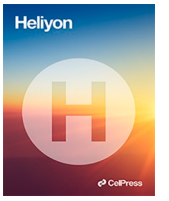
Heliyon 11 (2025) e41130

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Antibacterial properties of copper-tantalum thin films: The impact of copper content and thermal treatment on implant coatings

Bagdat Azamatov [a], Alexey Dzhes [a], Alexander Borisov [a], Daniyar Kaliyev [a], Bauyrzhan Maratuly [a], Amangeldi Sagidugumar [a], Myakinin Alexandr [b], Amanzhol Turlybekuly [b,c,*], Sergei Plotnikov [a]

[a] D. Serikbayev East Kazakhstan Technical University, Oskemen, 070000, Kazakhstan
[b] Institute of Materials Engineering LLP, Astana, 010000, Kazakhstan
[c] National Laboratory Astana, Nazarbayev University, Astana, 010000, Kazakhstan

A B S T R A C T

This study evaluates the antibacterial properties and physicochemical characteristics of –tantalum-copper (Ta-Cu) coatings deposited on titanium alloy substrates using high-power magnetron sputtering. Implant-associated infections, particularly those caused by bacterial adhesion and biofilm formation, pose significant challenges in the field of orthopedic and dental implants. To address these issues, Ta-Cu coatings with varying copper content (~3.0 wt%, ~10 wt%, ~17 wt% for TaCu-1, TaCu-2, and TaCu-3, respectively) and different thermal treatment conditions (400 °C, 500 °C, 600 °C) were assessed for their antibacterial efficacy against *Escherichia coli, Staphylococcus aureus, Salmonella enterica,* and *Pseudomonas aeruginosa*. The study utilized both the diffusion into agar method and the time-kill test to evaluate antibacterial activity. Results indicate that the TaCu-2 sample, particularly when annealed at 600 °C, demonstrated the highest bactericidal activity, especially against *E. coli* and *P. aeruginosa*. The findings highlight the critical role of optimizing both copper content and annealing temperature in enhancing the antibacterial properties of Cu-Ta coatings, making them promising candidates for preventing implant-associated infections.

1. Introduction

Implant-associated infections are the leading cause of post-surgery complications and the failure of dental and orthopedic implants. Additionally, aseptic loosening at the implant/tissue interface is an emerging issue that results in the softening of bone tissue, compromising the long-term stability and function of the implant [1–3].

Providing osteoconductive and, at the same time, antibacterial properties for implants is one of the possible solutions. Thus, developing implants that stimulate bone tissue growth and prevent bacteria growth is an emerging task that motivates researchers to modify implant surfaces [4–7].

Traditional coatings with osteoconductive bioceramics often lead to cracks, rapid wear, and long-term instability due to their inherently low shear strength. While surface topographies designed to enhance osteogenic differentiation can promote bone growth, they fail to prevent bacterial attachment and colonization, mainly because of the size difference between osteoblasts and bacteria. Therefore, there is an urgent need to develop smart implant surfaces that enhance both osteogenesis and antibacterial activity simultaneously.

Implant-associated infections are typically caused by bacterial adhesion and biofilm formation on the implant surface. Bacteria within biofilms can resist the host's immune responses and are less susceptible to antibiotics. Bacterial infections caused by the gram-

* Corresponding author. Institute of Materials Engineering LLP, Astana, 010000, Kazakhstan.
E-mail address: aturlybekuly@gmail.com (A. Turlybekuly).

https://doi.org/10.1016/j.heliyon.2024.e41130
Received 2 September 2024; Received in revised form 5 December 2024; Accepted 10 December 2024
Available online 12 December 2024

positive species *Staphylococcus aureus* and *Staphylococcus epidermidis* often result in the formation of persistent biofilms, which are challenging to eliminate with conventional antibiotics. Such periprosthetic infections are a common complication following joint replacements with titanium implants and can exacerbate the inflammatory response caused by the implant, leading to severe issues such as increased antibiotic dosage or the need for reoperation. This not only harms patients but also places a significant burden on healthcare systems worldwide[3,8–10].

With over 1.5 million primary joint replacements performed globally each year, despite antiseptic measures and antibiotic prophylaxis, these infections can occur in up to 2 % of patients after primary total hip replacement and up to 4 % after primary total knee replacement, with even higher rates following revision arthroplasty. Current treatments for periprosthetic joint infections, such as implant removal, thorough cleaning, and high-dose systemic antibiotics, may lead to secondary morbidity without guaranteeing a cure [11].

The periprosthetic bacterial infections preserve the biocompatibility and osseointegration of titanium implants, it is crucial to prevent bacterial biofilm formation on implant surfaces. Biofilms form when bacteria adhere to surfaces and secrete an extracellular polymer matrix, making bacterial adhesion the first and most critical step in this process. Once attached and biofilm is formed, bacteria become resistant to the host immune system and antibiotic treatment, contributing to persistent infections. *Staphylococcus epidermidis*, in particular, is known for its strong ability to form adherent biofilms on artificial surfaces like joint prostheses [12,13].

Current research is exploring ways to impart antibacterial properties to orthopedic devices by coating them with metal particles (such as copper, silver, zinc, cobalt, aluminum, and mercury ions), antibiotics, antibacterial solutions, antibacterial bioactive polymers, or nitric oxide-releasing coatings. However, challenges in implementing these technologies include cytotoxicity, poor adhesion, microstructural heterogeneity of the coatings, and the complexity of coating methods. Among antibacterial metals, copper has shown excellent antibacterial properties in vitro while maintaining an acceptable cytotoxicity profile. Copper is noted for its lower toxicity and higher cytocompatibility compared to other metals, and it is metabolized rather than accumulating in the human body.

A thin film containing copper can prevent early biofilm formation on implant surfaces and reduce the risk of periprosthetic infection. Additionally, copper is relatively inexpensive compared to silver and has excellent conductivity, making it suitable for various application methods, such as electrolytic deposition, thereby reducing overall technology costs. Studies have demonstrated that copper deposition on titanium surfaces results in films with antibacterial properties, as evidenced by reduced numbers of planktonic and adherent *Staphylococcus epidermidis* and *Staphylococcus aureus bacteria* [14,15].

However, copper (Cu) can be toxic to mammalian cells in a concentration-dependent manner and may cause adverse tissue effects in vivo. For instance, a study using titanium (Ti) samples coated with electroplated copper demonstrated stronger acute inflammatory responses compared to untreated controls during the first three days post-implantation in rats. Therefore, it is crucial to minimize these side effects to reduce the impact on peri-implant tissue while maintaining antibacterial properties. Various studies have explored different approaches to achieve non-cytotoxic antibacterial Cu coatings. These methods primarily involve controlling the copper content of the implant coating and embedding metallic copper nanoparticles into the matrix, preventing the release of toxic particles from the implant surface [12,14–18].

Wojcieszak et al. investigated the effect of surface properties of thin Cu-Ti films on antimicrobial activity and cell viability. They conducted a comprehensive analysis of the impact of material composition on the structure and surface properties of nanocrystalline bioactive coatings based on Cu and Ti, which were obtained via pulsed direct current magnetron sputtering. The study examined the antimicrobial activity of films with varying copper contents (25–75 wt%) against *E. coli* and *S. aureus* and found that all the films exhibited bactericidal properties. However, the cytotoxicity of the films was related to their Cu content. The bioactivity of the coatings was associated with ion migration processes and the oxidation state of copper ions, specifically the presence of Cu^{1+} ions. Since the experiments were only conducted over two days, there is no guarantee that Cu^{1+} will not later oxidize into the more stable Cu^{2+} form. The study highlighted the importance of copper content in influencing the viability of the L929 cell line, concluding that by controlling copper content, it is possible to create bioactive Cu-Ti coatings with the desired biological effects on various microorganisms using magnetron sputtering [19,20].

Kratochvíl et al. prepared Cu/C:F nanocomposites using physical vapor deposition on polyether ether ketone (PEEK) substrates. They found that these nanocomposites, with the optimal amount of Cu and C:F barrier thickness, exhibited strong antibacterial activity against *Escherichia coli (E. coli)* while remaining harmless to MG63 cells, thus demonstrating both antibacterial and biocompatible properties. The study revealed that the antibacterial efficiency of the coatings decreased as the C:F barrier thickness increased. The Cu/C:F nanocomposite effectively prevented biofilm formation on the PEEK substrate by reducing bacterial metabolic activity, which aligns with previous findings for Ag/C:F coatings with similar architecture [21].

Several works were dedicated to studying the potential of copper-based coatings to enhance titanium implants' antibacterial properties and durability. The findings demonstrated that copper-containing coatings obtained by micro-arc oxidation methods can significantly improve the resistance of titanium alloys' surfaces to bacterial contamination. However, the studies also highlight the need to balance this antibacterial effect with biocompatibility, as copper release can trigger moderate inflammatory responses in surrounding tissues [22–24].

Norambuena et al. have shown that two-component Cu-Ti thin films produced via magnetron sputtering, particularly pulsed magnetron sputtering, exhibit significant antimicrobial activity against *E. coli* and *S. aureus.* However, the antimicrobial effectiveness and cytotoxicity of these films depend on their Cu content, which varies widely according to different authors, ranging from 20 wt% Cu to 80 wt% Cu [25].

In recent years, coatings made from various metals, such as tantalum, niobium, and zirconium, have been used to modify the surface of biomedical products. The interest in coatings containing tantalum and niobium is due to their excellent corrosion resistance, good biocompatibility, and wear resistance.

Bahrami et al. investigated the mechanical properties and microstructural stability of Cu–Ta binary coatings. Coatings with a Ta content below 67 atomic percent (at.%) exhibited a notable increase in hardness, while their elastic moduli remained nearly unchanged. This effect may be related to enhanced toughness. It was found that the presence of Cu nanocrystallites within the amorphous Ta-rich Cu-Ta matrix could increase the ductility compared to glassy Cu-Ta coatings, and preserve after vacuum annealing at 550 °C, 1 h [26].

Guo et al. demonstrated that an amorphous copper-niobium alloy thin film, deposited from a CuNb (50/50 at.%) powder target using direct current (DC) magnetron sputtering, could achieve a hardness of 11.8 GPa and an elastic modulus of 198 GPa [27].

Tantalum (Ta) has been widely used as a bone graft substitute, in cranioplasty plates, suture wires, radiographic bone markers, and coatings for enhanced osseointegration in orthopedic and craniofacial surgery. Its popularity is due to its exceptional biocompatibility, corrosion resistance, and osteoconductivity. Nanostructured Ta surfaces have also been shown to influence fibronectin adsorption and direct cell-surface interactions, such as cell proliferation, focal contact assembly, and filopodia expression. Therefore, depositing a nanostructured Ta thin film on micropatterned Ti may further enhance new bone formation. Importantly, the immiscibility of Cu and Ta allows for two-phase coexistence, which prevents the formation of intermetallic compounds or solid solutions in the deposited metal films, thus enhancing antibacterial efficacy [28,29].

The primary objective of this study was to comprehensively evaluate the release of copper ions and the antibacterial efficacy of Ta-Cu coatings against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and Candida albicans in vitro. These coatings were applied to titanium alloy substrates, focusing on varying the copper content and optimizing the treatment parameters to achieve a superior antibacterial effect at lower Cu content (~3.0 wt%, ~10 wt%, ~17 wt%). A key innovation of this research is the application of high-power magnetron sputtering, a technique that closely mimics industrial implant manufacturing conditions. This approach enhances the relevance of our findings to real-world applications and allows for a more accurate determination of the optimal production conditions for these advanced coatings. The study underscores the critical importance of understanding and controlling the mechanical and chemical properties of the materials to design coatings that meet the stringent demands of biomedical applications, particularly in preventing implant-associated infections.

2. Materials and methods

Magnetron Sputtering. The experimental setup for thin film deposition and subsequent thermal treatment is illustrated in Fig. 1. Thin films were deposited using an EPOS-PVD-440 system (Beams&Plasmas, Russia) equipped with three DC magnetrons, each with dimensions of 472 mm × 132 mm x 18 mm. The TaCu thin films were deposited onto (100) Si wafers, which were pre-coated with a 100 nm titanium layer. The TaCu films were produced through co-sputtering from high-purity copper (99.999 %, Ulba LLP) and tantalum (99.999 %, Ulba LLP) targets. The DC magnetron power for the tantalum target was fixed at 2 kW, while the copper content in the films was controlled by varying the power supplied to the copper magnetron (0.33 kW, 0.46 kW, and 0.8 kW) across three different experiments. The resulting samples were designated as TaCu-1, TaCu-2, and TaCu-3, respectively. The deposition process was carried out for 60 min, with a base pressure of 7×10^{-9} bar and a working pressure of 1.8×10^{-6} bar. The substrate rotation speed was maintained at 8 rpm, and the distance between the magnetrons and the substrate was ~300 mm. Following deposition, the samples were annealed at temperatures of 400 °C, 500 °C, and 600 °C for 1 h under vacuum conditions, with a pressure of 2×10^{-5} bar. The resulting samples were designated as TaCu-#/temperature, i.e TaCu-1/400.

Samples characterization. The nanostructure and morphology of the samples were analyzed using a field emission scanning electron microscope (FESEM) (Zeiss, Germany), which is equipped with an energy dispersive (EDS) chemical composition analyzer from Oxford Instruments (UK). The phase composition was assessed through low-angle X-ray diffraction (XRD) with a Rigaku SmartLab diffractometer utilizing a Cu Kα X-ray source (Japan).

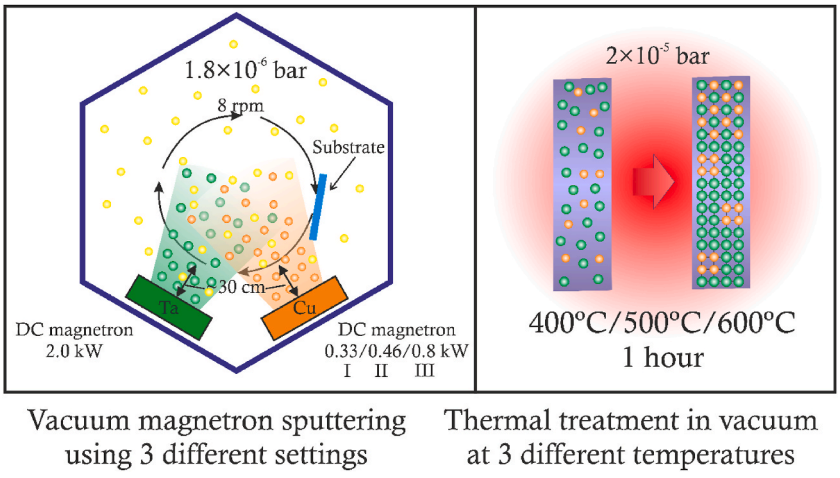


Fig. 1. Schematic illustration of the experimental design.

Diffusion into agar. The autoclaved Mueller-Hinton nutrient medium was poured into sterile Petri dishes, forming a layer 4 mm thick. The dishes were left at room temperature to solidify. A suspension of the test microorganisms (inoculum) was then prepared using a pure, daily culture grown on a solid nutrient medium. Several identical, well-isolated colonies were selected, and a small amount of material from the tops of the colonies was transferred with a loop into a test tube containing sterile physiological saline, adjusting the inoculum density to 0.5 according to the McFarland standard, equivalent to 1.5×10^8 CFU/ml. The inoculum was applied to the surface of the nutrient medium in the Petri dish using a pipette, distributing it evenly by shaking the dish, and then the excess was removed. The dishes were left slightly open to dry for 10 min at room temperature. Test materials were applied to the surface of the nutrient medium using sterile tweezers. After sample application, the Petri dishes were placed in an incubator and maintained at 35 °C for 24 h. After incubation, the zones of growth inhibition were measured on a matte surface with an accuracy of 1 mm. Antagonistic activity was categorized as follows: zero when the zone of growth inhibition was up to 5 mm, low for 5–10 mm, moderate for 10–17 mm, and high for 17 mm or more.

Method for Joint Cultivation of Target Microorganisms with Experimental Samples (Time-Kill Test). This test is detailed in document M26-A by CLSI [30]. Samples (1 ml) were added to test tubes containing a liquid nutrient medium inoculated with the target microorganisms at a concentration of 5×10^5 CFU/ml, and incubated in a shaker at 37 °C for 24 h. The medium containing the test strain without the addition of samples served as the control. The percentage of dead cells relative to the growth control was calculated at different time intervals (0, 1, 6, 10, and 24 h). For this, 100 μl of bacterial suspension were taken from each test tube, plated on nutrient agar, and incubated at 37 °C for 48 h to count the colony-forming units (CFU/ml) and determine the number of viable cells. The reduction in bacterial numbers was calculated using the following formula: R (%) = (A - B)/A x 100 %, where R represents the percentage reduction in bacterial count, A is the number of bacteria in the control group (without the test materials), and B is the number of bacteria in the test tubes with the experimental materials. A bactericidal effect is considered to be achieved if there is a 90 % reduction in cell count within 6 h, equivalent to 99.9 % reduction within 24 h [31]. Each experiment involved 4 samples and 4 test strains of microorganisms Gram-negative (*Salmonella enterica NCTC 6017, Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027*) Gram-positive (*Staphylococcus aureus ATCC 6538*) all obtained from the American Type Culture Collection.

Statistical analysis. The results are presented as means ± standard deviations. Comparisons between multiple groups were conducted using two-way ANOVA to evaluate the effects of treatment temperature, Cu concentration, and bacterial strain on the inhibition zones. When significant differences were detected, post-hoc analyses were conducted to identify specific group differences. A significance level of $P < 0.05$ was applied to determine statistical significance. All statistical analyses were performed using Python with the *statsmodels* and *scipy* libraries.

The ions release assessment. The samples were tested by immersion 5 × 5 mm substrates in 20 ml SBF solution for 7 days and kept at 37 °C. The SBF solution was prepared according to Kokubo et al. [32]. The assessment of released ions was performed at day-1, 3 and 7 by inductively coupled plasma mass spectrometer ICP-MS Agilent 7500cx (Agilent Technologies, USA).

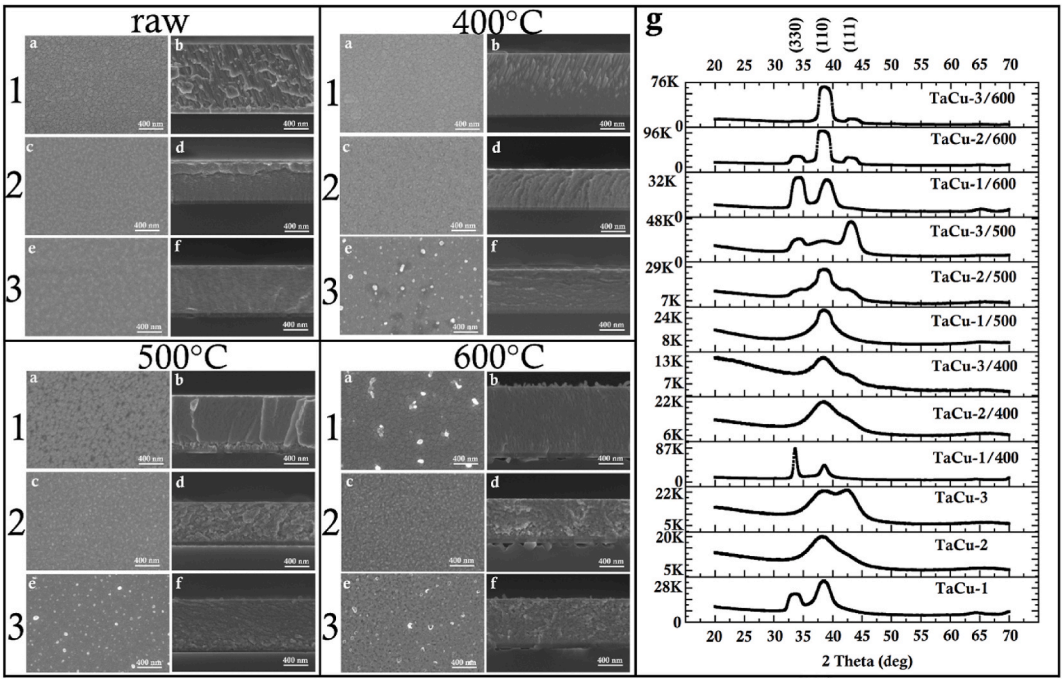


Fig. 2. The morphology and crystal structure of obtained thin films. SEM images of the thin films at different annealing temperatures: a) top view of the TaCu-1; b)cross-section of the TaCu-1; c) top view of the TaCu-2; d)cross-section of the TaCu-2; e) top view of the TaCu-3; f)cross-section of the TaCu-3; g) XRD patterns of the thin films.

3. Results and discussion

Fig. 1 shows the schematic design and fabrication of the thin films of the bicomponent TaCu with altering copper content. As it is shown in Fig. 2, the widths for TaCu-1, TaCu-2, and TaCu-3 were ~1.1 μm, ~0.7 μm, and ~0.8 μm, respectively. The high-power magnetron sputtering was applied to thin film deposition. As can be seen, the deposition parameters greatly influence the morphology. It is noted that the grain size decreases with an increase in copper content. At the same time, the thin films with increased copper content look more porose. These changes might be explained by hindering the movement of grain boundaries, leading to smaller grain sizes by copper atoms. This occurs because the additional copper atoms create more nucleation sites, where new grains can start growing, but they also inhibit the coalescence of these grains into larger structures. As a result, with more copper, the average grain size tends to decrease [33,34]. Moreover, incorporating copper can introduce stress and strain within the film due to differences in atomic sizes and lattice parameters between the base material and copper. This mismatch can lead to the creation of voids or defects, increasing the porosity [35,36].

The XRD graph shows (Fig. 2g) diffraction patterns for TaCu thin films, analyzed across various samples with differing copper content and annealed at 400 °C, 500 °C, and 600 °C. As the annealing temperature increases, the XRD peaks become sharper and more defined, indicating enhanced crystallinity. The peak at 33.5972° was identified as Copper Titanium (330) phase, 38.3454°as Tantalum (110), and the peak at 43.3102° as Copper (111), suggesting the presence of these compounds in the material [37]. The increased intensity of the XRD pattern for TaCu-1/400 might indicate the optimal annealing temperature for the formation of the crystalline structure of Copper Titanium (330) and Tantalum (110) body-centered cubic phases. The changes in peak positions and intensities suggest the development of different crystalline phases and structural modifications in response to the varying temperatures and copper content [38]. The broadening of XRD peaks indicates a considerable presence of amorphous phases, with higher temperatures leading to more well-formed and distinct phases. The differences between the samples with varying copper content suggest that the copper concentration plays a crucial role in determining the crystalline phases and their stability within the TaCu thin films.

The variation in the presence of the Copper Titanium (330) phase with changes in copper content and annealing temperature might be explained by temperature influences on phase stability. At room temperature, 400 °C, and 600 °C, higher copper content seems to disrupt the stability of the Copper Titanium (330) phase. This could be due to an excess of Cu atoms hindering the phase's nucleation and growth, with the higher Cu levels instead favoring the development of Cu-rich or amorphous regions rather than the stable Copper Titanium (330) structure [39].

In contrast, the intermediate annealing temperature of 500 °C may create optimal conditions for the Copper Titanium (330) phase to form and stabilize, even with higher Cu content. This temperature likely provides adequate thermal energy to allow atomic diffusion and ordering necessary for phase formation, without reaching the mobility levels at 600 °C, which could lead to phase dissolution or an amorphous state. Thus, 500 °C may offer a balanced environment that promotes the crystallization and growth of the Copper Titanium (330) phase, supported by favorable atomic arrangements in samples with increased copper content [39,40].

The SEM-EDS images of the cross-sectional views of three different samples with corresponding elemental analysis are provided in Fig. S1. The EDS spectra on the right side of each sample provide elemental composition analysis along the line scan indicated in the SEM images. In TaCu-1, the EDS data likely reveal the presence of elements associated with the smooth layers and columns, showing a consistent elemental distribution. In the samples TaCu-2 and TaCu-3, the EDS spectra may indicate a more mixed or uneven distribution of elements, reflecting the rougher and less uniform microstructure seen in the SEM images. These changes in microstructure and elemental distribution can significantly impact the material's performance as bactericidal agent.

Table 1 presents the elemental composition, in weight per cent (Wt.%), of TaCu thin film samples with varying copper content before and after annealing at 600 °C. The samples, labelled TaCu-1, TaCu-2, and TaCu-3, show an increasing amount of copper (3.2 %, 9.6 %, and 19.6 %, respectively) with a corresponding decrease in tantalum content (95.5 %, 89.1 %, and 79.1 %). Oxygen content remains consistent at 1.3 % across all samples. After annealing at 600 °C, denoted by the "/600″ suffix, the elemental compositions remain similar, with slight variations in tantalum and copper content and a slight increase in oxygen content for sample TaCu-3/600. The adsorbed oxygen might explain the presence of oxygen in EDS spectra.

The antibacterial activity of the TaCu thin films was assessed by two complementary testing methods: diffusion into the agar and the time-kill test. The diffusion into agar method involves placing a sample on a well on an agar plate inoculated with bacteria. As the substance diffuses through the agar, it inhibits bacterial growth, and the size of the inhibition zone around the sample measures the effectiveness. The main advantages of this method include its simplicity, ease of use, and the ability to assess antibacterial activity visually.

The comparative analysis of the antibacterial activity against four bacterial strains *E. coli*, *S. aureus*, *S. enterica*, and *Ps. aeruginosa* of

Table 1
The elemental composition assessed by EDS of the thin films.

Sample	Ti, wt.%	Ta, wt.%	Cu, wt.%	O, wt.%	Total, wt.%
TaCu-1	0	95.5 ± 0.2	3.2 ± 0.2	1.3 ± 0.1	100.0
TaCu-2	0	89.1 ± 0.2	9.6 ± 0.2	1.3 ± 0.1	100.0
TaCu-3	0.1	79.1 ± 0.2	19.5 ± 0.2	1.3 ± 0.1	100.0
TaCu-1/600	0.6 ± 0.01	94.7 ± 0.3	3.4 ± 0.2	1.3 ± 0.1	100.0
TaCu-2/600	0.4 ± 0.01	89.0 ± 0.4	9.4 ± 0.31	1.2 ± 0.2	100.0
TaCu-3/600	1.2 ± 0.1	78.5 ± 0.2	17.3 ± 0.1	3.0 ± 0.2	100.0

the TaCu thin films assessed by diffusion into agar are given in radar charts in Fig. 3. The TaCu-1 shows minimal antibacterial activity against the four bacterial strains, with slight variations depending on the annealing conditions Fig. 3a. There is a slight increase as the thermal treatment is applied (TaCu-1/400 to TaCu-1/600), but overall, the antibacterial activity remains low across all bacteria. The TaCu-2 displays the highest antibacterial activity among the three TaCu thin films, particularly against Gram-negative *E. coli*, *S. enterica* and *Ps. Aeruginosa,* Fig. 3b. A detailed statistical analysis is given in Table S1, supplementary data. However, the activity against Gram-positive *S. aureus* remains relatively lower than that of Gram-negative bacteria. The TaCu-3 exhibits slightly lower antibacterial activity than TaCu-2, especially against Gram-negative *E. coli* and *Ps. Aeruginosa*, however antibacterial activity against Gram-positive *S. aureus* remains relatively same with TaCu-2/600 Fig. 3c. The images of bacterial growth inhibition radius in agar for all samples and numerical values are given in Table S1 in supplementary data.

Even though diffusion into agar primarily provides qualitative or semi-quantitative data, the substance's diffusion can be inconsistent, potentially leading to variable results. Additionally, this method may be unsuitable for substances with low infusibility in agar. In contrast, the time-kill test offers a more detailed quantitative analysis of antibacterial activity by measuring the reduction in bacterial count over time. In this method, the test substance is incubated with bacteria in a liquid medium, and samples are taken at different time intervals to determine the number of viable bacteria, which is then plotted to assess the rate of bacterial killing. This method is particularly useful for understanding the kinetics of bacterial killing, making it valuable for pharmacodynamic studies. However, it is more labor-intensive and requires more complex data analysis than the diffusion method. The time-kill test is ideal for in-depth studies where a detailed understanding of bactericidal activity is needed. However, high-throughput screening may be less practical due to its time and resource requirements.

The results of time-kill tests for the thin films against four bacterial strains: *Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica,* and *Staphylococcus aureus* are given in Fig. 4. The inhibition rate (%) is plotted over time (hours), providing insights into the bactericidal effectiveness of these samples. The inhibition rate of *E. coli* increases significantly over time, with a marked difference at the 24-h mark, see Fig. 4a. Among the samples, TaCu-2/600 shows the highest inhibition rate, approaching ~75 % after 24 h, indicating bactericidal solid activity against *E. coli*. TaCu-3/500 and TaCu-3/600 also exhibit notable inhibition, but the effectiveness of TaCu-1 is relatively lower across all conditions.

Similar to *E. coli*, the inhibition rate against *P. aeruginosa* increases over time, but the overall effectiveness is slightly lower compared to *E. coli*. TaCu-2/600 demonstrated the highest inhibition rate, followed by TaCu-3/600, see Fig. 4b. The lower inhibition rates of TaCu-1 suggest that its bactericidal activity against *P. aeruginosa* is weaker than that of the other samples.

The inhibition rates against *S. enterica* are generally lower across all samples and conditions, with most samples showing modest activity even after 24 h, as shown in Fig. 4c. However, TaCu-2/600 demonstrated the highest inhibition rate, though it is significantly lower than its effectiveness against *E. coli* and *P. aeruginosa*. This indicates that *S. enterica* is more resistant to these samples, particularly to TaCu-1.

The inhibition rates against *S. aureus* exhibit a trend close to that observed for *S. enterica*, with moderate activity overall. TaCu-2/600 and TaCu-3/600 show relatively higher inhibition rates, but the effectiveness is still below that observed for *E. coli* and *P. aeruginosa*. The lower performance of TaCu-1 suggests it is the least effective sample across all bacterial strains.

The time-kill test results suggest that the antibacterial activity of the TaCu samples varies significantly depending on the bacterial strain, sample composition, and treatment conditions. The detailed statistical analysis is given in Table S2, supplementary data. TaCu-2/600 consistently demonstrates the highest bactericidal activity across all strains, particularly against Gram-negative and Gram-positive bacteria. This indicates that the composition of TaCu-2 annealed at 600°C for 1 h was optimal for antibacterial effectiveness. On the other hand, *S. aureus* appears more resistant to these samples, as reflected by their lower inhibition rates. The lower effectiveness of TaCu-1 across all conditions suggests that it is less suitable for applications requiring vigorous bactericidal activity. Overall, the time-kill test highlights the importance of both sample composition and treatment conditions in determining antibacterial efficacy, with TaCu-2/600 emerging as the most potent combination.

Thus, the materials exhibited antibacterial activity against the tested bacteria, as evidenced by the suppression of their specific growth rates and reduced colony-forming units. Notably, the bactericidal effect of the samples was more pronounced against Gram-positive bacteria than Gram-negative bacteria. This difference is likely due to the presence of an outer membrane in Gram-negative bacteria, which acts as a barrier and provides greater resistance to the components in the material.

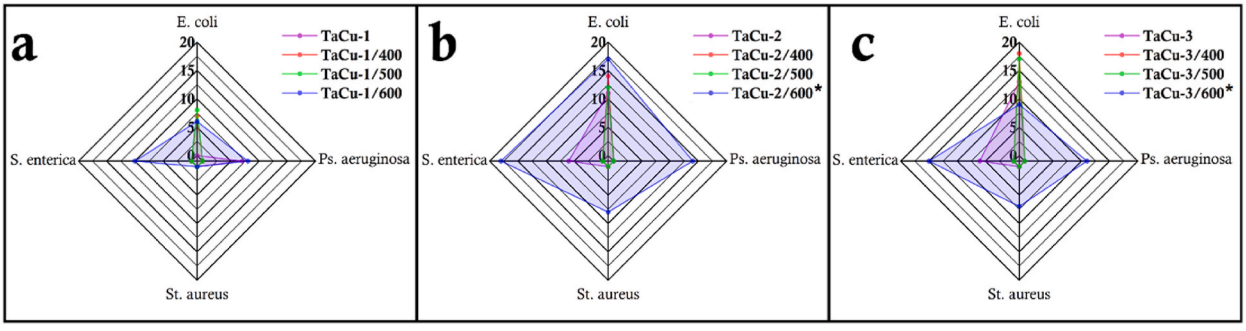


Fig. 3. The assessment of antimicrobial activity of the TaCu thin films by "agar diffusion" experiment. * - significant difference ($p \leq 0.05$).

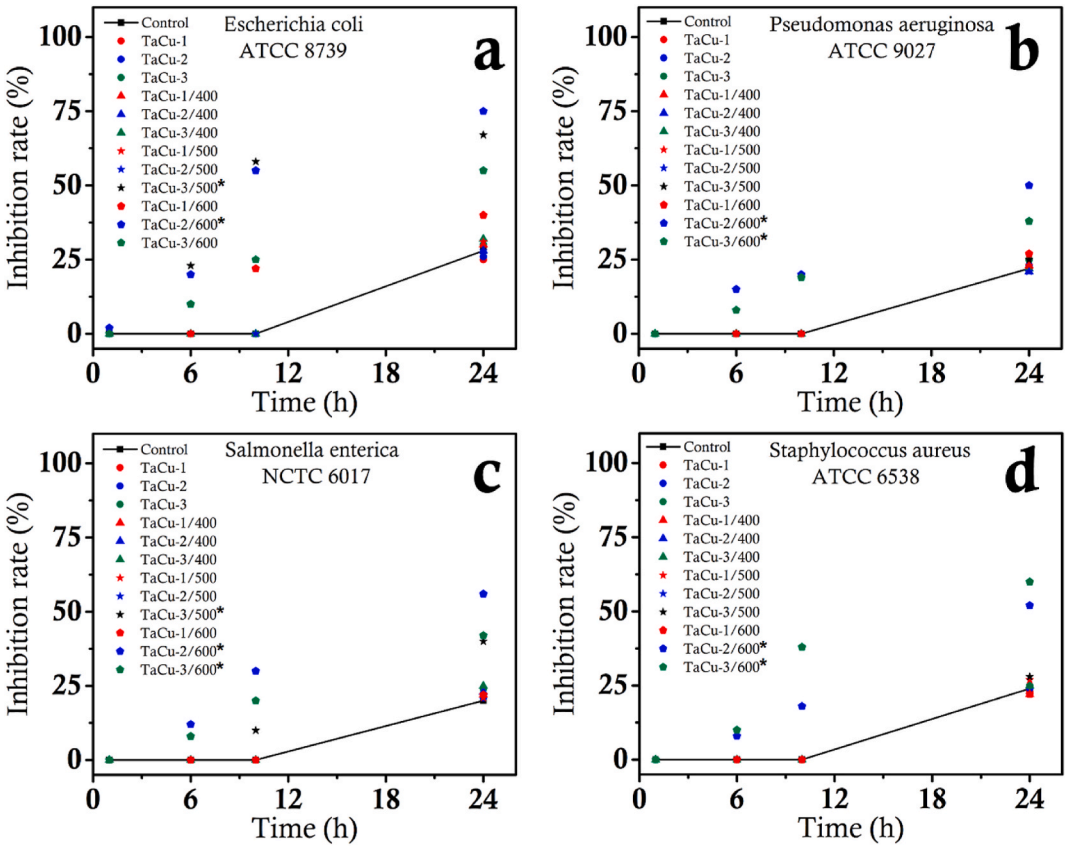


Fig. 4. The time-kill test of thin films toward: a) *Escherichia coli;* b) *Pseudomonas aeruginosa;* c) *Salmonella enterica;* d) *Staphylococcus aureus*. * - significant difference ($p \leq 0.05$).

The evaluation of TaCu thin films with varying copper content and thermal treatment conditions has revealed significant insights into their antibacterial properties and physicochemical characteristics. The findings suggest that both copper content and annealing temperature play crucial roles in determining the effectiveness of these coatings against bacterial strains, particularly *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enterica,* and *Pseudomonas aeruginosa.*

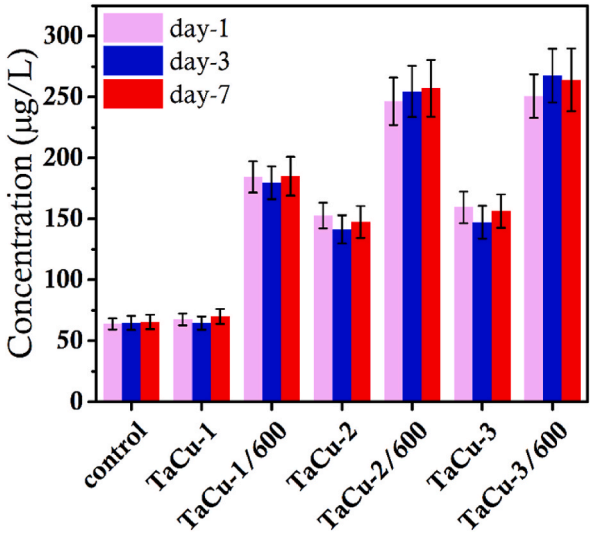


Fig. 5. Copper ions released by time in SBF.

The ion release test in simulated body fluid (SBF) was done to confirm the prime role of copper in the origin of antibacterial properties [41]. As shown in Fig. 5, the TaCu-1 shows almost the same concentration after 1-day immersion in SBF as control. At the same time, TaCu-2 and TaCu-3 demonstrated a gradual increase with ~2 times the higher concentrations of copper ions (152.7 and 159.6 μg/L, respectively). However, after thermal treatment, the thin films demonstrate a doubled release of copper compared with non-treated pairs. The elevated copper ion release, especially from TaCu-2/600, could lead to increased reactive oxygen species (ROS) generation and disruption of bacterial cell membranes, which are widely recognized for their effectiveness against Gram-negative and Gram-positive bacteria [42,43]. These findings correlate with the antibacterial assessments, where samples with enhanced copper release demonstrated higher inhibition of bacterial growth.

The study showed that the antibacterial effectiveness of the TaCu thin films was more pronounced against Gram-negative bacteria (*E. coli* and *P. aeruginosa*) than Gram-positive bacteria (*S. aureus* and *S. enterica*). This difference is likely due to the structural differences between Gram-negative and Gram-positive bacteria, with the former possessing an outer membrane that provides an additional barrier against antibacterial agents. The lower performance of TaCu-1 across all bacterial strains suggests that a lower copper content may not be sufficient to achieve the desired antibacterial effects, particularly under lower thermal treatment conditions.

Fig. 6 illustrates the antibacterial mechanisms of TaCu thin films where copper ions and reactive oxygen species (ROS) provide bactericidal activity against Gram-negative and Gram-positive bacteria. For Gram-negative bacteria (left side), which have a thinner peptidoglycan layer and an outer membrane made of lipopolysaccharides, copper ions and reactive oxygen species (ROS) can more easily penetrate the cell, leading to a high flow of both copper ions and ROS into the cell. This results in significant protein and DNA disruption. In contrast, Gram-positive bacteria (right side) have a thicker peptidoglycan layer that restricts the flow of copper ions and ROS into the cell [42,43]. This thicker barrier leads to lower intracellular concentrations of copper ions and ROS, resulting in reduced disruption of proteins and DNA, making Gram-positive bacteria less susceptible to the antibacterial effects of the TaCu thin film. The differences in susceptibility between the two types of bacteria are primarily due to the structural variations in their cell walls. Copper ions exhibit less bactericidal activity against Gram-positive bacteria than Gram-negative bacteria primarily due to differences in their cell wall structures. Gram-positive bacteria have a thick peptidoglycan layer that acts as a barrier, sequestering copper ions and limiting their ability to disrupt the cytoplasmic membrane. In contrast, Gram-negative bacteria, with their thinner peptidoglycan layer and permeable outer membrane, allow copper ions to more easily penetrate and exert toxic effects. Additionally, Gram-positive bacteria may possess more effective efflux pumps and resistance mechanisms that reduce copper ion concentration within the cell, further diminishing their susceptibility [42,44].

The diffusion into agar method visually assessed the antibacterial activity, with results supporting the time-kill test findings. However, the method's limitations, such as potential inconsistencies in substance diffusion, highlight the need for complementary testing methods like the time-kill test to obtain a more comprehensive understanding of antibacterial efficacy.

Overall, the results indicate that TaCu-2, particularly when annealed at 600 °C, offers a promising balance of antibacterial effectiveness and physicochemical stability. These findings underscore the importance of optimizing the composition and processing conditions of TaCu thin films to maximize their potential in medical applications, particularly in preventing implant-associated infections. Further studies should explore these coatings' long-term stability and biocompatibility in vivo to ensure their safe and effective use in clinical settings.

4. Conclusion

The investigation into the antibacterial properties of Cu-Ta thin films has revealed that both copper content and thermal treatment conditions significantly impact their effectiveness against bacterial strains. The TaCu-2 thin film, with the copper content (~10 wt%) and annealed at 600 °C, consistently demonstrated superior bactericidal activity, particularly against Gram-negative bacteria such as *E. coli, S. enterica* and *P. aeruginosa*. This enhanced performance is attributed to the optimized release of copper ions and the generation

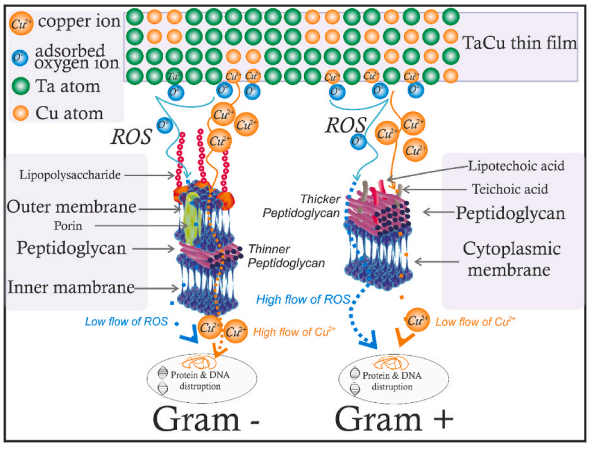


Fig. 6. The possible antibacterial mechanism of TaCu thin films.

of reactive oxygen species (ROS), which collectively disrupt bacterial cell membranes and interfere with vital cellular functions. Conversely, Gram-positive bacteria like *S. aureus* exhibited more resistance, likely due to their structural differences. The study underscores the importance of tailoring the composition and processing parameters of Cu-Ta coatings to maximize their antibacterial efficacy, thereby providing a potential solution for reducing implant-associated infections. Future research should focus on assessing the long-term stability, biocompatibility, and clinical applicability of these coatings to ensure their safe and effective use in medical implants.

CRediT authorship contribution statement

Bagdat Azamatov: Writing – original draft, Funding acquisition, Conceptualization. **Alexey Dzhes:** Writing – review & editing. **Alexander Borisov:** Data curation. **Daniyar Kaliyev:** Data curation. **Bauyrzhan Maratuly:** Data curation. **Amangeldi Sagidugumar:** Writing – review & editing, Formal analysis. **Myakinin Alexandr:** Writing – review & editing, Writing – original draft. **Amanzhol Turlybekuly:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Sergei Plotnikov:** Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research has been funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP14871715 “Development of technology for applying Ti-Cu-Ta and Ti-Cu-Nb based bactericidal coatings of medical implants by magnetron sputtering”).

We would like to dedicate this work to the memory of Professor **Sergei Viktorovich Plotnikov**, whose guidance, expertise, and passion for scientific discovery greatly enriched this research. Professor Plotnikov was a dedicated mentor and a visionary scientist who contributed significantly to the field of materials science. His untimely passing during the research project is an immense loss to the scientific community of Kazakhstan and everyone who had the privilege of working with him.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e41130.

References

[1] J. Li, L. Tan, X. Liu, Z. Cui, X. Yang, K.W.K. Yeung, P.K. Chu, S. Wu, Balancing bacteria–osteoblast competition through selective physical puncture and biofunctionalization of ZnO/Polydopamine/Arginine-Glycine-Aspartic acid-cysteine nanorods, ACS Nano 11 (2017) 11250–11263, https://doi.org/10.1021/acsnano.7b05620.

[2] D. Campoccia, L. Montanaro, C.R. Arciola, The significance of infection related to orthopedic devices and issues of antibiotic resistance, Biomaterials 27 (2006) 2331–2339, https://doi.org/10.1016/j.biomaterials.2005.11.044.

[3] N.J. Shah, Md.N. Hyder, J.S. Moskowitz, M.A. Quadir, S.W. Morton, H.J. Seeherman, R.F. Padera, M. Spector, P.T. Hammond, Surface-mediated bone tissue morphogenesis from tunable nanolayered implant coatings, Sci. Transl. Med. 5 (2013), https://doi.org/10.1126/scitranslmed.3005576.

[4] X. Chen, J. Zhou, Y. Qian, L. Zhao, Antibacterial coatings on orthopedic implants, Mater. Today Bio. 19 (2023) 100586, https://doi.org/10.1016/j.mtbio.2023.100586.

[5] M.M. Hasani-Sadrabadi, S. Pouraghaei, E. Zahedi, P. Sarrion, M. Ishijima, E. Dashtimoghadam, N. Jahedmanesh, S. Ansari, T. Ogawa, A. Moshaverinia, Antibacterial and osteoinductive implant surface using layer-by-layer assembly, J. Dent. Res. 100 (2021) 1161–1168, https://doi.org/10.1177/00220345211029185.

[6] A. Sagidugumar, D. Dogadkin, A. Turlybekuly, D. Kaliyev, Calcium phosphate coatings deposited on 3D-printed Ti–6Al–4V alloy by plasma electrolytic oxidation, Coatings 14 (2024) 696, https://doi.org/10.3390/coatings14060696.

[7] D. Akilbekova, A. Turlybekuly, Patient-specific 3D bioprinting for in situ tissue engineering and regenerative medicine, in: 3D Print Med, Elsevier, 2023, pp. 149–178, https://doi.org/10.1016/B978-0-323-89831-7.00003-1.

[8] M. Zhu, J. Fang, Y. Li, C. Zhong, S. Feng, X. Ge, H. Ye, X. Wang, W. Zhu, X. Lu, F. Ren, The synergy of topographical micropatterning and Ta|TaCu bilayered thin film on titanium implants enables dual-functions of enhanced osteogenesis and anti-infection, Adv. Healthcare Mater. 10 (2021), https://doi.org/10.1002/adhm.202002020.

[9] X. Zheng, M. Huang, C. Ding, Bond strength of plasma-sprayed hydroxyapatite/Ti composite coatings, Biomaterials 21 (2000) 841–849, https://doi.org/10.1016/S0142-9612(99)00255-0.

[10] L. Sun, C.C. Berndt, K.A. Gross, A. Kucuk, Material fundamentals and clinical performance of plasma-sprayed hydroxyapatite coatings: a review, J. Biomed. Mater. Res. 58 (2001) 570–592, https://doi.org/10.1002/jbm.1056.

[11] M. Reed, Executive Summary Professor Mike Reed, Chair of the NJR Editorial Committee Mr Tim Wilton, NJR Medical Director, 2024. https://reports.njrcentre.org.uk/AR-Executive-Summary. (Accessed 14 August 2024).

[12] A. Ghimire, J. Song, Anti-periprosthetic infection strategies: from implant surface topographical engineering to smart drug-releasing coatings, ACS Appl. Mater. Interfaces 13 (2021) 20921–20937, https://doi.org/10.1021/acsami.1c01389.

[13] A. Turlybekuly, A. Pogrebnjak, L. Sukhodub, L. Sukhodub, O. Bondar, ZnO doped nanosized composite material based on hydroxyapatite and sodium alginate matrix, in: NAP-2018, 2018 IEEE 8th International Conference on "Nanomaterials: Applications & Properties," Zatoka, Ukraine, 2018, p. 4, 04NNLS36-1-04NNLS36.
[14] E. Zhang, X. Zhao, J. Hu, R. Wang, S. Fu, G. Qin, Antibacterial metals and alloys for potential biomedical implants, Bioact. Mater. 6 (2021) 2569–2612, https://doi.org/10.1016/j.bioactmat.2021.01.030.
[15] B.F. Finina, A.K. Mersha, Nano-enabled antimicrobial thin films: design and mechanism of action, RSC Adv. 14 (2024) 5290–5308, https://doi.org/10.1039/D3RA07884A.
[16] S. Mahmoudi-Qashqay, M.-R. Zamani-Meymian, S.J. Sadati, Improving antibacterial ability of Ti-Cu thin films with co-sputtering method, Sci. Rep. 13 (2023) 16593, https://doi.org/10.1038/s41598-023-43875-4.
[17] J.H. Hsieh, T.H. Yeh, C. Li, C.H. Chiu, C.T. Huang, Antibacterial properties of TaN–(Ag,Cu) nanocomposite thin films, Vacuum 87 (2013) 160–163, https://doi.org/10.1016/j.vacuum.2012.02.016.
[18] J.-F. Tang, P.-Y. Huang, J.-H. Lin, T.-W. Liu, F.-C. Yang, C.-L. Chang, Microstructure and antimicrobial properties of Zr-Cu-Ti thin-film metallic glass deposited using high-power impulse magnetron sputtering, Materials 15 (2022) 2461, https://doi.org/10.3390/ma15072461.
[19] M. Osękowska, D. Wojcieszak, D. Kaczmarek, M. Mazur, A. Obstarczyk, B. Szponar, Multifunctional nanocrystalline Cu–Ti thin films enhance survival and induce proliferation of mouse fibroblasts in vitro, Coatings 11 (2021) 300, https://doi.org/10.3390/coatings11030300.
[20] D. Wojcieszak, M. Osekowska, D. Kaczmarek, B. Szponar, M. Mazur, P. Mazur, A. Obstarczyk, Influence of material composition on structure, surface properties and biological activity of nanocrystalline coatings based on Cu and Ti, Coatings 10 (2020) 343, https://doi.org/10.3390/coatings10040343.
[21] J. Kratochvíl, J. Štěrba, J. Lieskovská, H. Langhansová, A. Kuzminova, I. Khalakhan, O. Kylián, V. Straňák, Antibacterial effect of Cu/C:F nanocomposites deposited on PEEK substrates, Mater. Lett. 230 (2018) 96–99, https://doi.org/10.1016/j.matlet.2018.07.082.
[22] A. Hoene, C. Prinz, U. Walschus, S. Lucke, M. Patrzyk, L. Wilhelm, H.-G. Neumann, M. Schlosser, In vivo evaluation of copper release and acute local tissue reactions after implantation of copper-coated titanium implants in rats, Biomed. Mater. 8 (2013) 035009, https://doi.org/10.1088/1748-6041/8/3/035009.
[23] J. Tang, F. Wei, L. Zhao, L. Yang, J. Li, Z. Sun, C. Yang, W. Zhang, B. Liu, Superior antibacterial properties of copper-doped titanium oxide films prepared by micro-arc oxidation, Ceram. Int. 50 (2024) 1370–1378, https://doi.org/10.1016/j.ceramint.2023.10.225.
[24] G. Li, Y. Wang, L. Qiao, R. Zhao, S. Zhang, R. Zhang, C. Chen, X. Li, Y. Zhao, Preparation and formation mechanism of copper incorporated micro-arc oxidation coatings developed on Ti-6Al-4V alloys, Surf. Coat. Technol. 375 (2019) 74–85, https://doi.org/10.1016/j.surfcoat.2019.06.096.
[25] G.A. Norambuena, R. Patel, M. Karau, C.C. Wyles, P.J. Jannetto, K.E. Bennet, A.D. Hanssen, R.J. Sierra, Antibacterial and biocompatible titanium-copper oxide coating may Be a potential strategy to reduce periprosthetic infection: an in vitro study, Clin. Orthop. Relat. Res. 475 (2017) 722–732, https://doi.org/10.1007/s11999-016-4713-7.
[26] A. Bahrami, C.F. Onofre Carrasco, A.D. Cardona, T. Huminiuc, T. Polcar, S.E. Rodil, Mechanical properties and microstructural stability of CuTa/Cu composite coatings, Surf. Coat. Technol. 364 (2019) 22–31, https://doi.org/10.1016/j.surfcoat.2019.02.072.
[27] C. Guo, J. Zhou, Y. Yu, L. Wang, H. Zhou, J. Chen, Microstructure and tribological properties of Ti–Cu intermetallic compound coating, Mater. Des. 36 (2012) 482–489, https://doi.org/10.1016/j.matdes.2011.10.033, 1980-2015.
[28] M. Al Deeb, A.A. Aldosari, S. Anil, Osseointegration of tantalum trabecular metal in titanium dental implants: histological and micro-CT study, J. Funct. Biomater. 14 (2023) 355, https://doi.org/10.3390/jfb14070355.
[29] G. Huang, S.-T. Pan, J.-X. Qiu, The clinical application of porous tantalum and its new development for bone tissue engineering, Materials 14 (2021) 2647, https://doi.org/10.3390/ma14102647.
[30] CLSI, Methods for Determining Bactericidal Activity of Antimicrobial Agents, 1998.
[31] M. Balouiri, M. Sadiki, S.K. Ibnsouda, Methods for in vitro evaluating antimicrobial activity: a review, J. Pharm. Anal. 6 (2016) 71–79, https://doi.org/10.1016/j.jpha.2015.11.005.
[32] T. Kokubo, H. Takadama, How useful is SBF in predicting in vivo bone bioactivity? Biomaterials 27 (2006) 2907–2915, https://doi.org/10.1016/j.biomaterials.2006.01.017.
[33] S.-C. Yang, D.-P. Tran, C. Chen, Recrystallization and grain growth in Cu-Cu joints under electromigration at low temperatures, Materials 16 (2023) 5822, https://doi.org/10.3390/ma16175822.
[34] A.M. Engwall, Z. Rao, E. Chason, Origins of residual stress in thin films: interaction between microstructure and growth kinetics, Mater. Des. 110 (2016) 616–623, https://doi.org/10.1016/j.matdes.2016.07.089.
[35] M. Ohring, Materials Science of Thin Films: Deposition and Structure, second ed., Academic, San Diego, 2002.
[36] A.D. Pogrebnyak, A.P. Shpak, N.A. Azarenkov, V.M. Beresnev, Structures and properties of hard and superhard nanocomposite coatings, Phys. Usp. 52 (2009) 29–54, https://doi.org/10.3367/UFNe.0179.200901b.0035.
[37] W. Qin, L. Fu, J. Zhu, W. Yang, J. Sang, D. Li, L. Zhou, Electrical and thermal properties of Cu-Ta films prepared by magnetron sputtering, Appl. Surf. Sci. 443 (2018) 97–102, https://doi.org/10.1016/j.apsusc.2018.02.262.
[38] S. Srinivasan, S. Sharma, S. Turnage, B.C. Hornbuckle, C. Kale, K.A. Darling, K. Solanki, Role of tantalum concentration, processing temperature, and strain-rate on the mechanical behavior of copper-tantalum alloys, Acta Mater. 208 (2021) 116706, https://doi.org/10.1016/j.actamat.2021.116706.
[39] K. Koike, K.D. Clarke, A.J. Clarke, Microstructural evolution and mechanical properties of heavily cold-rolled and subsequently annealed Cu-3 wt.%Ti alloys with nano-lamellar structure, JOM 71 (2019) 4789–4798, https://doi.org/10.1007/s11837-019-03838-5.
[40] Q. Lin, S. Yang, W. Jing, C. Li, C. Wang, Z. Jiang, K. Jiang, Buckling and delamination of Ti/Cu/Si thin film during annealing, J. Electron. Mater. 43 (2014) 3351–3356, https://doi.org/10.1007/s11664-014-3238-7.
[41] M.N. Capela, D.M. Tobaldi, C. Oliveira, A. Pereira, A.S. Duarte, M.P. Seabra, M.H.V. Fernandes, Bioactivity and antibacterial activity against E-coli of calcium-phosphate-based glasses: effect of silver content and crystallinity, Ceram. Int. 43 (2017) 13800–13809, https://doi.org/10.1016/j.ceramint.2017.07.100.
[42] I. Salah, I.P. Parkin, E. Allan, Copper as an antimicrobial agent: recent advances, RSC Adv. 11 (2021) 18179–18186, https://doi.org/10.1039/D1RA02149D.
[43] M. Yang, Y. Liu, Y. Liao, C. Tang, Z. Wen, A. Fazal, R. Yang, J. Qi, Z. Hong, Y. Li, Y. Yang, Excess copper promotes catabolic activity of gram-positive bacteria and resistance of gram-negative bacteria but inhibits fungal community in soil, Environ. Sci. Pollut. Control Ser. 29 (2022) 22602–22612, https://doi.org/10.1007/s11356-021-17510-6.
[44] T.N. Nguyen, Q.H. Do, T.T.D. Vu, T.T. Nguyen, D.T. Nguyen, T.B.N. Nguyen, T.T.H. Tran, T.K.O. Vu, T.H.L. Nghiem, T.M.T. Hoa, T.M.H. Nguyen, P.N. Bui, M. H. Nguyen, D.M. Pham, T.C.T. Tran, Enhancement of antibacterial activity by a copper(II) and zinc(II) in chelation with ethylenediaminetetra-acetic acid and urea complex, Chem. Pap. 76 (2022) 7163–7176, https://doi.org/10.1007/s11696-022-02361-3.