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Darya Alontseva[1*], Bagdat Azamatov[1,2], Alexander Borisov[2], Bauyrzhan Maratuly[2], Yuliya Safarova (Yantsen)[3], Sergii Voinarovych[4], Alexey Dzhes[2], Leszek Łatka[5]**

[1] School of Digital Technologies and Artificial Intelligence, D. Serikbayev East Kazakhstan Technical University, Ust-Kamenogorsk, Kazakhstan
[2] Smart Engineering Competence Centre, D. Serikbayev East Kazakhstan Technical University, Ust-Kamenogorsk, Kazakhstan
[3] Laboratory of Bioengineering and Regenerative Medicine, National Laboratory Astana, Nazarbayev University, Astana, Kazakhstan
[4] E.O. Paton Electric Welding Institute of NAS of Ukraine, Kyiv, Ukraine
[5] Department of Metal Forming, Welding and Metrology, Faculty of Mechanical Engineering, University of Science and Technology, Wrocław, Poland

***** *dalontseva@ektu.kz*
*** leszek.latka@pwr.edu.pl*

ANTIBACTERIAL Ti-Cu AND Ta-Cu COATINGS FOR ENDOPROSTHESES APPLIED BY MAGNETRON SPUTTERING ONTO Ti6Al4V ALLOY

ABSTRACT

The paper presents new results of manufacturing coatings by magnetron sputtering to improve the functional properties of joint endoprostheses. The antibacterial properties of Ti-Cu and Ta-Cu coatings deposited by DC multi-magnetron sputtering on Ti6Al4V alloy substrates subjected of gas-abrasive treatment have been investigated. The roughness of the substrate was measured by optical profilometry. The coating hardness and elastic modulus were estimated by nanoindentation methods; the adhesion characteristics were assessed by Rockwell test. Scanning electron microscopy with energy-dispersive X-ray analysis verified the application of coatings with 25 at.% Cu, at thicknesses of 2 μm and 10 μm to roughened Ti6Al4V alloy. All coatings demonstrated sufficient adhesion, whereas Ta-Cu coatings generally revealed higher hardness, while the elastic modulus decreased with increasing coating thickness. Staphylococcus aureus strains were used for in vitro study of the antibacterial properties of Ti-Cu and Ta-Cu coatings. The largest zones of inhibition of bacteria S. aureus 23 mm were observed for 10 μm Ta-Cu coating thickness. The release dynamics of Cu ions from Ta-Cu and Ti-Cu coatings into physiological solution analyzed over seven days via inductively coupled plasma mass spectrometry, matched the inhibition zone growth. The Ti-Cu and Ta-Cu coatings of 2 μm thickness provided weaker antibacterial effect. The optimal parameters of magnetron sputtering of antibacterial Ti-Cu and Ta-Cu coatings on Ti6Al4 alloy substrates were selected. These findings support the potential of these coatings in developing endoprosthesis implants with enhanced antimicrobial and wear-resistant properties

Keywords: *magnetron sputtering; antibacterial coatings; titanium implants*

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INTRODUCTION

According to Medical Implant Market Research Report (2021), based on National Institutes of Health research worldwide, the number of people aged 65 years and older was approximately 703 million in 2019 and is expected to reach 1.5 billion by 2050, representing 16% of the world's population [1]. As the population ages, chronic diseases such as age-related injuries, osteoarthritis and osteoporosis are becoming more common. Therefore, the number of primary total hip replacement surgeries is projected to increase, and the number of such surgeries will continue to increase as the population ages [2]. In addition, the prevalence of diabetes, the increase in the number of people with excess body weight due to unhealthy lifestyle and other health problems lead to an increasing number of young patients suffering from joint and bone defects, which threatens their health and significantly reduces the quality of human life in general [3, 4]. If the reason for the deterioration in the quality of life of a sick person is a damaged or diseased joint that causes severe pain and limited mobility, then surgical replacement of the joint with an appropriate implant may be necessary to treat this condition. Accordingly, the global orthopedic implant market size was estimated at US$48 billion in 2022 and is projected to reach around US$72.08 billion by 2032 [1] Both the aging and younger populations require extended implant life to spare patients from further revision surgeries. Currently, some of the most common causes of implant failure are aseptic loosening and infection, accounting for approximately 20% and 18% of implant failures, respectively [5].

Implant-related infections are the most common and challenging problem in clinical orthopedics and are the most common cause of implant failure. Bacterial infections, primarily caused by Gram-positive species like Staphylococcus aureus and Staphylococcus epidermidis, often form resilient biofilms that are challenging to eradicate with standard antibiotics [6]. The formation of biofilms on implants is an important feature of periprosthetic infection, which is involved in its occurrence and development, and should be taken into consideration for the prevention and treatment of implant-related infection [7-9]. Once formed, biofilm is difficult to eliminate, ultimately leading to chronic infections. Antibiotics, traditionally the most common strategy for treating infections, are usually not effective for periprosthetic infections because they cannot penetrate the biofilm. In addition, antibiotics can cause a number of problems, such as cytotoxicity and delayed bone regeneration. As a result, if a periprosthetic infection occurs, there is a high probability of repeat surgery to replace the implant.

To prevent the formation of biofilms on the surface of the implant, one of the important strategies is to give the implant antibacterial properties. Technologies for applying active antibacterial metal coatings to the surface of the implant are currently being developed [10-12]. Some metals (Cu, Ag, etc.) have bactericidal properties; their nanoparticles (as well as their oxides and nitrides) are widely used in medicine [13-15]. In recent years, two-component Cu-Ti, Cu-Ta, Cu-Nb, Ti-Ta coatings and multi-component Cu-Ta-Nb, Ti-Ta-Nb coatings due to their excellent physical and chemical properties and biocompatibility, have attracted great attention when modifying the surface of biomedical materials [16-19]. Coatings made from these metals can simultaneously have many properties, such as being bioactive and promoting osseointegration, suppressing microbial colonization, and having sufficient mechanical strength. In particular, Stranak et al [20] established the concept that a thin film Ti-Cu coating on an implant could provide a local antibacterial environment while remaining relatively nontoxic to the human osteoblast cell line. While pure copper is cytotoxic and Cu coatings are not wear-resistant, the authors of papers [21-24] report that doping Cu coatings with Ti and Ta can significantly reduce cytotoxicity and increase the strength, adhesion to the substrate and wear resistance of these coatings compared to coatings from pure Cu.

In studies [20, 22], magnetron sputtering (MS) was employed to fabricate Ti-Cu thin films that demonstrated significant antimicrobial efficacy against *Escherichia coli* and *Staphylococcus aureus*. The antimicrobial activity and cytotoxicity of these coatings are closely tied to the copper content in the films, with optimal Cu concentrations reported to range between 20 and 80 wt%. Norambuena et al. [22] highlighted that TiO and TiCuO coatings containing 20%, 40%, and 80% Cu, which were deposited using high-power pulsed magnetron sputtering (HiPIMS) on Ti6Al4V alloy discs, exhibited the highest effectiveness at 80 wt% Cu. At this concentration, a notable reduction in biofilm density and planktonic cells was observed, coupled with minimal cytotoxicity toward human osteoblasts in vitro. This antimicrobial potency is largely attributed to the controlled release of Cu ions in biological fluids, which disrupts microbial growth.

One of the primary advantages of the MS technique is its ability to produce high-purity thin films under vacuum conditions. Research [20, 23- 25] indicates that Ti-Cu MS coatings typically range from tens to several hundred nanometers in thickness, such as 64–122 nm [20] and 420–880 nm [23], and are applied to meticulously polished surfaces. For instance, Wang et al. achieved extremely smooth surfaces on Ti6Al4V alloy with an average roughness (Ra) of 0.03 ± 0.02 μm [24]. However, in the context of orthopedic endoprosthetic implants, higher surface roughness is generally preferred, as it enhances the contact area between the implant and bone, promoting improved fixation and osseointegration. Research suggests that the ideal roughness for titanium orthopedic endoprosthetic implants lies within a broad Ra range of 0.07 to 100 μm [26, 27], with Jahani B. and Wang H. identifying the optimal range to be 20–25 μm [27]. Thus, research indicates that the optimal surface roughness of titanium implants depends on the specific application and desired properties. For implants where greater corrosion and lower ion release are prioritized (for example, titanium plates, which are removed after healing), smoother surfaces with lower roughness are recommended [20, 23-25]. Conversely, for applications where osteo-integration is critical, such as endoprosthetic implants, surfaces with greater roughness have been shown to enhance bone-implant interaction and promote better osseointegration [26, 27]. These contrasting requirements demonstrate that the concept of an 'ideal' surface roughness is context-dependent, and the selection of surface roughness must align with the implant's intended function.

Copper-based coatings, specifically Cu and Cu-O films deposited on titanium via MS, have demonstrated excellent antimicrobial properties [22, 28, 29]. However, both the biocompatibility and antibacterial effectiveness of these coatings are highly sensitive to the deposition parameters and thickness of the film. For example, Ilievskaya et al. [28] showed that the antimicrobial activity of Cu-O MS coatings on commercially pure titanium (cp Ti) against *Escherichia coli* increased with the thickness of the coating, resulting in up to a 79.6% reduction in bacterial viability. The thickness of these coatings ranged from 41 to 125 nm, with deposition times of 5, 10, and 15 minutes, respectively. Despite the antimicrobial benefits, thicker coatings exhibited cytotoxicity to MG-63 osteoblast-like cells, reducing their viability by up to 60% compared to uncoated cp Ti. The release of Cu ions from the coatings deposited over 15 minutes further reduced the viability of MG-63 cells within 24 hours, indicating a potential trade-off between antimicrobial efficiency and cytotoxicity. Therefore, when developing two-component Ta-Cu and Nb-Cu coatings on titanium substrates via MS, it is crucial to optimize the balance between biocompatibility and antimicrobial efficacy. This can be achieved by carefully controlling the copper content and managing the release dynamics of Cu ions to ensure both effective microbial suppression and safe interaction with human cells.

Thus, in recent years, new ideas have emerged on the composition and materials of coatings and various technologies have been used for applying antibacterial coatings to orthopedic implants to extend the life of the implants and meet the needs of patients, of which the most promising and widespread is MS. Coatings produced in vacuum by MS have high purity, which is especially important for medical products. Therefore, MS coatings, their composition, microstructure and properties, as well as coating manufacturing technology are the focus of contemporary research. It is important to note that MS technology is also successfully combined with such advanced technologies as additive manufacturing (AM) of complex parts from Ti6Al4V alloy and physical vapor deposition (PVD) of ceramic coatings on them [30], which highlights the growing interest in these technologies for biomedical applications. On the other hand, Ti13Nb13Zr alloy is also deep investigated [31-33].

Our choice of composition and coating materials was guided by the critical insights presented in studies [18-20, 23]. These factors included, firstly, the superior bioactivity of Ta and Ti in comparison to the bioinert Ti6Al4V alloy, and secondly, the optimal proportions of Cu in coatings recognized for their potent bactericidal properties. Utilizing Confocal Dual Magnetron Co-Sputtering technology, as employed by the authors in [20, 21], we sought to refine the MS parameters by drawing comparisons with their results.

Some alternative method on the field of surface engineerins is modification by laser treatment which is used also for different titanium alloys [34-36]. It could improve mechanical properties as well as its biomedical ones.

The goal of this study was to evaluate the antibacterial properties of single-layer Cu-Ta and Cu-Ti coatings in relation to Staphylococcus aureus (in vitro), assuming that these MS coatings on titanium alloy substrates have greater potential for clinical use as functional coatings on large joint endoprostheses. In light of different opinions regarding the influence of various factors on the bactericidal effectiveness of coatings, our approach is focused on achieving 25 at.%. Cu content in the MS Ta-Cu and Ti-Cu coating and subsequent assessment of the influence of the coating thickness on its antibacterial properties.

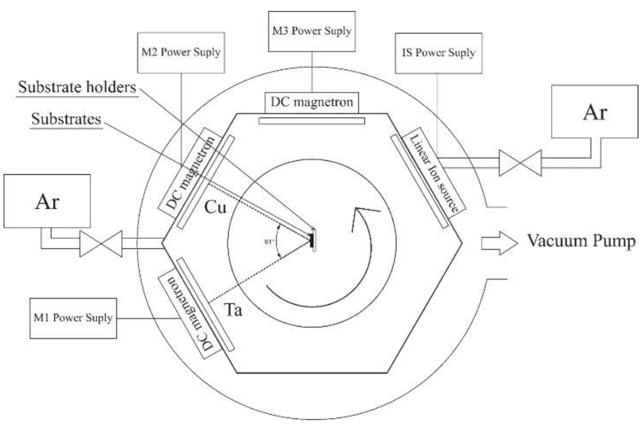
MATERIALS AND METHODS

For MS, rectangular metal targets made of pure Cu, Ta and Ti with dimensions of 472x132x18 mm were used. As a substrate for MS, specimens were used in the form of disks of 2 mm thickness, cut from rods of Ti6Al4V alloy with a diameter of 10 mm, using a CTX 510 ecoline computer numerical control (CNC) machine (DMG MORI AG, Bielefeld, Germany). The Ti6Al4V alloy is a Grade 5 titanium alloy according to the International Organization for Standardization (ISO) 5832-3 [31]. The alloy composition was also additionally verified by scanning electron microscopy (SEM) with EDX analysis, which confirmed the compliance of the alloy elemental composition with the specified standard.

MS of Ta-Cu and Ti-Cu coatings onto Ti6Al4V alloy substrate was carried out using an EPOS-PVD-440 installation (Beams&Plasmas, Novosibirsk, Russia), equipped with three direct current (DC) magnetrons (Magnetron-479) and an II-400 ion source (Beams&Plasmas, Novosibirsk, Russia). Before MS of coatings onto discs of Ti6Al4V alloy, the substrate surface was subjected to gas abrasive blasting treatment followed by cleaning. The gas abrasive surface treatment was carried out by a Contracor ECO abrasive blasting machine (Comprag Group GmbH, Germany) using normal grade A14 electrocorundum at a compressed air pressure of 0.6 MPa (the parameters specified in the previous paper [16]).

Immediately before MS, all specimens were cleaned in distilled water in a Stegler 22DT ultrasonic bath (Shenzhen Bestman Instrument Co., Ltd., China) for 30 minutes and dried in air for 40-60 minutes, then cleaned with an II-400 ion source installed in the vacuum chamber of the magnetron installation, with the following parameters: base pressure in the chamber: 7·10-4 Pa; working pressure in the chamber: 0.18 Pa; plasma gas (Ar) flow rate was 0.124 standard liter per minute [slpm]; discharge voltage: 280 V, discharge current: 1.7 A, distance from ion source to the substrate was 150 mm; the processing time was 20 minutes, after which MS was carried out.

Monolayer Ta-Cu and Ti-Cu coatings were deposited using simultaneous co-sputtering with two DC magnetrons and substrate rotation, as shown in Figure 1a, i.e., dual-MS was used. Argon (Ar) was used as the chamber gas; the base pressure in the chamber was 7·10-4 Pa; the operating pressure in the chamber was 0.2 Pa. The specimens were placed on a substrate holder in the form of a disk with a diameter of 190 mm (Fig. 1b). The axis of rotation of the substrate holder coincided with the axis of symmetry of the vacuum chamber. The substrate was not heated, the rotation speed of the substrate holder was 7.5 revolutions per minute.



a)

b)

Fig. 1. Magnetron sputtering process illustration: schematic view of multi-magnetron sputtering system (a) and specimen arrangement in the chamber (b)

To obtain coatings of the desired composition (25 at.%. Cu content), MS parameters were selected based on an analysis of literature sources given in the Introduction section, and determined the elemental composition of the coatings using energy dispersive X-ray (EDX) analysis, adjusting MS parameters as necessary. The selected MS parameters for Ti-Cu and Ta-Cu coatings are given in Table 1.

The MS process for Ti-Cu and Ta-Cu coatings on Ti6Al4V alloy substrates was conducted over 90 and 180 minutes. Consequently, the coatings were categorized into two distinct groups, as detailed in Table 1: Group 1 encompasses coatings that were sputtered for 90 minutes, resulting in thinner layers, whereas Group 2 includes coatings sputtered for 180 minutes, yielding thicker layers. Within each group, there were five specimens.

Before MS, the structural and phase composition of the Ti6Al4V alloy substrate was studied by X-ray diffraction analysis (XRD) using an X'Pert PRO diffractometer (PANalytical, the Netherlands). The XRD analysis confirmed that the Ti6Al4V alloy specimens have a typical two-phase α + β composition consisting of a Ti-α phase with a hexagonal lattice and a Ti-β phase with a cubic crystal lattice.

Table 1. *Parameters of magnetron sputtering of Ti-Cu and Ta-Cu coatings*

Coating Composition and Group	Sputtering material	Parameters/settings				
		Current I(A)	Voltage U (V)	Duration, t (min)	Sputtering distance, H (mm)	Plasma gas (Ar) flow rate, slpm
Ti-Cu (Group 1)	Ti	3.0	300	90	210	0.062
	Cu	0.3	380			
Ti-Cu (Group 2)	Ti	3.0	300	180		
	Cu	0.3	380			
Ta-Cu (Group 1)	Ta	3.0	315	90		
	Cu	0.6	400			
Ta-Cu (Group 2)	Ta	3.0	315	180		
	Cu	0.6	400			

Investigations of the structural-phase composition of the substrate are important for information on how it affects the structural-phase composition of the thin MS film on the surface. However, in this case, when magnetron sputtering was applied to the substrate after gas-abrasive treatment carried out with parameters suitable for thermal (microplasma) spraying of coatings, it is obvious that the surface condition has a greater effect on the coating microstructure than the phase composition of the substrate, and also leads to the need to form relatively thick coatings.

A substrate with high roughness was specifically chosen to replicate the surface conditions commonly encountered in biomedical implants, where rough surfaces are often required to promote better adhesion of coatings and enhance osteointegration. High roughness provides a larger surface area for coating deposition and can improve mechanical interlocking, which is critical for ensuring the durability and functionality of the coatings under physiological conditions.

The selected coating thicknesses were strategically chosen to guarantee a continuous layer on rough substrates, it was taken into consideration that the antibacterial efficacy of the coatings could be affected by the proportion of specific surface areas between the coated and uncoated regions of the substrate. The thicknesses of 2 μm and 10 μm were selected based on findings from our previous paper [32], where it has been demonstrated that antibacterial properties of MS Cu and Cu-Ti coatings emerge only when the sputtering time corresponds to thicknesses 2 μm and more. This occurs because a continuous coating fully covers the rough substrate, ensuring that no protrusions of the underlying material are exposed. On the other hand, Cu-based coatings, while exhibiting strong antimicrobial properties, may become cytotoxic to human osteoblast-like cells at higher concentrations or thicknesses, so we limited the coating thickness to 10 μm.

The microstructure examination, thickness evaluation, and elemental composition analysis of the coatings were performed using a BX51 light microscope (OLYMPUS, Tokyo, Japan) and a JSM-6390LV scanning electron microscope (JEOL, Tokyo, Japan). The electron microscope was equipped with an EDX attachment (INCA ENERGY, Oxford Instruments, UK) and the JSM-6060 (JEOL, Tokyo, Japan).

The surface characteristics of the specimens were examined at high resolution using a non-contact optical profilometer (Huvitz HDS-2520, Gyeonggi, Republic of Korea). Subsequently, 3D surface topographies and roughness properties were analyzed using Mountains® 9 software (Digital Surf, Besancon, France). The key roughness parameters for a given surface area were determined based on the mean areal roughness (Sa), calculated with a standard deviation of ±0.1 μm. The choice of the Sa parameter was due to its ability to provide more accurate and realistic roughness measurements compared to the arithmetic mean roughness (Ra), which only captures deviations along a single line within the area of interest. Each scan covered an area of approximately 1400 x 900 μm^2, and surface roughness was measured at three randomly selected locations on each specimen to ensure reproducibility.

The mechanical properties, specifically hardness and elastic modulus, were evaluated with a nanoindenter UNAT from ASMEC (Radeberg, Germany) at a force of 10 mN. This setting was chosen to maintain the penetration depth within 10-15% of the coating's thickness to avoid influence of substrate. The tests utilized a Berkovich diamond indenter. Each sample underwent fifteen measurements. The hardness (H) and elastic modulus (E) values were calculated from the load-displacement curve by applying the Oliver and Pharr method [33]. Prior to these tests, calibration was conducted using standard materials like fused quartz (SiO2) and sapphire (Al_2O_3).

In order to characterize adhesion of all coatings a Revetest Scratch Tester from Anton Paar (Graz, Austria) with Rockwell indenter was used. After indentation scanning electron microscope MIRA IV from Tescan (Brno, Czech Republic) was used to evaluate each coating. Backscattered electron (BSE) mode was applied in order to detect contrast between areas with different chemical compositions. On each sample three measurements were produced.

The release of Cu ions from magnetron-sputtered (MS) coatings on Ti6Al4V alloy was quantified using an ICP-MS Agilent 7500cx inductively coupled plasma mass spectrometer (Agilent Technologies, Wilmington, DE, USA). The specimens were immersed in a 0.9% sodium chloride (NaCl) saline solution, then placed in a low-temperature oven (SNOL 67/350, Umega Group AB, Ukmergė, Lithuania) and maintained at a constant temperature of 37°C for 24 hours to mimic physiological conditions. Cu^{2+} ion concentrations ($\mu g \cdot l^{-1}$) in the saline solution were measured after 1, 2, 3, 4, 7, and 10 days, using five specimens from each MS Ta-Cu coating group.

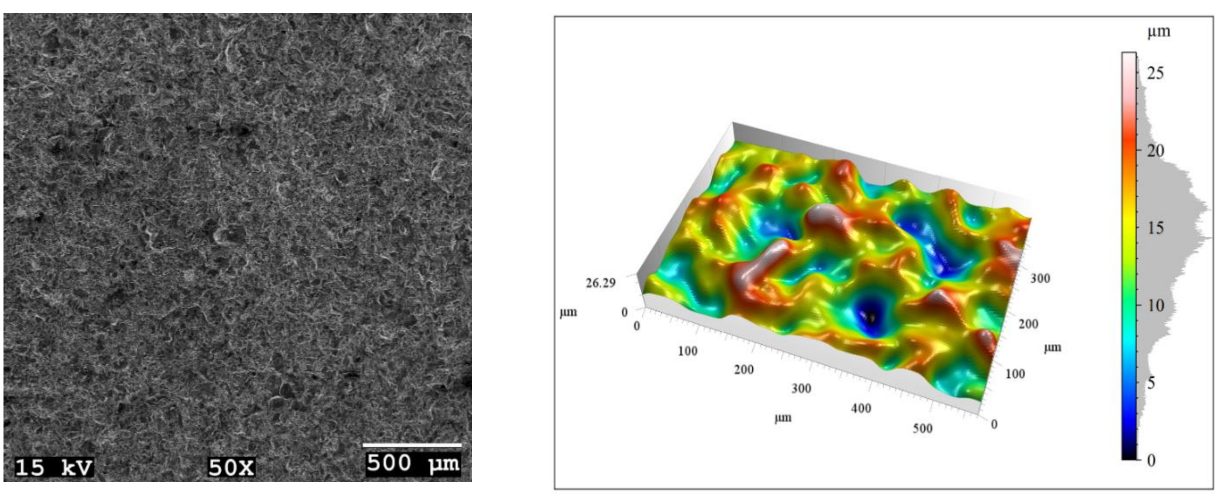
The antibacterial assay using the agar disc diffusion method is an established technique for evaluating the antimicrobial properties of materials, including titanium-based coatings. The material being tested, typically a disc-shaped sample, is placed onto an agar plate that has been uniformly inoculated with bacterial cultures. The agar disc diffusion assay is particularly useful for assessing the ability of coatings or surface modifications to inhibit the growth of bacteria, making it valuable for testing materials used in biomedical implants where resistance to bacterial infection is crucial. The bacterial strain Staphylococcus aureus (209P B-RM 005 from Biobank of Industrial Microorganisms RCM) was cultured in nutrient broth with a cell concentration of $3x10^7$ colony-forming units per milliliter ($CFU \cdot ml^{-1}$). Based on McFarland standards, this concentration corresponds to a bacterial turbidity standard of 0.5. Bacterial suspension was then spread evenly across the surface of nutrient agar in a Petri dish to create a "lawn" of bacteria.

A biologically neutral polypropylene (PP) disk served as the control in the experiment. Prior to testing, all samples were sterilized using a UV lamp. Either coated or uncoated, titanium discs are carefully placed onto the inoculated agar surface. As the discs come into contact with the agar, any antibacterial compounds on the surface of the titanium will diffuse into the surrounding agar, potentially creating an inhibition zone where bacterial growth is suppressed. The plates were incubated at 37°C for 24 hours, after which the diameter of the inhibition zone around each disc was measured. The antibacterial activity of the samples against S. aureus strains was assessed by measuring the inhibition zones of bacterial growth in millimeters after 24 hours. The reported results represent the average of four independent measurements. The size of this inhibition zone is a direct indicator of the antibacterial efficacy of the material being tested. Larger inhibition zones indicate more effective antibacterial activity, while the absence of an inhibition zone suggests that the material lacks significant antimicrobial properties.

The agar disc diffusion assay is particularly useful for assessing the antibacterial activity of titanium used in medical implants because it mimics the initial phases of bacterial colonization on the surface of the implant. Titanium and tantalum are susceptible to bacterial colonization, which can lead to infections. By applying antibacterial coatings, such as copper-containing, the performance of titanium implants in preventing infections is improved, thereby reducing the need for antibiotics and minimizing complications after surgery. While the method is relatively simple, it provides valuable insights into the antimicrobial capabilities of modified titanium surfaces. The test allows comparing different formulations, concentrations, and thicknesses of antibacterial coatings. It also helps identify optimal conditions that ensure both effective bacterial inhibition and minimal cytotoxicity, which is crucial for maintaining the biocompatibility of the titanium implant. The results from these assays are critical for further development and improvement of biomaterials used in medical devices and implants.

RESULTS AND DISCUSSION

Figure 2 presents an SEM image of the Ti6Al4V alloy substrate surface following gas abrasive treatment, along with the corresponding surface roughness measurements.

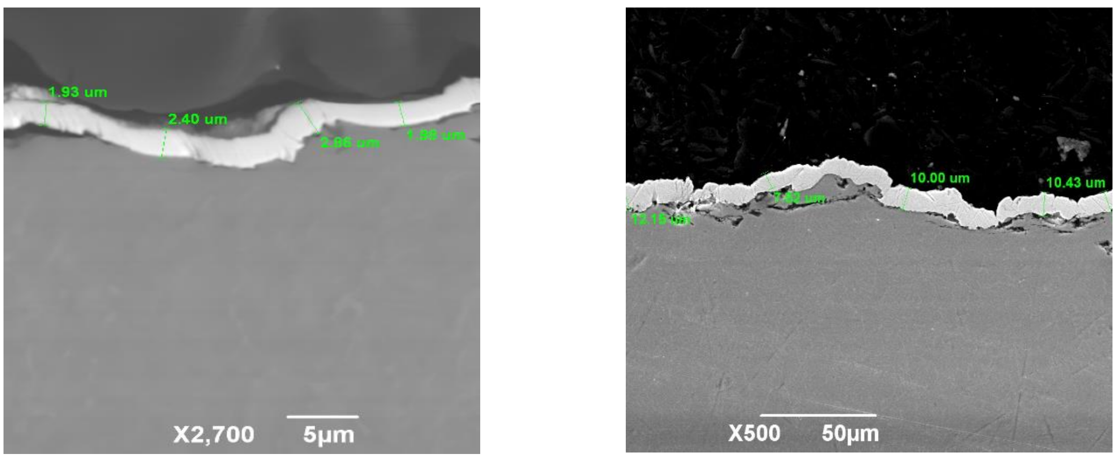


a) b)

Fig. 2. SEM image (a) and 3D surface topography (b) of the Ti6Al4V alloy after gas abrasive treatment

The mean areal roughness (Sa) was measured at 4.6 ± 1 µm, which aligns well with previously reported data in the literature [26, 27]. The grit-blasted specimens in this study demonstrated roughness values similar to those found by Lewallen et al. (Ra 5.74 ± 0.19 µm) and notably higher than the roughness of bead-blasted substrates (Ra 1.10 ± 0.18 µm) [26]. Surface topography plays a vital role in determining the mechanical and biological properties of biomedical titanium alloys, as emphasized in prior research [34]. Gas abrasive treatment of the Ti6Al4V alloy resulted in unevenly distributed valleys and peaks across the surface, with maximum heights of approximately 12.5 ± 1 µm and 14.0 ± 1 µm, respectively. This surface topography creates favorable conditions for cell adhesion and protein adsorption, thereby enhancing the long-term stability of implants. Additionally, the microstructural complexity significantly contributes to the antibacterial properties of metallic biomaterials [34].

The results of measuring the thickness of MS Ta-Cu coatings on Ti6Al4V alloy substrates subjected to gas abrasive treatment are presented in Figure 3. The image of the titanium alloy is uniform dark gray, Ta-Cu coatings are light gray. Since the specimens were sealed in epoxy resin, its image is also shown in Fig. 3 on top of the rough surface of the specimen.

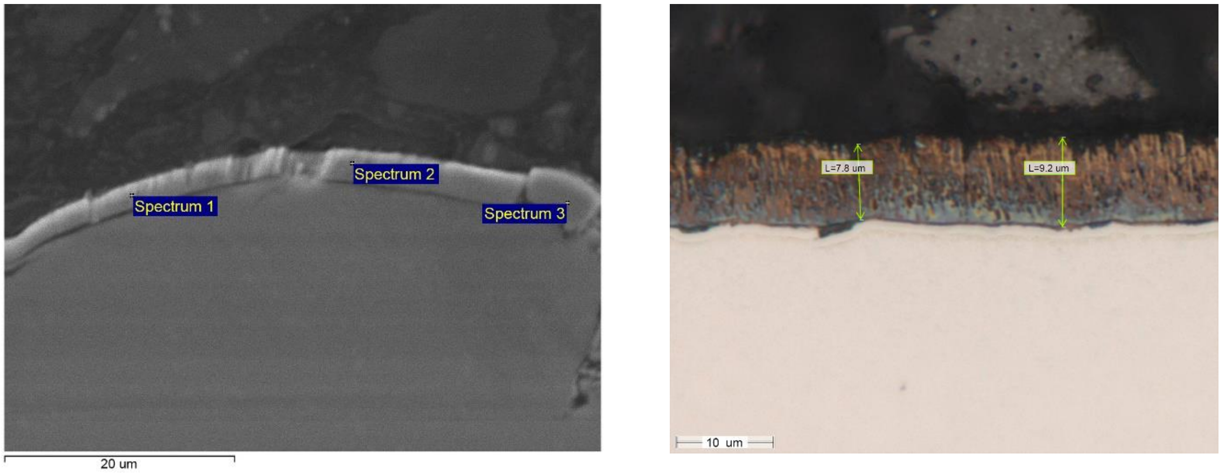


a) b)

Fig. 3. SEM images of Ti6Al4V alloy after gas-abrasive treatment coated with MS Ta-Cu coating: Group 1 (a) and Group 2 (b)

According to the Fig. 3a, the average thickness of Ta-Cu coatings of Group 1 (Table 1) was 2.2 ± 0.5 µm. At the same time, the average thickness of Ta-Cu coatings of Group 2 (Table 1) was 10.5 ± 0.5 µm (Fig. 3 b). The similar results have been obtained for Ti-Cu coatings (Fig.4a, b)

From Fig. 3 and Fig.4, it can also be concluded that DC MS makes it possible to obtain coatings of the same thickness on the rough surface of a titanium alloy after gas abrasive treatment, which is very promising for the development of technologies for applying multilayer coatings with lower layers made of biocompatible materials by microplasma spraying and upper layers made of bactericidal coatings with Cu by MS. Thus, the known dependence of the coating thickness on the MS time makes it possible in the future to form coatings of a given thickness from certain materials by varying the sputtering parameters.



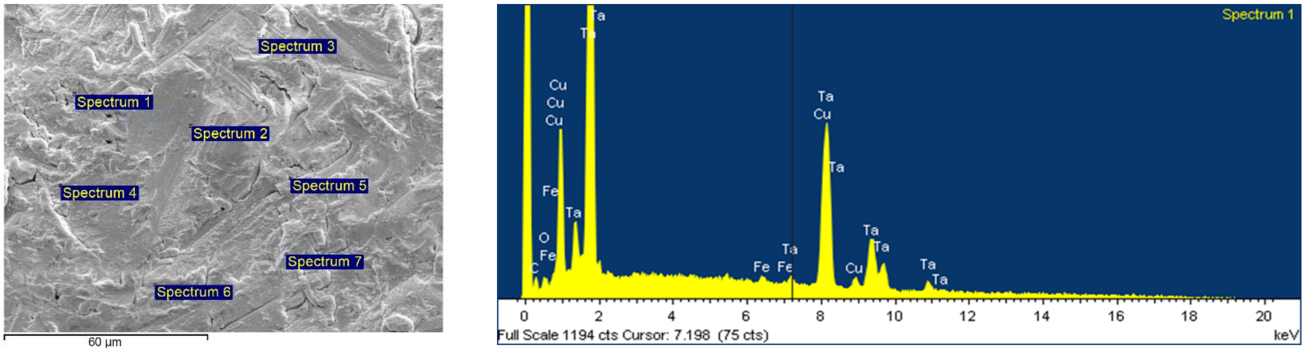
a) b)

Fig. 4. The cross-section of Ti-Cu coating on a Ti6Al4V alloy substrate: coating of Group 1 with areas of EDX analysis (a); coating of Group 2 (b)

The MS parameters presented in Table 1 were selected to provide the desired coating composition with 25 at. % Cu. It was assumed that Ti and Ta have similar sputtering ratio (0.4 and 0.5, respectively), while for Cu the ratio is about 1.65. That is, Cu is sputtered better than Ti and Ta, while Ta is sputtered slightly better than Ti. The sputtering rate could be defined as the ratio of the number of atoms lost from a surface to the number of incident energetic particles striking the surface. Thus, the sputtering yield is proportional to the number of knocked-out target atoms per argon atom. It was assumed that the desired content of 25 at% Cu was achieved in coatings with the composition of 90 wt.% Ta-10 wt.% Cu and 70 wt.% Ti - 30 wt.% Cu. The results of the EDX analysis indicated that the desired coating ratio was achieved, as illustrated in Fig.4a and Table 2 and Fig. 5 and Table 3.

Table 2. *Data (in wt./%) of the normalized results of EDX analysis of all elements in the spectra of the Ti-Cu coating shown in Fig. 5 (a)*

Spectrum	In stats.	O	Cu	Ti	Total
Spectrum 1	Yes	2.58	34.66	62.76	100.00
Spectrum 2	Yes	2.37	25.82	71.81	100.00
Spectrum 3	Yes	1.61	22.83	75.56	100.00
Max.		2.58	34.66	75.56	
Min.		1.61	22.83	62.76	



a) b)

Fig. 5. SEM image of the Ta-Cu coating surface indicating areas of EDX analysis, that is, the location of the spectra (a) and the results of spectrum analysis (b)

Table 3. *Data (in wt.%) of the normalized results of EDX analysis of all elements in the spectra of the Ta-Cu coating shown in Fig. 6 (a)*

Spectrum	In stats.	O	Fe	Cu	Ta	Total
Spectrum 1	Yes	2.13	0.84	13.89	83.14	100.00
Spectrum 2	Yes	2.29		4.84	92.87	100.00
Spectrum 3	Yes	3.09	0.97	10.94	84.99	100.00
Spectrum 4	Yes	2.77		3.95	93.28	100.00
Spectrum 5	Yes	2.92	0.65	4.02	92.41	100.00
Spectrum 6	Yes	2.81		4.79	92.40	100.00
Spectrum 7	Yes	2.65		4.32	93.02	100.00
Max.		3.09	0.97	13.89	93.28	
Min.		2.13	0.65	3.95	83.14	

For each measurement, from 3 to 7 spectra were taken from the surface or the cross-section of five samples of each group, which were then statistically processed. This assessment was quite rough and was suitable only for the first stages of the research. In the future, it is planned to continue the research on the selection of parameters and conditions of DC magnetron sputtering to ensure the production of thin coatings of uniform and controlled thickness and composition.

The mechanical properties of magnetron sputtered Ti-Cu and Ta-Cu coatings are presented in Table 4.

Table 4. *Mechanical properties of magnetron sputtered Ti-Cu and Ta-Cu coatings*

Coating	Hardness (GPa)	E-Modulus (GPa)
Ti-Cu (Group 1)	4.9±0.8	129.3±24.1
Ti-Cu (Group 2)	4.3±0.7	121.5±27.7
Ta-Cu (Group 1)	6.60±0.95	122.7±21.1
Ta-Cu (Group 2)	5.75±1.24	117.9±25.3

The Ta-Cu coatings revealed an overall higher hardness than the Ti-Cu coatings (Table 4) and whereas the lowest values of elastic modulus were observed in the thicker Ta-Cu coating, which is consistent with the literature values [35]. In both types of coatings, the modulus of elasticity decreased with increasing coating thickness (Table 4). Coatings with lower thickness showed a higher hardness than the thicker ones, which can be related to the mobility of the adatoms during the film growth [36].

The damage to the coating was compared with a defined adhesion strength quality determined by Daimler Benz Adhesion Test VDI 3198 [37]. Accoridng to it HF1-HF4 define a sufficient adhesion whereas HF 5 and HF 6 represent insufficient adhesion. SEM images of the surface of MS coatings after indentation to characterize the adhesion of coatings to the substrate are presented in Figure 6.

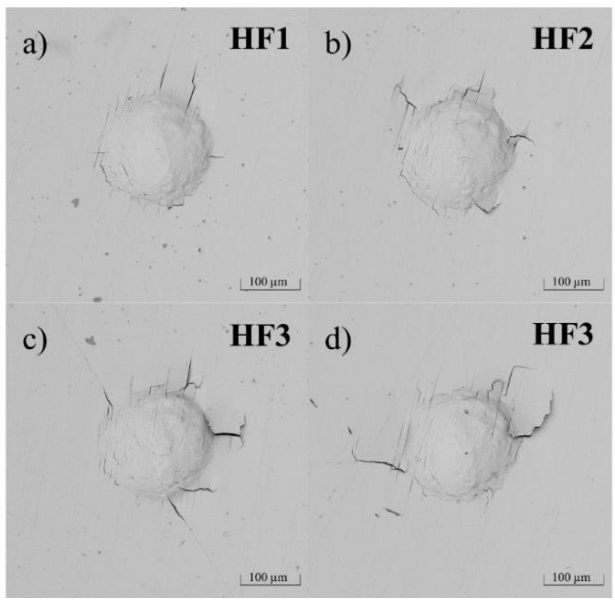


Fig. 6. Surface of magnetron sputtered coatings after Rockwell test: Ti-Cu Group 1 (a); Ti-Cu Group 2 (b); Ta-Cu Group 1 (c) and Ta-Cu Group 2 (d)

All coatings revealed sufficient adhesion whereas thinner Ti-Cu and Ta-Cu coatings (Fig. 6 a, c) revealed better adhesion. In case of thick Ti-Cu and Ta-Cu coatings (Fig. 6 b, d), no significant delamination is observed, but the cracks are very wide and parts of the coatings are close to be removed. Increasing the coating thickness leads to an acceleration of the impact depth and and earlier film fracture under the exerted loads.

It should be noted that in general for coatings of implants-endoprostheses that are not subject to wear in the human body, but grow together with the host bone, a decrease in elastic modulus is desirable to prevent aseptic loosening of the implant [12, 38]. However, the calculated data of elastic modulus in Table 4 are still significantly higher than the known values of the elastic moduli of human bones [39]. Further studies are required to clarify the nature of the change in elastic modulus in these coatings with an increase in coating thickness and its effect on biocompatibility. As noted in the second section of this paper, an increase in the thickness of the Cu-based coating can lead to an increase in its cytotoxicity to osteoblast cells, i.e., reduce the biocompatibility of the coating.

The in vitro antibacterial activity of the coatings was assessed using the agar well diffusion technique, which measures the ability of a material to inhibit bacterial growth. According to the results presented in Figure 7, Ta-Cu Group 2 demonstrated the most effective antibacterial performance against Staphylococcus aureus, achieving an inhibition zone of 23.0 ± 0.3 mm. This suggests that the composition and thickness of the Ta-Cu Group 2 coating are particularly effective in creating an environment hostile to bacterial proliferation. Ta-Cu Group 1 and Ti-Cu Group 2 coatings produced similar levels of antibacterial efficacy, each demonstrating an inhibition zone of 17.7 ± 0.25 mm. This indicates that these coatings, while not as effective as Ta-Cu Group 2, still possess considerable antibacterial properties capable of inhibiting bacterial growth to a significant extent. These results highlight the role of both coating composition and thickness in determining antibacterial effectiveness. In contrast, the thinner Ti-Cu Group 1 coating did not demonstrate significant antibacterial activity, with no substantial inhibition zone observed against Staphylococcus aureus. This lack of efficacy suggests that reducing the coating thickness may diminish its ability to effectively release antibacterial ions or maintain surface conditions that inhibit bacterial growth (Fig. 7).

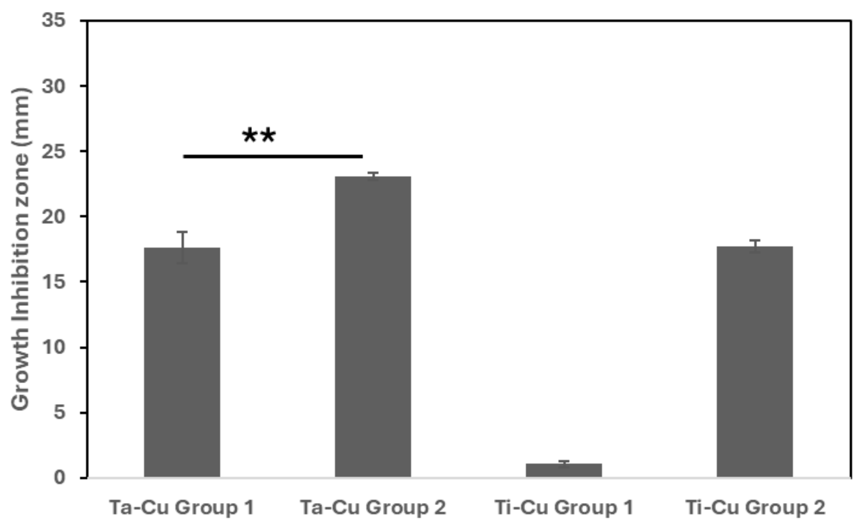


Fig. 7. Evaluation of Inhibitory Zones for Various Coatings Against S.aureus Growth (* - p value≤ 0.05 compared to the Ta-Cu Group 1)

The superior performance of Ta-Cu Group 2 indicates that a combination of optimal copper content and coating thickness is critical for effective antibacterial activity. The authors of the article [28] reported a similar relationship between antimicrobial properties and coating thickness showing that the antimicrobial activity increases with the thickness of the MS Cu-containing coating.

Since thin Ti-Cu coatings from Group 1 did not demonstrate pronounced antibacterial activity, the dynamics of copper ion release was determined only for Ti-Cu coatings of Group 2, while measurements were carried out for Ta-Cu coatings from both groups. The results of measuring the concentration of Cu^{2+} released into a 0.9% NaCl solution over 7 days are presented in Figure 8.

Figure 8 demonstrates that the thickness of the coatings directly influences the release rate of Cu ions into saline solution, which is crucial for their antibacterial effectiveness. The data show a nonlinear decrease in ion concentration over time, indicating a deceleration in the release process.

When comparing the time-dependent Cu ion release profiles from Ta-Cu and Ti-Cu coatings to those from MS Ti-Cu films [20, 28, 29], a slightly different pattern emerges: Ti-Cu films exhibit a rapid release of Cu ions within the first 24 hours, which contributes to their cytotoxic effects. For thicker coatings (in the micrometer range), the release profile shows a steady decline, whereas for thinner films (in the nanometer range), the release curve drops sharply [20, 28, 29]. This controlled release of Cu ions is critical for preventing periprosthetic infections in the immediate postoperative period. The sustained release pattern observed in Figure 7 suggests that this approach could effectively prevent infections in the crucial weeks following surgery, when they are still at risk of developing.

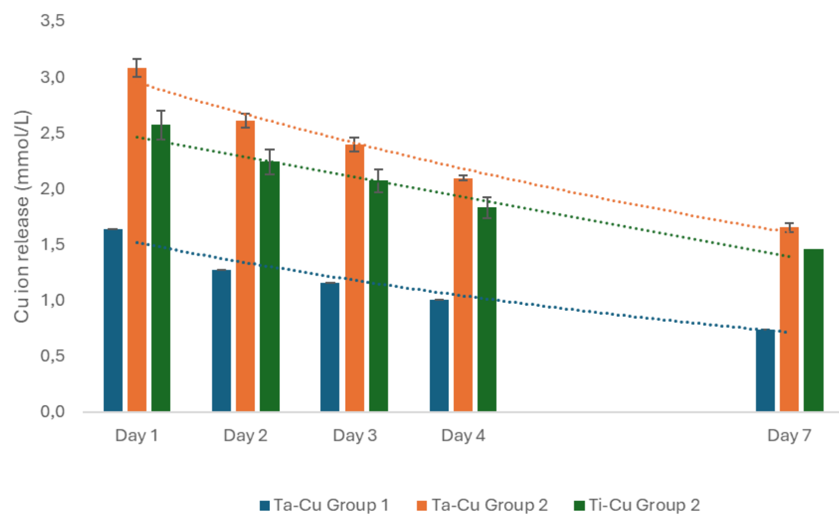


Fig. 8. Release of Cu ions into a 0.9% NaCl solution within 7 days

Notably, the Cu ion release from micrometer-thick Ta-Cu and Ti-Cu coatings is significantly (5-6 times) higher than the concentrations reported for thin Ti-Cu films in previous studies [20, 28, 29]. This discrepancy can be partly attributed to the increased surface roughness of the coated samples. Zietz et al. [29] reported that rougher surfaces result in higher Cu concentrations in the surrounding liquid due to the larger contact area; for instance, a corundum-blasted surface released approximately three times more Cu ions within 24 hours compared to a polished surface. Additionally, the coating thickness and the Cu weight percentage likely contribute to these elevated concentrations, raising concerns about potential cytotoxicity to osteoblast cells. As noted in the "Introduction", Ilievska et al. [28] showed that Cu ion release from Cu-O films applied for 5 or 10 minutes remained within the safe range for MG-63 cell viability, whereas coatings applied for 15 minutes were cytotoxic, reducing cell viability to about 60%, unlike pure Ti. Therefore, achieving a balance between cytotoxicity and biocompatibility requires thorough in vitro testing, particularly given that excessive Cu ion release can be detrimental to cell health. According to ISO 10993-5 [40], a reduction in cell viability greater than 30% compared to the control is considered significantly cytotoxic. Based on the data in Figure 8, it may be necessary to reduce both the coating thickness and the Cu weight percentage to lower the concentration of released Cu ions. Further research is essential to address these concerns effectively.

Future research perspectives

The next stages of the research are planned to include testing the corrosion resistance of the coatings in a simulated physiological fluid. The research will delve into the effects of composition, specifically the atomic percentage of Cu, and coating thickness on the bactericidal and biocompatibility properties of Ta-Cu and Ti-Cu coatings, with an emphasis on understanding the mechanisms involved. Additionally, the study will assess the phase composition of the coatings to determine its impact on the release of Cu ions into saline solution.

To provide a comprehensive understanding of antibacterial activity over time, upcoming in vitro experiments will quantify bacterial inhibition zones at each testing stage. Measuring the diameter of these zones, where bacterial growth is successfully suppressed, will offer a direct indication of the effectiveness of the antimicrobial coatings. These measurements will be correlated with the concentration of Cu^{2+} ions released into the surrounding medium.

Further research will investigate the kinetics of Cu ion release, focusing on factors such as coating thickness, surface roughness, and the composition of the base material. This will include evaluating the long-term durability of the antimicrobial effect, which is particularly important for endoprosthesis applications where sustained infection prevention is critical.

Future research will also be aimed at establishing the usability and reproducibility of the thickness of layers deposited by MS on non-flat elements, with the prospect of magnetron sputtering coatings on implants with complex geometries tailored to anatomical shapes.

CONCLUSIONS

The study has shown that dual direct current magnetron sputtering (DC MS) enables precise deposition of Ta-Cu and Ti-Cu coatings, particularly with a composition of around 25 at.% Cu and a specified thickness of 2 μm and 10 μm onto Ti6Al4V alloy surfaces. These alloy surfaces were pre-treated with gas abrasion to achieve a mean roughness of 4.6 ± 1 μm, effectively preparing them for targeted coatings. All coatings revealed sufficient adhesion, whereas thinner Ti-Cu and Ta-Cu coatings showed better adhesion than thicker ones. Ta-Cu coatings overall demonstratedhigher hardness than Ti-Cu coatings, and the elastic modulus decreased for both coating compositions with increasing coating thickness. This approach demonstrates the capability to uniformly apply coatings with specified compositions and thikness on substrates of varying textures and porosities, making it highly versatile for surface preparation in advanced biomedical applications.

In this research, MS coatings with a thickness of 10 μm exhibited different levels of antimicrobial effectiveness. Ta-Cu coatings achieved a maximum inhibition zone of 23.0 ± 0.3 mm against Staphylococcus strains, while Ti-Cu coating Grop 2 produced a maximum inhibition zone of 17.7 mm. When the coating thickness was reduced to 2 μm, Ta-Cu coatings remained effective, inhibiting bacterial growth up to 17.7 mm. However, Ti-Cu coatings of the same thickness and untreated Ti6Al4V alloy substrates did not show any inhibitory effect against bacteria. These findings emphasize three main points: first, thicker Ta-Cu and Ti-Cu coatings are more effective at resisting bacterial infections; second, achieving optimal antimicrobial efficacy for MS coatings on textured surfaces may require coating thickness in the micrometer range to maintain integrity; and third, Ta-Cu coatings provide superior protection against bacterial infections in endoprostheses.

The release profile of Cu^{2+} ions from Ta-Cu and Ti-Cu coatings on Ti6Al4V alloy, observed over a 7-day period, followed a similar monotonically decreasing pattern for both 10 μm and 2 μm thicknesses. The majority of ions were released during the first 24 hours, indicating a direct relationship between coating thickness and antimicrobial effectiveness.

These findings highlight the potential of combining magnetron sputtering and microplasma spraying technologies to develop endoprosthesis implants with improved resistance to bacterial colonization.

However, further research is necessary to strike a balance between biocompatibility and antibacterial performance, as the substantial release of Cu ions suggests potential cytotoxic effects on osteoblast cells. This concern may be mitigated by reducing both the Cu content and the coating thickness.

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