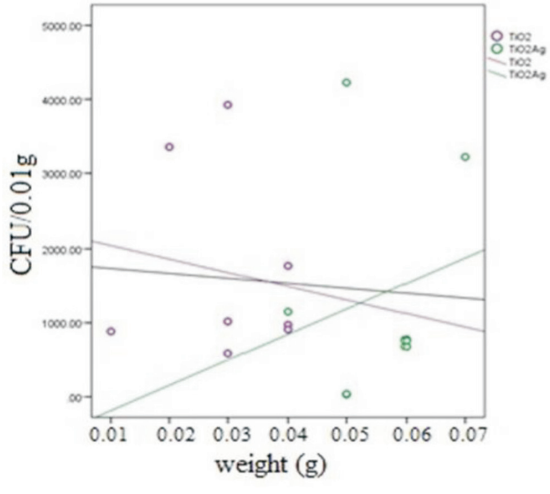

Fig. 10. Impact of biomaterial weight on *C. albicans* colonisation intensity.

form biofilms on artificial surfaces are linked to bacteria that have polysaccharide or protein adhesins. Among Gr+ bacteria, coagulase positive and negative staphylococci are the most common causative agents of BAI (Ribeiro *et al*., 2012). In order to prevent the development of BAI, it is necessary to establish preventive measures, for example, to ensure sterility during surgery or to use prophylactic antimicrobials. In accordance with the recommendations of the American Association of Orthopaedic Surgeons and National Surgical Infection Prevention Project, antimicrobials should be administered to the patient one hour before surgery. Compliance with these recommendations has decreased the number of BAI by 20%. Nevertheless, the use of systemic antimicrobials also has negative implications such as development of resistance and dysbacteriosis. (Smith and Chetter, 2015).

Nowadays, composite materials or coatings, such as hydroxyapatite or titanium dioxide coatings, are becoming increasingly popular due to their ability to reduce adhesion and colonisation of microorganisms to the surface of the implant. Studies on other coatings and their properties, such as silver coatings are also underway. (Wilding *et al.,* 2016; Eto *et al*., 2016; Taga *et al*., 2018). Our research showed that biomaterials containing silver have antibacterial properties both against Gr+ and Gr- bacteria when compared to biomaterials without silver particles. Studies investigating the possible use of biomaterials impregnated with local antimicrobials have also been conducted (Kroica *et al*., 2016). This approach has demonstrated that such biomaterials retain antibacterial properties up to two weeks, therefore precluding bacterial adhesion and the subsequent formation of biofilms on the surface of the biomaterial.

Since the most common causative agents of BAI are bacteria, antibacterial coatings are also considered. Such local antimicrobial coatings have a number of benefits, for example, a reduced risk of side effects that is associated with the use of systemic antimicrobials, such as dysbacteriosis, which is one of the most common causes of fungal infections (Goodman *et al*., 2013; Skadins *et al*., 2017).

Fungi are not typical implant-induced infectious agents, but are still found in the population. Over the years and with the development of technologies, implantable medical devices have also been used in different patient groups. Examples include those undergoing organ transplantation, and in patients with other comorbidities, whose immune system may be suppressed, thereby increasing the general risk of fungal infections (Chue *et al*., 2011; McCarty and Pappas, 2016). In order to avoid infections associated with implantable medical devices, it is important to choose a biomaterial that not only fulfils the structural and mechanical requirements but also ensures antimicrobial and anti-fungal properties. It is therefore important to clarify the inclination of certain biomaterials to attract and enable colonisation of the most common agents on their surface, including *Candida albicans*. Since implantable medical equipment is widely used nowadays, the range of infections associated with these devices is very wide. The causative agent depends on both the biomaterial used and its predisposition to enable the attachment and colonisation of pathogens on its surface, as well as the location of the implant in the body and the protective functions of the patient's body. The most common causes, accounting for approximately 65% of all implant-associated infections, are *Staphylococcus aureus* and *Staphylococcus epidermidis* (Smith and Chetter, 2015; McCarty and Pappas, 2016; Oliveira, *et al*., 2018).

In dentistry, dysfunction of dental implants due to infections is also relatively rare (about 1%), regardless of the fact that dental implants are placed in a non-sterile environment, since the oral cavity contains a wide spectrum of the normal microbiota as well as opportunistic pathogens. This could be explained by various substances that are also present in the oral cavity, such as albumin, which may impede the attachment of microorganisms to implants. Almost all implant-associated infections are caused by bacteria or fungi. Bacterial infections are not only caused by Gram-positive bacteria (most commonly staphylococci), but also by enterococci, choline bacteria, and Gram-negative bacteria such as *Pseudomonas spp*., while the most common causes of fungal infections are *Candida spp*., viruses, protozoa and helminths are rarely associated with implant infections (Bürgers *et al*., 2010; Busscher *et al*., 2012; Oliveira, *et al*., 2018).

In Orthopaedics, *Candida* spp. associated infections in regard to implants are relatively rare. In the case of tinnitus infections, about 1% are caused by *Candida spp*. fungi, most often *C. albicans* (Chue *et al*., 2011).

In dentistry, *Candida albicans* is a cause of infections because it is common in the oral cavity. It is closely linked to dental implants associated with dental implant associated infections. The incidence is up to 67% in patients with dentures. *C. albicans* has also been identified as an opportunistic pathogen for peri-implant damage (Bürgers *et al*., 2010). However, despite the frequent occurrence of *C. albicans* in dental prostheses and implants and their ability to cause infections, research in this area is relatively limited and the current results are not conclusive. The ability of *C. albicans* to attach, colonise and form biofilms on various materials is still unclear (Liad *et al*., 2012; Nascimento *et al*., 2013).

CONCLUSIONS

The intensity of adhesion of *P.aeruginosa* is significantly higher than the adhesion of *S. epidermidis* to the surface of TiO_2 ceramics, originally synthesised for this research. There is no significant difference between the intensity of colonisation of *P. aeruginosa* and *S. epidermidis* of the surface of TiO_2 ceramics. With an increase in weight of the originally synthesised TiO_2 ceramics, the intensity of colonisation of *P. aeruginosa* and *S. epidermidis* decreased. Based on the results of this research, it is possible to conclude that the intensity of adhesion and colonisation of TiO_2 and TiO_2Ag biomaterials is low.

A tendency towards a slight increase in the intensity of *C. albicans* adhesion and colonisation was observed in TiO_2Ag biomaterials in comparison to TiO_2 biomaterials, yet a statistically significant difference was not found. No correlation between the weight of the sample and the intensity of adhesion and colonisation was observed, but the samples used in this research had only minor differences in weight. In order to establish such correlation, further research using biomaterial samples within a larger range of weight is required.

Authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

Albrektsson, T., Johansson, C. (2002). Osteoinduction, osteoconduction and osseointegration. *Eur. Spine J*., **2**, 96–101.

Busscher, H. J., van der Mei, H. C., Subbiahdoss, G., Jutte, P. C., van den Dungen, J. J. A. M., Zaat, S. A. J., Schultz, M. J., Grainger, D. W. (2012). Biomaterial- associated infection: Locating the finish line in the race for the surface. *Sci. Transl. Med.*, **4**, 153rv10.

Bürgers, R., Hahnel, S., Reichert, T. E., Rosentritt, M., Behr, M., Handel, T. G. G., Gosau, M. (2010). Adhesion of *Candida albicans* to various dental implant surfaces and the influence of salivary pellicle proteins. *Acta Biomaterialia*, **6**, 2307–2313.

Chue, A. L., Moran, E., Atkins, B. L., Byren, I. (2011). Orthopaedic device associated Candidal infection. A retrospective review of patient characteristics and outcome. *J. Infect.*, **63**, 167–171.

Eldesoqi, K., Seebach, C., Nguyen Ngoc, C., Meier, S., Nau, C., Schaible, A., Marzi, I., Henrich, D. (2013). High calcium bioglass enhances differentiation and survival of endothelial progenitor cells, inducing early vascularization in critical size bone defects. *PLoS One*, **14**, e79058.

Eto, S., Kawano, S., Someya, S., Miyamoto, H., Sonohata, M., Mawatari, M. (2016). First clinical experience with thermal-sprayed silver oxide-containing hydroxyapatite coating implant. *J. Arthroplasty*, **31**, 1498–1503.

Flemming, H. C., Wingender, J. (2010). The biofilm matrix. *Nat. Rev. Microbiol*, **8**, 623–633.

Goodman, S. B., Yao, Z., Keeney, M., Yang, F. (2013). The future of biologic coatings for orthopaedic implants. *Biomaterials*, **34**, 3174–3183.

Hall-Stoodley, L., Costerton, J.W., Stoodley, P. (2004). Bacterial biofilms: From the natural environment to infectious diseases. *Nat. Rev. Microbiol*. **2**, 95–108.

Huebsch, N., Mooney, D. J. (2009). Inspiration and application in the evolution of biomaterials. *Nature*, **462**, 426–432.

Hyun, J. S., Tran, M. C, Wong, V. W., Chung, M. T., Lo, D. D., Montoro, D. T., et al. (2013). Enhancing stem cell survival *in vivo* for tissue repair. *Biotechnol Adv*, **31**, 736–743.

Kroica, J., Skadins, I., Salma, I., Reinis, A., Sokolova, M., Rostoka D., Berza N. (2016). Antibacterial efficiency of hydroxyapatite biomaterials with biodegradable polylactic acid and polycaprolactone polymers saturated with antibiotics. *Proc. Latvian Acad. Sci., Section B*, **70** (4), 220–226.

Lewis, K. (2001). Riddle of biofilm resistance. *Antimicrob. Agents Chemother*, **45**, 999–1007.

Liad, J., Hirotab, K., Gotoa, T., Yumotoc, H., Miyake, Y., Ichikawaa, T. (2012). Biofilm formation of *Candida albicans* on implant overdenture materials and its removal. *J. Dentistry*, **40**, 686–692.

McCarty, T. P., Pappas, P. G. (2016). Invasive candidiasis. *Infect. Dis. Clin. North Amer.*, **30**, 103–124.

Nascimento, C., Pita, M. S., Pedrazzi, V., Ferreira de Albuquerque Junior, R., Ribeiro, R. F. (2013). *In vivo* evaluation of *Candida* spp. adhesion on titanium or zirconia abutment surfaces. *Arch. Oral Biol.*, **58**, 853–861.

O`Gara, J. P., Humphryes, H. (2001). *Staphylococcus epidermidis* biofilms: Importance and implications. *J. Med. Microbiol*., **7**, 582–587.

Oliveira, W. F., Silva, P. M. S., Silva, R. C. S., Silva, G. M. M., Machado, G., Coelho. M. T. S. Correia, L. C. B. B. (2018). *Staphylococcus aureus* and *Staphylococcus epidermidis* infections on implants. *J. Hospital Infect.*, **98**, 111–117.

Post, J. C. (2001). Direct evidence of bacterial biofilms in otitis media. *Laryngoscope*, **111**, 2083–2094.

Ratner, B. D, Bryant, S. J. (2004). Biomaterials: Where we have been and where we are going. *Annu. Rev. Biomed. Eng.*, **6**, 41–75.

Ribeiro, M., Monteiro, F. J., Ferraz, M. P. (2012). Infection of orthopedic implants with emphasis on bacterial adhesion process and techniques used in studying bacterial-material interactions. *Biomatter*, **2** (4), 176–194.

Sampedro, M. F., Patel, R. Infections associated with long-term prosthetic devices. *Infect. Dis. Clin. North Amer.*, **21**, 785–819.

Skadins, I., Kroica, J., Salma, I., Reinis, A., Sokolova, M., Rostoka, D. (2017). The level of inflammatory cytokines and antimicrobial peptides after composite material implantation and contamination with bacterial culture. *Key Eng. Mater.*, **721**, 245–250.

Smith, G., Chetter, I. (2015). Infection in prosthetic material. *Surgery*, **33**, 559–564.

Taga, T., Kabata, T., Kajino, Y., Inoue, D., Ohmori, T., Yamamoto, T., Takagi, T., Tsuchiya, H. (2018). Comparison with the osteoconductivity and bone-bonding ability of the iodine supported titanium, titanium with porous oxide layer and the titanium alloy in the rabbit model. *J. Orthop. Sci*., **23**, 585–591.

Trampuz, A., Piper, K. E., Jacobson, M. J., Hanssen, A. D., Unni, K. K., Osmon, D. R., Mandrekar, J. N., Cockerill, F. R., Steckelberg, J. M., Greenleaf, J. F., Patel, R. (2007). Sonication of removed hip and knee prostheses for diagnosis of infection. *New Engl. J. Med.*, **357**, 654–663.

Wilding, C. P., Cooper, G.A., Freeman, A. K., Parry, M. C., Jeys, L. (2016). Can a silver-coated arthrodesis implant provide a viable alternative to above knee amputation in the unsalvageable, infected total knee arthroplasty? *J. Arthroplasty*, **31**, 2542–2547.

Williams, D. F. (1986). *Definitions in Biomaterials: Proceedings of a Consensus Conference of the European Society for Biomaterials, Chester, England, March 3–5, 1986.* Elsevier, Amsterdam, p. 30.

Zimmerli, W., Trampuz, A., Ochsner, P. E. (2004). Prosthetic-joint infections. *New Engl. J. Med.*, **351**, 1645–1654.

Received 3 January 2019
Accepted in the final form 23 April 2019

MIKROORGANISMU ADHĒZIJA UN KOLONIZĀCIJA UZ PORAINIEM TIO_2 UN TIO_2 AR SUDRABU BIOMATERIĀLIEM

Kaulaudu transplantācija ir viena no biežāk veiktajām transplantācijām pasaulē un šajā nozarē ir vērojams nozīmīgs biomateriālu pielietošanas pieaugums. Kaulaudu aizvietotājiem ir plašs pielietojums traumatoloģijā, ortopēdijā, kā arī sejas-žokļu ķirurģijā un zobārstniecībā, taču relatīvi maz biomateriāliem raksturīga perfekta strukturālo, mehānisko, kā arī antimikrobiālo īpašību kombinācija. Šī iemesla dēļ biomateriālu asociētas infekcijas joprojām paliek viena no visbiežāk sastopamajām ar biomateriālu implantāciju saistītām komplikācijām. Šī pētījuma mērķis ir noteikt un salīdzināt *Staphylococcus epidermidis*, *Pseudomona aeruginosa* un *Candida albicans* adhēzijas un kolonizācijas intensitāti uz oriģināli sintezētas porainas TiO_2 un TiO_2Ag keramikas.