SHORT COMMUNICATION

Susceptibility of Vascular Implants to Colonization *in vitro* by *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*

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Abstract

We compared association of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* with nine vascular implants after co-culture. Vascular implants were composed of various materials such as warp knitted polyester (with or without gelatin and silver ions), expanded polytetrafluoroethylene and biological materials – surface treated porcine pericardial patch and Omniflow II. The lowest overall number of associated bacteria was detected for polytetrafluoroethylene implants and porcine pericardial patch. The highest overall number of associated bacteria was detected for Omniflow II implant. The major source of variation, *i.e.* primary factor influencing colonization, is the implant type (56.22%), bacterial species is responsible for only 1.81%, and interaction of those two factors – 13.09% of variation.

K e y   w o r d s: biofilm, graft infection, Omniflow II, porcine pericardial patch, vascular implant

Application of synthetic vascular implants has considerably improved treatment results in vascular surgery. However, implanting artificial bypasses might bring about complications of which graft infection seems to pose a major threat. The incidence of the infection is 7% (Herrera *et al*., 2009) and it is correlated with a high mortality rate (up to 75% in the aortic segment) and an extremely high risk (up to 70%) of a complete or partial loss of the limb. In most cases, the vascular implant gets contaminated by microorganisms during an operation. A surgeon makes the patient's vessels accessible by incising or puncturing the inguinal region. Being naturally colonized by numerous microorganisms, this region is considered one of the major risk factors for infection of vascular implant (Antonios *et al*., 2006).

Staphylococcus aureus strains and coagulase negative *Staphylococcus* spp. are responsible for 70–90% infections of vascular bypasses (Bozoglan *et al.*, 2016). They produce glycocalyx, which on the one hand, facilitates their adhesion to the surface of the prosthesis, and on the other hand, it is a component of biofilm which serves as protection against phagocytes, antibodies and antibiotics (Bandyk *et al.*, 1991). Of gram-negative bacteria, *Pseudomonas aeruginosa* is the most common etiological factor contributing to vascular implant infections. It causes about 10% of such infections. Infections caused by *P. aeruginosa* often result in the occurrence of false aneurysms and dissection of the vascular wall in the infection site. Esterase and alkaline protease, produced by *P. aeruginosa*, are responsible for these processes (Chiesa *et al.*, 2002).

Gram-positive cocci, including *Enterococcus faecalis*, have recently become highly contributive factors in infections of vascular implants. Several years ago, these bacteria were considered relatively harmless and were characterized as possessing slight virulence traits. Despite being commonly found in human environment, they contributed only to a small percentage of infections. A characteristic feature of *Enterococci* is their natural resistance to cefalosporins, thus introduction of cefalosporins to perioperative prophylaxis on a great scale led to a great dominance of *Enterococci* in intestinal flora. This, in turn, resulted in an increase in the number of dangerous infections induced by these bacteria in hospitalized patients. Pathogenicity of *Enterococci* is associated with production of proteins

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which facilitate adhesion and colonization of the surface of an artificial prosthesis as well as formation of protective biofilm (Bronk and Samet, 2008).

Unsuccessful treatment of infected vascular implants makes scientists implement new strategies in order to lessen the risk of infections. Infection-resistant implantable material might appear to be that innovation. Such material should be characterized with low thrombogenicity and immune indifference.

Implantable materials, currently used in vascular surgery, can be divided into three groups: synthetic, biosynthetic and biological. Professional literature does not, however, contain collective comparative studies on susceptibility of various implantable materials to infections.

Vascular prostheses, made of polyethylene terephthalate (Dacron) and polytetrafluoroethylene (PTFE) have been commonly applied for more than 50 years. PTFE is commonly believed to be less susceptible to colonization than Dacron. This observation was earlier confirmed in *in vitro* studies (Schmitt *et al.*, 1986), which revealed that most bacteria are prone to adhere to Dacron, rather than to PTFE. Bacterial adhesion to synthetic material, which vascular implants are made of, is closely associated with its colonization, which increases the risk of postoperative infections/complications in patients. However, clinical studies, comparing complications after implanting Dacron or PTFE, did not confirm that one material outdoes the other. Patency indices for bypasses manufactured from both the synthetic materials, and the number of infection-induced complications in both the groups were similar (Post *et al.*, 2001; Jensen *et al.*, 2007).

Scientists had high hopes that biosynthetic prostheses would make infection-resistant implantable material. One of such prostheses is a composite bioprosthesis, Omniflow II. Its complex structure consists of polyester mesh (a supportive element and framework for biological component) which is coated with ovine collagen (facilitating permeation of the graft wall through the recipient's vessels and tissues). The ability of Omniflow II prostheses to easily heal in the recipient's body has been known for more than 20 years (Werkmeister *et al.*, 1995). This property makes them an alternative for autologous material in the process of creating a vascular bypass (Dünschede *et al.*, 2015) or arteriovenous access for dialysis (Palumbo *et al.*, 2009). Application of Omniflow II prostheses in treatment of synthetic prosthesis infections is an interesting observation. In 2012 Töpel *et al.* (2012) presented the first benefits of Omniflow II prostheses which replaced infected bypasses, located below inguinal ligaments. In 2015 Krasznai *et al.* (2016) presented reconstruction of infected synthetic bypasses with Omniflow II prostheses in the aortoiliac segment. Most authors claim that Omniflow II prostheses are highly beneficial since they are resistant

to re-infections and degenerative changes. In a study conducted on rat models, Bozoglan *et al.* (2016) compared PTFE and Omniflow II biosynthetic prostheses in terms of their resistance to *S. aureus* infections and the authors arrived at completely different conclusions. They confirmed that Omniflow II prosthesis is more susceptible to infections than PTFE prosthesis.

For dozens of years scientists have also been conducting clinical studies on application of biological prostheses. They include patches, implants and valves produced from bovine or porcine pericardium, prepared according to the No-React technology (multistage detoxification process with the use of glutaraldehyde). Due to this technology, biological material is biocompatible with recipient's tissues and the biocomponent is protected against calcification. Biological material which has been prepared with the application of the above technology gets covered with endothelium within 6 weeks. The endothelial coating provides a natural barriers against infections. After conducting studies for longer than a decade, Musci *et al.* (2013) presented positive findings regarding application of biological prostheses in treating infectious endocarditis. Those positive results might encourage for a wider use of such prostheses. In a different study, Avsar *et al.* (2013) presented early results of using bovine internal mammary arteries, also prepared in the No-React technology. A number of patients were implanted 33 femoropopliteal bypasses, 100% of which remained patent one year following the surgery. The authors did not observe infections of the grafts, either.

The aim of our study was to compare susceptibility of selected synthetic and biological implantable materials, applied in vascular surgery, to colonization *in vitro* by *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *E. faecalis*.

Despite the fact that, *in vitro* experiments using abiotic medical devices and laboratory cultures lack the component of host produced proteins and specific protein-interactions, co-culture allows comparison of colonization using controlled condition by quantification of implant related bacteria.

In the study we tested 9 types of implants (numbered in the text 1 through 9), all types and their manufacturers are listed in Table I. The implants were made from various materials such as warp knitted polyester (with additional surface covering substances such as gelatin and silver ions), expanded polytetrafluoroethylene and two biological implants-surface treated porcine pericardial patch and Omniflow II.

To determine number of bacteria associated with implants, we used strains that represent the most common causes of vascular implant infections *i.e. S. aureus* (ATCC 29213), *S. epidermidis* (ATCC 14990), *P. aeruginosa* (ATCC 27853) and *E. faecalis* (ATCC 29212). Overnight cultures of each of the strains were diluted

in 75 ml of fresh TSB medium in 300 ml flask and fragments of sterile implants were placed in the diluted culture. Implants were incubated with bacteria for 24 h at 37°C with gentle mixing (100 rpm) to assure contact of the whole implant surface with the bacterial culture. After 24 h bacterial culture was decanted and implants were washed five times with 100 ml of sterile buffered saline (PBS). After the wash, each implant fragment was transferred to 7 ml of sterile PBS in a glass tube and bacteria associated with the implant were detached by sonication for 5 minutes on ice (Branson Sonifier 250, micro tip, output control 6, constant duty cycle). PBS with detached bacteria was serially diluted, plated on TSA solid medium and colony forming units (CFU) from 2–3 dilutions for each experimental variant were

counted the next day. The experiment was repeated independently for at least 3 fragments for each implant/bacterial species combinations. To calculate the number of bacteria per cm^2 of the implant, we used the formula

$$CFU/cm^2 = \frac{(CFU \text{ per 1 ml}) \times 7}{(\text{implant surface in } cm^2) \times 2}$$

Outliers from the datasets were removed using Grubbs' test (Grubbs, 1950) and the datasets were compared using 1 or 2 way ANOVA, to analyze one (species or prosthesis type) or two factor influence (species and prosthesis type).

We detected high colonization rate of all types of devices (Fig. 1) with median ranging from $1,94 \times 10^6$ CFU/cm^2 (implant no. 3) to almost 100 times higher value
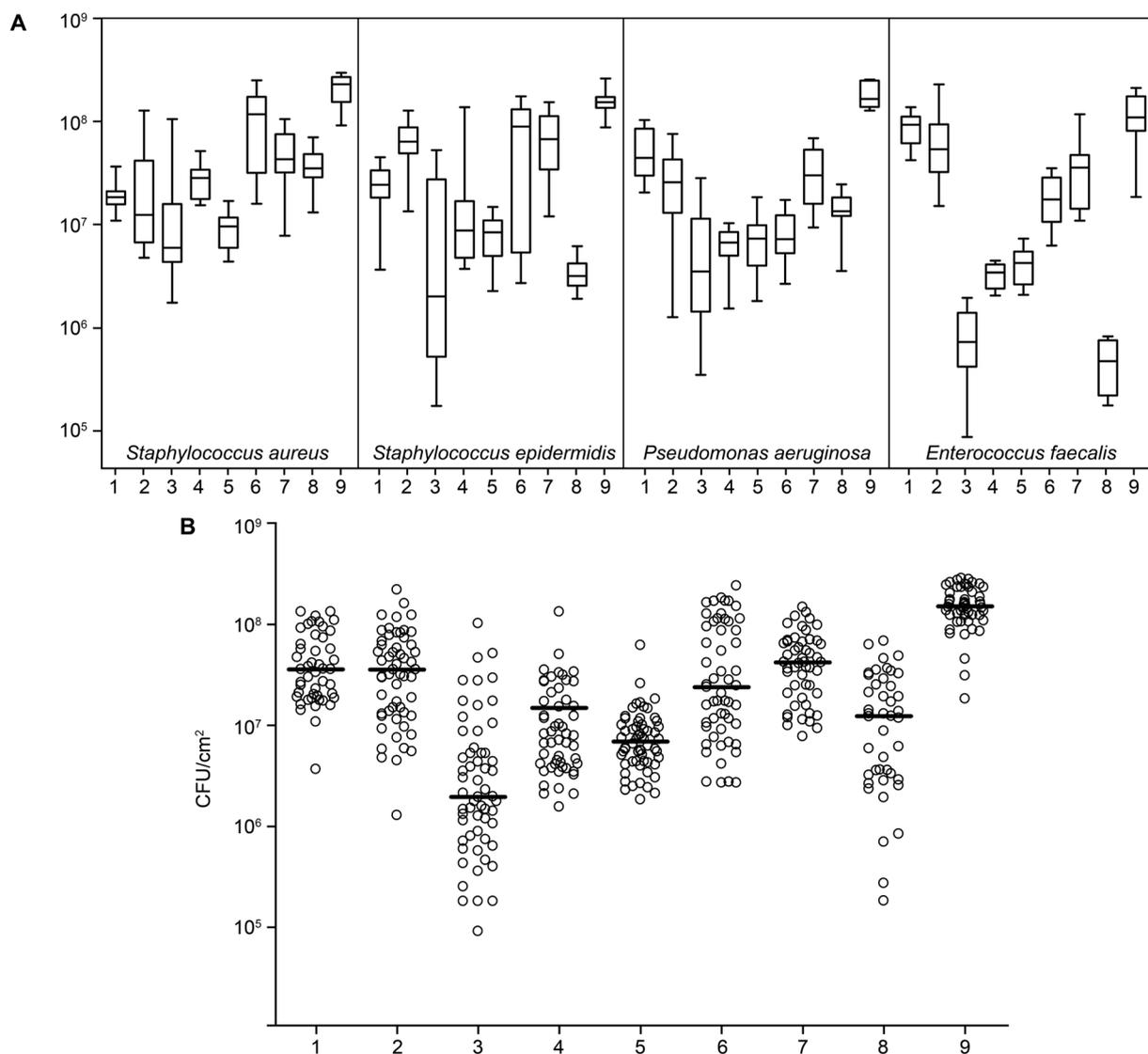

Fig. 1. Number of bacteria recovered from implants after 24 h incubation.
A. Results obtained individually for each bacterial species for implants 1–9 (description in Table 1 and in text). **B** combined data for all tested bacterial species. Each dot represents single bacterial count, from either separate implant or calculated from single dilution of bacteria. Statistical significance of observed differences in colonization was assessed using one way ANOVA with nonparametric Kruskal-Wallis test and Dunns multiple comparison post-test, * denotes $p < 0,05$, ** $p < 0,01$, ***$p < 0,001$. Statistically significant differences in colonization were detected for pairs 1 *vs* 3 (***), 1 *vs* 4 (***), 1 *vs* 5 (***), 1 *vs* 8 (***), 1 *vs* 9 (***), 2 *vs* 3 (***), 2 *vs* 4 (***), 2 *vs* 5 (***), 2 *vs* 8 (**), 2 *vs* 9 (***), 3 *vs* 6 (***), 3 *vs* 7 (***), 3 *vs* 8 (*), 3 *vs* 9 (***), 4 *vs* 6 (**), 4 *vs* 7 (***), 4 *vs* 9 (***), 5 *vs* 6 (***), 5 *vs* 7 (***), 5 *vs* 9 (***), 6 *vs* 8 (*), 6 *vs* 9 (***), 7 *vs* 8 (***), 7 *vs* 9 (***), 8 *vs* 9 (***)
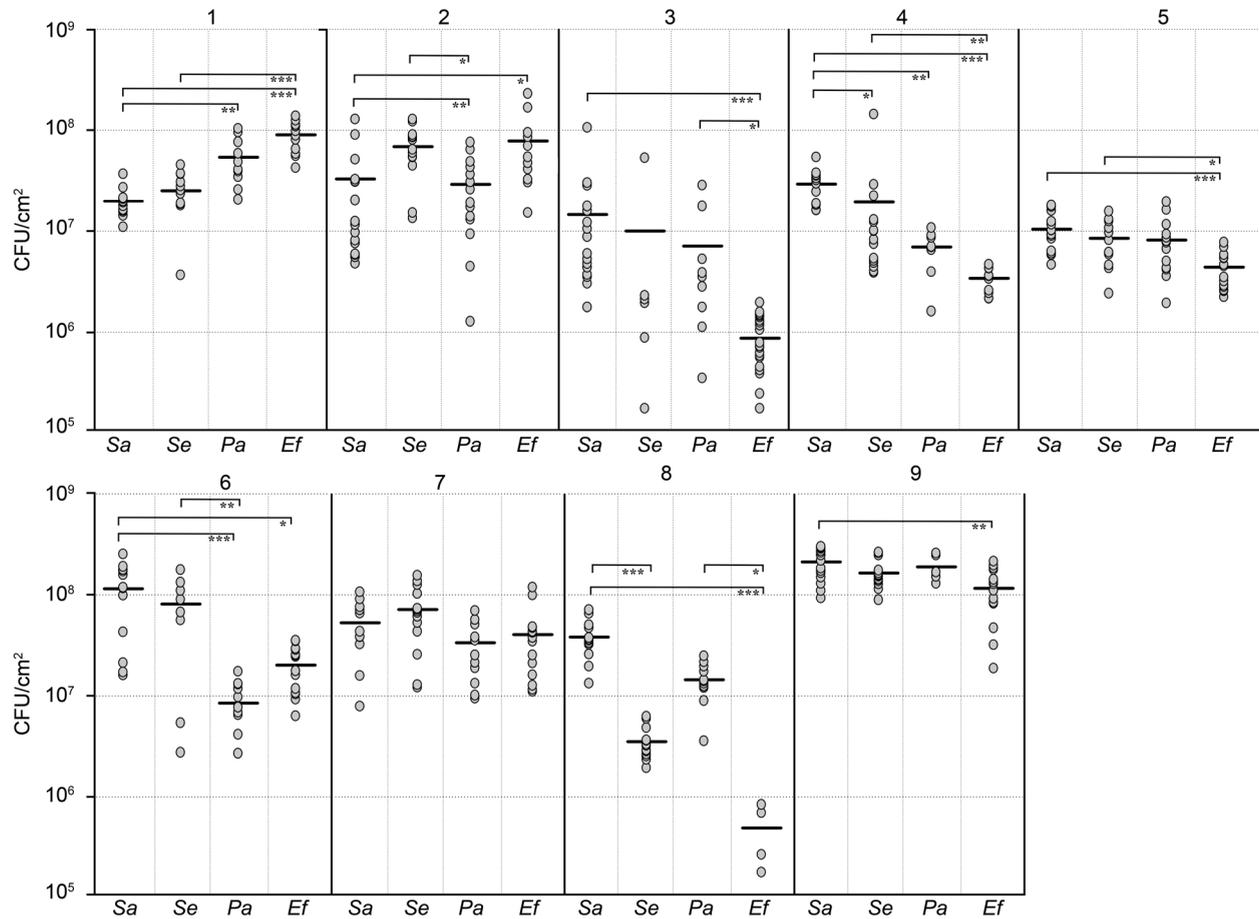
Fig. 2. The influence of bacterial species on the implant colonization.

Numerals 1–9 denote implant type (as in Table I and in text), *Sa* – *S. aureus*, *Se* – *S. epidermidis*, *Pa* – *P. aeruginosa*, *Ef* – *E. feacalis*. Each gray dot represents single bacterial count, from either separate implant or calculated from single dilution of bacteria. Statistical significance of observed differences in colonization was assessed using one way ANOVA with nonparametric Kruskal-Wallis test and Dunns multiple comparison post-test, * denotes $p < 0.05$, ** $p < 0.01$, *** $p < 0.00.1$

Table I
List of implants used in the study.

No.	Implant type	Material	Manufacturer
1	DALLON H	conventional warp knitted polyester vascular graft	TRICOMED SA
2	Gelsoft Plus	gelatin impreganted, conventional warp knitted polyester vascular graft	VASCUTEC Ltd. a TERUMO Company
3	IMPRA	expanded polytetrafluoroethylene (ePFTE)	BARD Peripheral Vascular, Inc.
4	VIABAHN ENDOPROSTHESIS	self-expanding endoluminal endoprosthesis consisting of an expanded polytetrafluoroethylene lining with an external nitinol support extending along its entire length	W.L.GORE &ASSOCIATES, Inc.
5	FLUENCY PLUS	self-expanding Nitinol Stent encapsulated with expanded polytetrafluoroethylene	BARD Peripheral Vascular, Inc.
6	Zenith Flex AUI AAA Endovascular Graft	full-thickness woven polyester fabric sewn to self-expanding stainless steel Cook-Z stents with braided polyester and monofilament polypropylene suture	WILLIAM COOK EUROPE ApS
7	Silver Graft	warp-knitted, double-velour vascular polyester prosthesis, impregnated with modified bovine gelatine (Polygelin) and coated with a layer of silver on its surface	B. Braun Melsungen AG Vascular Systems
8	NO-REACT PATCH	porcine pericardial patch	BioIntegral Surgical, Inc
9	Omniflow II Vascular Prosthesis	a composite biosynthetic material comprised of polyester mesh incorporated within a cross-linked ovine fibrocollagenous tissue matrix	Bio Nova International Pty Ltd

$1,56 \times 10^8$ CFU/cm^2 for Omniflow II, based on combined data recorded for all tested species (p < 0.0001). The lowest overall colonization was detected for implants no. 3 (Impra), 5 (Fluency) and 8 (porcine patch), regardless of the species (Fig. 1A and B). It confirms claims of porcine patch manufacturer that No-React® proprietary treatment with glutaraldehyde significantly lowers infection rate after implantation. Low colonization rate for both PFTE implants is also not surprising as lower attachment to hydrophobic surface can be expected.

The highest overall colonization rate was observed for Omniflow II (9) implant (Fig. 1), as shown by Bozoglan et al. (2016).

Increased susceptibility of Dacron to colonization in vitro was usually caused by its greater porosity than that of PTFP. Smoothing the Dacron surface with gelatin makes it less susceptible to infections in a mouse model (Yasim et al., 2006). For polyester implants, we did not observe the influence of the treatment with gelatin or gelatin and silver ions on the number of implant associated bacteria (1 vs 2, and 1 vs 7, Fig. 1B).

Two way ANOVA analysis of the whole dataset revealed that the major source of variation, i.e. primary factor influencing colonization, is the implant type (56,22% of total variation, p < 0.0001) and bacterial species is responsible for only 1,81% of total variation (p < 0.0001). Interaction of those two factors is a source of 13,09% of variation (p < 0.0001).

In a conclusion, a factor that the most influences colonization is the type and the material of the implant. We show that PFTE (No. 3) is colonized to much less extent than Omniflow II. The number of bacteria of different species associated with various implants varies. For example in our experiment, the best colonizer is S. aureus. Aseptics and a perioperative antibiotic therapy still remain the most common methods of combating infections of vascular prostheses (Giacometti et al., 2000).

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