In vitro study of the antibacterial properties of amorphous copper-doped carbon coatings

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Abstract. A deposition technology of amorphous carbon coating (a-C), doped with copper (C-Cu), has been developed. The C-Cu coating deposition was performed with the simultaneous operation of arc- and pulse-arc sources with copper and graphite cathodes, respectively. There was studied the antibacterial activity of the a-C and C-Cu against E. coli, S. aureus and P. aeruginosa. The copper-doped coating has bactericidal activity against P. aeruginosa. The number of bacterial colonies is five times lower in compare with a-C and Ti.

1. Introduction
The number of infectious complications after surgical interventions using endoprostheses is steadily increasing. The introduction of various metal structures is often accompanied by the occurrence of implant-associated infections. Microorganisms attach to the surface of the endoprosthesis, accumulate, forming a biofilm. Existing medical technologies to treatment deep infection involve the use of temporary prostheses (spacers) or revision implants in combination with antibiotic-containing bone cements. This approach does not exclude the occurrence of a relapse, since the complete death of microcolonial forms of bacteria requires about a hundred times higher concentrations of antibiotics [1–3].

To improve the mechanical properties and corrosion resistance of metal implants, as well as to increase the biological and antibacterial activity of their surface, biocompatible coatings are used. Diamond-like amorphous carbon coatings (a-C), due to their good corrosion resistance, wear resistance, and biocompatibility, attract much attention in the field of biomedicine [4]. However, the a-C coatings do not possess sufficiently antibacterial activity, since amorphous carbon can only interact with bacteria that have been adhered to its surface. Copper is an important micronutrient that attracts attention owing its antibacterial activity and role in wound healing [5–6]. The released copper ions tend to interact with cells or bacteria in a wide range. Therefore, a-C coatings, doped with copper, can provide better antibacterial activity.

The objective of this work was to study the antibacterial properties of coatings based on amorphous carbon and amorphous carbon doped with copper.

2. Materials and methods
An UVNIPA-1-001 industrial installation was used to deposit coatings on titanium disks of 20 mm in diameter and a thickness of 3 mm. Before coating deposition, the discs were washed in an ultrasonic
bath in a mixture of distilled water and alcohol. The final cleaning was carried out by etching with Ar$^+$ ions (E = 4 keV, P = 4.2×10$^{-2}$ Pa, 30 min). All surface of the discs were covered.

A-C and similar layers in a two-layer coating were deposited using a pulse-arc source with a graphite cathode. a-C, doped with copper, was deposited with the simultaneous operation of an arc plasma source with a copper cathode and a pulse-arc source with a graphite cathode. The copper concentration was determined by X-ray energy dispersive spectroscopy using scanning electron microscope QUANTA-200.

The study of antibacterial activity of bare titanium (control sample) and titanium with coatings was provided in the microbiological laboratory of the Tyumen State Medical University. By using S. aureus (ATCC 25923), E. coli (ATCC 25922), and P. aeruginosa (ATCC 27863) the antibacterial activity of samples was evaluated. The coated titanium disks and the control samples were seeded in vials with meat-peptone broth (MPB) with a concentration of 200 bacterial cells (BC) in 1 ml of the culture medium. Bacteria on various surfaces were then cultured in a 37 °C incubator for 3 and 6 hours. After this procedure medium with bacteria in the amount of 0.1 ml were placed on Petri dishes with a solid medium "Mueller-Hinton agar" and cultured in an incubator at t = 36°C for 24 hours.

The number of bacterial colonies after incubation was counted using an optical microscope. The number of colonies was taken as an indicator of the activity of bacterial cells. There was used the arithmetic mean of the indicator for five samples to describe the assessment of the sample. The significance of the difference between the mean values of the two samples was assessed using the STUDENT TEST function. The difference was considered significant at p ≤ 0.05.

### 3. Results and discussion

Two type of the coatings were deposited on titanium disks: amorphous carbon (a-C) or amorphous carbon, upper layer of which is alloyed with copper ([a-C/Cu]). The results of the bacteriological tests are presented in table 1. The characteristics of the samples are shown in table 2.

**Table 1.** The number of colonies of bacterial strains after incubation of medium taken from vials with samples incubated in MPB with BC for three and six hours.

| Sample      | Bacterial cells | Incubation time of MPB with BC with samples /time of incubation of MPB with BC without sample |
|-------------|-----------------|-------------------------------------------------------------------------------------------|
|             | S. aureus       | E. coli         | P. aeruginosa                                      |
|             | Incubation time of MPB with BC with samples /time of incubation of MPB with BC without sample |
|             | h               | 3/24            | 6/24                   | 3/24            | 6/24                   | 3/24            | 6/24                   |
| Ti (control)| 7               | 23              | >200                   | 5               | 49                     | >200                   | 5               | 25                     | >200                   |
| Ti+[a-C]    | 8               | 25              | >200                   | 5               | 53                     | >200                   | 6               | 22                     | >200                   |
| Ti+[a-c/Cu]| 7               | 27              | >200                   | 4               | 50                     | >200                   | 3               | 5                      | 54                     |

The presence of a statistically significant difference (p ≤ 0.05) in the antibacterial activity of [a-C/Cu] was established against P. aeruginosa strains only. At the same time, the ability to suppress the growth of S. aureus and E. coli was not found for both a-C and [a-C/Cu] (p ≥ 0.05).

Early, the authors of ref. [7] investigated the antibacterial properties of diamond-like carbon coating and that coating doped with germanium. The coatings were deposited by the HC-PECVD technique. Both coatings showed a significant effect on P. aeruginosa. There was a 90% decrease in
P. aeruginosa biomass compared to the control. They did not find an inhibitory effect of both coatings on S. aureus also. It has been suggested that antibacterial effect is related to the difference between the thickness of the cell wall and the composition of gram-positive (S. aureus) and gram-negative (P. aeruginosa) bacteria. In our experiment, we did not find antibacterial effect of the diamond-like carbon coating (a-C) against P. aeruginosa, E. coli and S. aureus. This is most likely owing the difference in the composition and structure of a-C and a-C:H films deposited by PVD and HC-PECVD techniques.

Table 2. Characteristics of coatings deposited on titanium disks.

| Sample     | Coating     | Thickness of coating (thickness of separate layer) | Copper content at.% |
|------------|-------------|---------------------------------------------------|---------------------|
| Ti+[a-C]   | a-C         | 200                                               | -                   |
| Ti+[a-C/C-Cu] | [a-C/C-Cu] | 700 (500/200)                                    | 15.3                |

When amorphous carbon, doped with copper, contacts the biological medium, Cu²⁺ ions are released into the surrounding biological environment [5]. Copper ions are not cytotoxic and can effectively kill bacteria if its concentration is within 7 mg/L [8]. In most of the experiments, reported in the literature, the C-Cu coated samples were active against S. aureus and E. coli after incubation of the samples in a bacterial medium for at least 24 hours. In our experiment, the incubation of the samples in the medium with bacteria was 3 and 6 hours, which is most likely not enough to release copper ions in a sufficient amount to inhibit bacterial growth.

4. Conclusions
The copper-doped [a-C/C-Cu] coating keeps down the growth of colonies of P. aeruginosa already after three hours of incubation. Compared to titanium and a-C coating, the number of colonies is reduced by a factor of five. The coating has a bactericidal effect, which makes it promising for use in implantology.

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