Adhesion of *Staphylococcus Aureus* to Implants with Different Physicochemical Characteristics

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Adhesion of pathogenic *Staphylococcus aureus* (strain 209) to BT1-0 titanium disks (12 mm in diameter) with different coatings and noncoated was studied *in vitro* by photocolorimetry. Transparency of bacterial suspension in normal saline was evaluated after 2-h culturing with the implants at 37°C. The decrease of *S. aureus* content in the suspension due to its adsorption on implants was negligible and increased by 0.9-5.5% in comparison with the control (adhesion to glass). When the specimens were placed into bacterial suspension, the density of staphylococcal adsorption on the surface considerably increased (by 9-53%) in comparison with the control, which attested to active participation of the implants in bacterial adsorption. The degree of bacterial adhesion to the implants decreased in the following order: disk with calcium phosphate ceramic coating—disk with calcium phosphate X-ray amorphous coating—disk without coating—disk with cermet coating. The adhesion of Staphylococcus is a stochastic process depending on the sum of implant characteristics, in particular, on the phase composition of the coating, electric conductivity, and Ca/P ionic ratio. The authors conclude that the formation of antibacterial properties of coating by saturating them with antibiotics or impregnation with metals, specifically silver ion implantation, is justified, because it reduces the postimplantation infection risk.

Key Words: *staphylococci; adhesion; ceramic coating; photocolorimetry*

Postimplantation infectious complications deteriorating the results of treatment remain an important problem of traumatology and orthopedics [10]. It is well known that implants can potentiate infection due to synergism of proinflammatory effect of bacterial cells and foreign surface of implanted devices on the host [5]. Bacterial adhesion to artificial surface is a first important step in infection of implanted constructions. Chemical modification of the implant surface prevents adhesion of bacterial cells. Implants with protective ceramic coating improving their biocompatibility [1, 5,7] are now widely used in medical practice. On the other hand, there is no ideal material completely compatible with human organism and corresponding to bone tissue characteristics. Adherence to the biomimetic principle of the implant formation (calcium phosphate coating, surface macrorelief, *etc.*) improves the osteointegration, but is paralleled by increased risk of infection because of physical protection of bacteria from the host immune system [5]. Adhesion of bacteria to polymers, plastic, metals, and alloys, including titanium, was demonstrated [8,9], but surprisingly little is known about their interactions with calcium phosphate materials playing an important role in traumatology and orthopedics.

Staphylococci, specifically *S. aureus* are the most active bacteria contaminating implants and possessing high affinity to the bone tissue [3,5]. We studied

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S. aureus adhesion to coatings applied onto titanium carrier by various methods.

MATERIALS AND METHODS

Experiments were carried out on pathogenic *S. aureus* strain 209 from the collection of Department of Microbiology of Siberian Medical University (Tomsk). Strain 209 exhibits typical morphological, biochemical, hemolytic, and plasma-coagulating properties of this bacterium. Three types of coating were applied onto BT1-0 titanium disks (0.5 mm thick, 12 mm in diameter,): cermet (anodic coating with spark erosion, coating No. 1), ceramic calcium phosphate (slip casting, coating No. 2), and calcium phosphate coating formed by anode-spark method (coating No. 3) in hydroxyapatite-containing electrolyte as described previously [2]. Four disks of each type were used in experiments; each disk was incubated in a vial (bottom diameter 2 cm, volume 10 ml) at 37°C in bacterial suspension in normal saline (10^9 bacterial cells/ml, 5 ml) for 2 h.

The surface structure of the studied coatings (Table 1) varied from relatively smooth (noncoated disks), microroughness and micropores (coating No. 1), clear-cut micropores (coating No. 3), and macropores reaching the titanium carrier (coating No. 2). Different structure of the coatings ruled out the use of routine methods for evaluation of bacterial adhesion to surface (Lederberg impressions or scanning electron microscopy). Therefore after removal of the implants bacterial adhesion was evaluated by indirect (photocolorimetrical) method on a KFK-2 device by transparency (T) of bacterial suspension at λ=540 nm in cuvettes with optic path of 10 mm against normal saline. Bacterial suspension without disks (3 flasks) after 2-h culturing served as the control (adhesion to glass).

The results of the experiment were expressed in percent of the control **(1)**, in percent of the initial transparency **(2)**, and in absolute values by the calibration curve (Fig. 1):

$$\Delta T_1=(T1/T2-1)\times100\% \quad \textbf{(1)},$$

$$\Delta T_2=(T3/T4-1)\times100\% \quad \textbf{(2)},$$

where T1 is transparency of bacterial suspension after removal of disks (experiment), T2 transparency of the suspension after adhesion to glass (control), T3 transparency of the suspension in the experiment or in the control, and T4 initial transparency of bacterial suspension (before culturing).

Surface porosity of the coating was evaluated using Adobe PhotoShop 5.0 software on digital images obtained by optic (slip coating, ×50) and scanning electron microscopy (×2500 and ×5000). Phase composition of the coatings and calcium to phosphorus ratio were studied by X-ray diffraction and X-ray microspectral analysis. Electroconduction ($1/R_n$) of a coating was estimated from the transition resistance (R_n) measured in 0.9% NaCl on a P-5848 potentiostat as described previously [2].

The results were statistically processed using Student's *t* test and Mann—Whitney's *U* test.

TABLE 1. Physicochemical Characteristics of Implant Surface and *S. aureus* Adhesion after 2-h Culturing in 0.9% NaCl (*X*, *X*±*m*)

Parameter	Control (no disks)	Disks			
		noncoated	cermet	calcium phosphate	
				amorphous	ceramic
Porosity, %	—	0	7.16±0.73°	14.90±0.13°	5.35±0.70°
Ca, %/P, %	—	0/0	0.93/16.83	18.39/40.20	37.01/44.31
Conduction, $(m\Omega\times cm^2)^{-1}$	—	0.385±0.010	0.069±0.003°	0.213±0.029°	0.294±0.023°
dT, %					
compared to initial level	8.48 (3)	10.31* (4)	9.48 (4)	11.47* (4)	14.40*+ (4)
compared to control	—	1.6*	0.9	2.8*	5.5*+
Elimination of bacteria from suspension, 10^6 bacterial cells	328.5	383.5*	359	417.5*	502.5*+
Density of bacterial adsorption to surface, $10^6/cm^2$	104.6	122.1*	114.3	133*	160*+

Note. Significant differences: *from the control (*U* test); +from noncoated titanium disks (*U* test), °from noncoated titanium disks (Student's *t* test). The number of disks is given in parentheses.

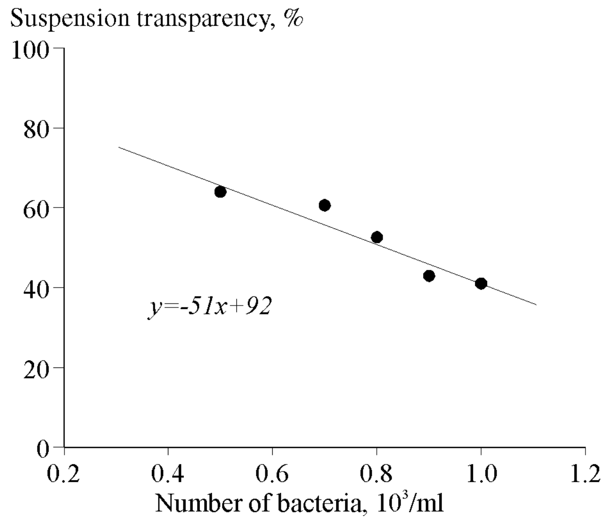

Fig. 1. Calibration curve of the number of staphylococci in normal saline.

RESULTS

The mean increment of transparency in the control (adhesion to glass) after 2-h adsorption of Staphylococcus was 8.48% (7.23-9.38%) of the initial level (Table 1). According to the calibration curve it corresponded to adsorption of 65.7×10^6 bacteria from 1 ml of suspension (Fig. 1). The flask bottom area was 3.14 cm^2, so the density of *S. aureus* precipitation on the glass from the total volume of suspension was 104.6×10^6 bacterial cells/cm^2.

Implants with different coating submerged into bacterial suspension increased its transparency in comparison with the initial value; adhesion of bacteria from the suspension also increased due to their fixation on the disks (Table 1) and reached 0.61-3.48% of their initial number. These values were notably higher than with implants without barrier coating (up to 40% adhering streptococci) [9]; the biocompatibility of constructions with modified surface was higher [2].

Bacterial adhesion to the implants decreased in the following series: coating No. 2—coating No. 3—no coating—coating No. 1. Only in the last case the differences from the control (by transparency and absolute values) were insignificant, whereas calcium phosphate coating sharply activated the studied process. Adhesion of bacteria from the suspension was the maximum with coating No. 2 (Table 1), being notably higher than in the control (by 53%) and with noncoated samples. Hence, the presence of disks in the suspension considerably increased the density of Staphylococcus precipitation on their surfaces in comparison with the control (Table 1), which proved active participation of the implants in bacterial adsorption.

Bacterial adhesion to metal surfaces directly depended on their electric conductivity [9]. This correlation was also observed for implants with barrier cermet or calcium phosphate layers (Fig. 2, *a*). However, self-passivated titanium alloy was characterized by the highest conductivity of the surface due to the differences in the slight oxide film and median adhesion of Staphylococcus (Table 1).

A possible explanation of this phenomenon is well-developed surface relief of modified implants increasing the contact area with the bacterium and protecting the bacterium from the host immune system [5]. Adsorption of *S. aureus* increased with increasing porosity of the coatings formed by the electrochemical method (Table 1). On the other hand, a virtually twofold increase (compared to coating No. 3) of the number of staphylococci adhering to the slip coating was associated with leveling of the surface relief. R. A. Bos *et al.* [4] noted that microorganisms poorly adhere

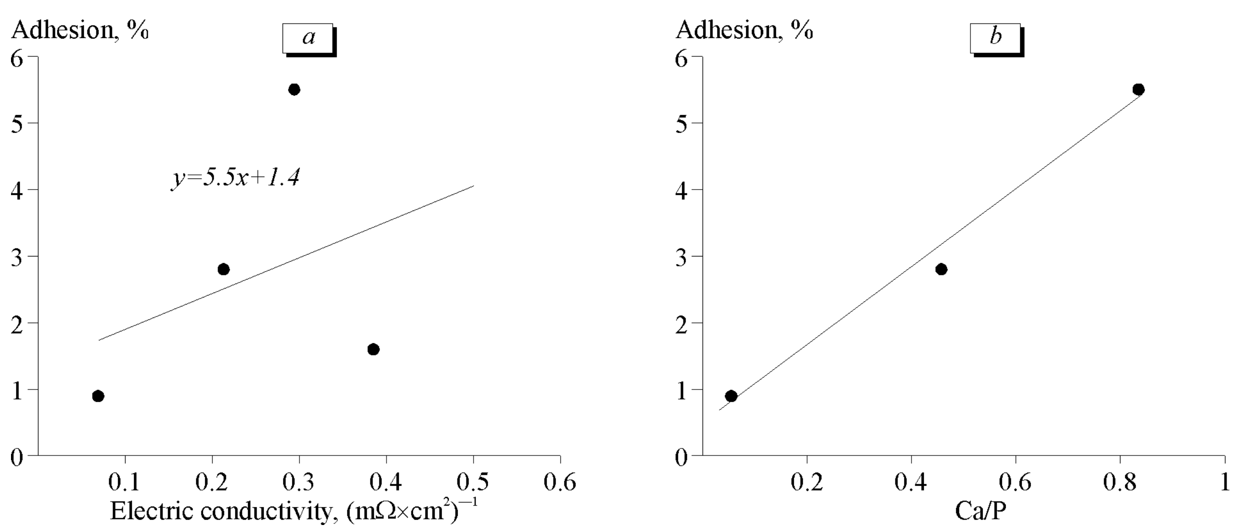

Fig. 2. Relationship between *Staphylococcus aureus* adhesion to implants and electric conductivity of their coating (*a*) and Ca/P ratio (*b*).

to scratches and warts. Presumably, the surface relief is not the key factor in the process of bacterial adhesion.

Chemical composition of coating is an important factor for bacterial colonization. For cermet layer the main phase is titanium oxide (anatase, rutile) with low content of titanium phosphate $Ti_4P_6O_{23}$. For electrochemical calcium phosphate coating the phase composition included titanium, phosphorus, and calcium compounds: titanium hydroxyphosphate $(TiO)_2P_2O_7$, titanium phosphate $Ti_4P_6O_{23}$, calcium titanate $CaTi_4O_9$, calcium titanophosphate $CaTi_4(PO_4)_6$, calcium phosphate CaP_4O_{11}, tricalcium phosphate (vitlokite) Ca_3PO_4. Slip coating consisted mainly from hydroxyapatite and tricalcium phosphate with increased Ca/P ratio (Table 1). *S. aureus* possess affinity for bone tissue [3,6] and hydroxyapatite [11]. This factor can underlie more intensive adhesion of the bacterium to amorphous, and more so, to cermet Ca/P coating in comparison with metal ceramics (Table 1; Fig. 2).

Hence, adhesion of *S. aureus* is a stochastic value depending on the sum of the implant surface characteristics, specifically, on electric conductivity and Ca/P ratio (coincidence with bacterial adhesion in 3 of 4 cases) and to a lesser extent on surface porosity (in 2 of 4 cases).

Despite low relative adsorption of Staphylococcus on the implants (Table 1), the absolute values for samples with different coating varied from 30.5 to 174×10^6 bacterial cells on the surface area of 2.26 cm^2. The dose sufficient for infection of the wound is 10^6 microbial bodies. The implanted materials can decrease the threshold dose causing infective inflammation to 10^2 bacterial cells depending on their biocompatibility [5]. Therefore, despite high clinical efficiency of implants with ceramic coating even under conditions of the pin tract infection, the contamination during orthopedic and traumatological operations remains possible. In the light of our data, the formation of antibacterial properties of coatings by saturation with antibiotics [5] or metal impregnation, *e. g.* ionic implantation of silver, seems to be justified.

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