THE EFFECT OF TITANIUM DIOXIDE MODIFICATION ON THE COPPER POWDER BACTERICIDAL PROPERTIES

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Abstract

The bactericidal and bacteriostatic effects of copper have been known for a long time. However, the coatings apart from biological activity should fulfil a number of other requirements, such as tightness, scratch resistance or aesthetic appearance. Researchers have been working on creating durable coatings meeting these requirements for a long time. Scientific research indicates a high interest in active coatings. Nano-scale additives are used, with the aim to modify the material's performance at the atomic level. Composite coatings allow us to provide the materials multifunctionality, and in addition, can enhance their mutual action. There are many methods for creating such materials. One of the techniques of applying composite coatings is the Cold Spray method, in which the coating is made of a powder. The main purpose of the modification is to obtain a bactericidal and bacteriostatic effect, but also a durable and wear-resistant coating. The paper proposes modifications of copper powder with amorphous submicron titanium dioxide in order to increase its biological activity. The modified powder can be used to create coatings by various methods including thermal methods. The work presents a material analysis of Cu and TiO_2 powders and results of bactericidal tests carried out on a Cu-TiO_2 composite powder. The experiment included Escherichia coli and Staphylococcus aureus. The studies have shown a positive effect of the addition of TiO_2 on bactericidal properties against both Staphylococcus aureus (Gram-positive bacteria) and Escherichia coli (Gram-negative bacteria) when mixed with copper at 1:9 ratio.

Keywords: *composite powder, Cu, TiO_2, bactericidal tests*

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Introduction

Copper is known as an important micronutrient required in very small amounts for the survival of most aerobic organisms but at high concentrations can become toxic and inhibit microbial growth. Bogdanovic et al. [1] show that copper nanoparticles (Cu NPs) (diameter 5.3 nm) were able to reduce more than 98% of all tested strain (include *E. coli* and *S. aureus*) after 2 h of contact and Cu NPs has higher reduction rate for *E. coli* than *S. aureus*. Argueta-Figueroa et al. [2] also confirmed that Cu NPs have the antibacterial activity against *E. coli* and *S. aureus* (minimal inhibitory concentration, MIC = 1000 µg/ml) and cause more membrane damages of the *E. coli* as compared to *S. aureus*. The results of the experiment with another Gram-negative bacteria (*Salmonella*) showed that under dry incubation conditions bacterial cells are extremely vulnerable to copper [3]. He X. et al. [4] also shows that CuO/TiO_2 coating has high antibacterial activity against *S. aureus* in contrast to pure titanium and TiO_2 coating. It can be explained that the addition of copper is the crucial factor to endow copper/TiO_2 coating with the antibacterial effect against *S. aureus*. TiO_2 coating with the addition of copper enhanced antibacterial activity against *E. coli* and *S. aureus* [5].

The bactericidal and bacteriostatic effects of copper have been known for a long time, since then, researchers have been working on coatings that have these properties. The coatings apart from biological activity should fulfil a number of other requirements, such as tightness, scratch resistance or aesthetic appearance. The production of active composite materials with bactericidal activity is a very hot topic nowadays. The problem is, that currently known and applied Cu-TiO_2 protective layers are thick, heterogeneous and unstable. The composites based on copper and titanium dioxide have been tested several times, in most cases coatings in the form of nanopowders or thin films have been made. Sol-gel deposited Cu-TiO_2 films are not mechanically stable, in many cases their preparation is not reproducible, do not present uniformity but only low adhesion. Films obtained by a direct current magnetron sputtering, as reported, avoid the disadvantages of Cu-films prepared by sol-gel methods [6-8]. Ultra-thin copper/titanate coatings can be produced, among others, by the Highly Ionized Pulsed Plasma Magnetron Sputtering method (HIPMS), in which the bactericidal effect is activated by visible light. The study shows the first complete report on ultrathin TiO_2/Cu nano-particulate films leading to fast bacterial loss of viability. The TiO_2/Cu sputtered films induced complete bacteria *E. coli* inactivation in the dark, which was not observed in the case of TiO_2. When Cu was present, the bacterial inactivation was accelerated under low-intensity solar light and this may have a positive practical impact on biological technology. Disadvantages of HIPMS lead scientists to look for new technologies to complement these shortcomings. Cold spray technology, due to the low temperature during spraying, does not cause physicochemical changes in the modified material, also the plastic strain energy accompanying the formation of the coating does not cause phase changes in the coating [9].

In this study, we propose modifications of copper powder with amorphous submicron titanium dioxide in order to increase its biological activity. The modified powder can be used to create coatings by various methods including thermal methods. The work presents an analysis of Cu and TiO_2 powders and results of bactericidal tests carried out on a Cu-TiO_2 composite powder.

Materials and Methods

A dendritic commercial copper powder with a particle size of 10-100 µm obtained by the electrolytic method was used for the tests. The use of the dendritic form of the powder assures its better binding to the surface. However, such high surface area has a direct effect on the accelerated oxidation of the powder surface. The powder is characterized by different structure and size of dendritic particles as shown by scanning electron microscopy (SEM) observations (FIG. 1 a-d).

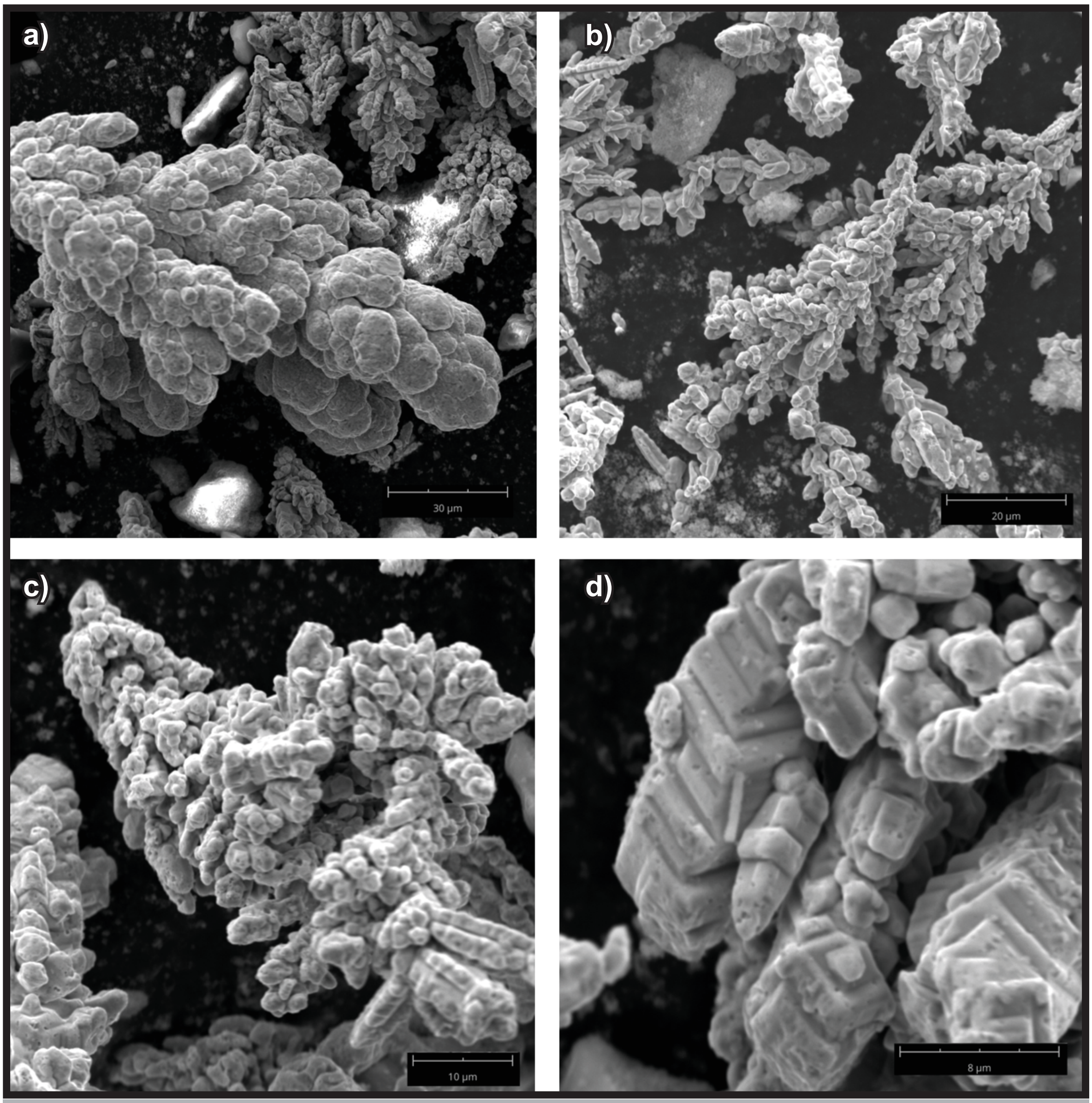

FIG. 1. a-d Different types of morphology of copper powder produced by electrolytic method studied by SEM.

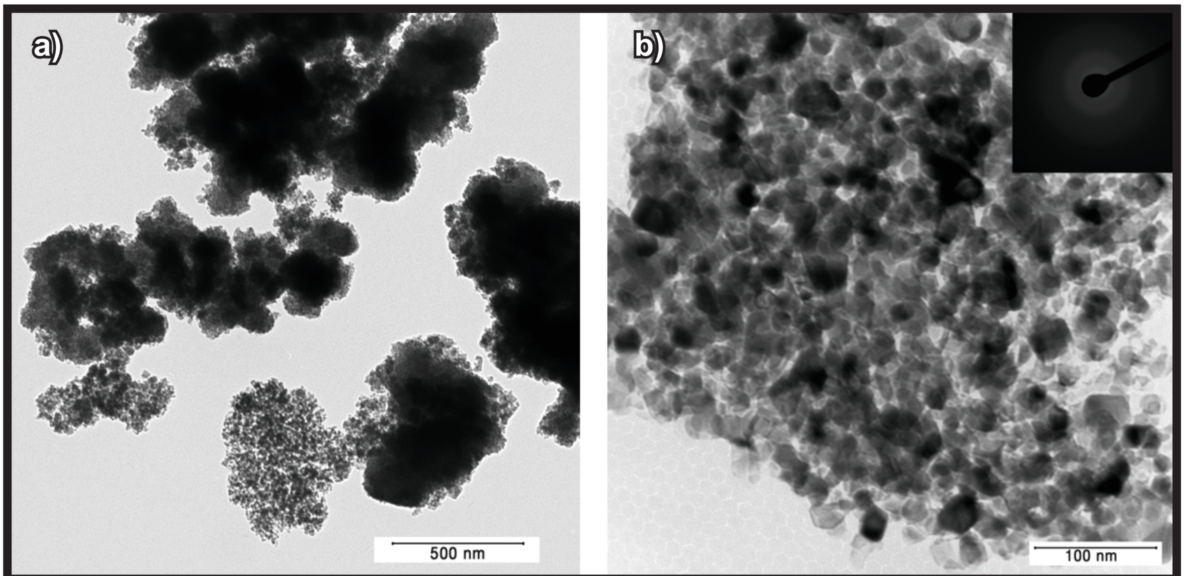

FIG. 2. a-b Morphology of titanium dioxide powder studied by TEM.

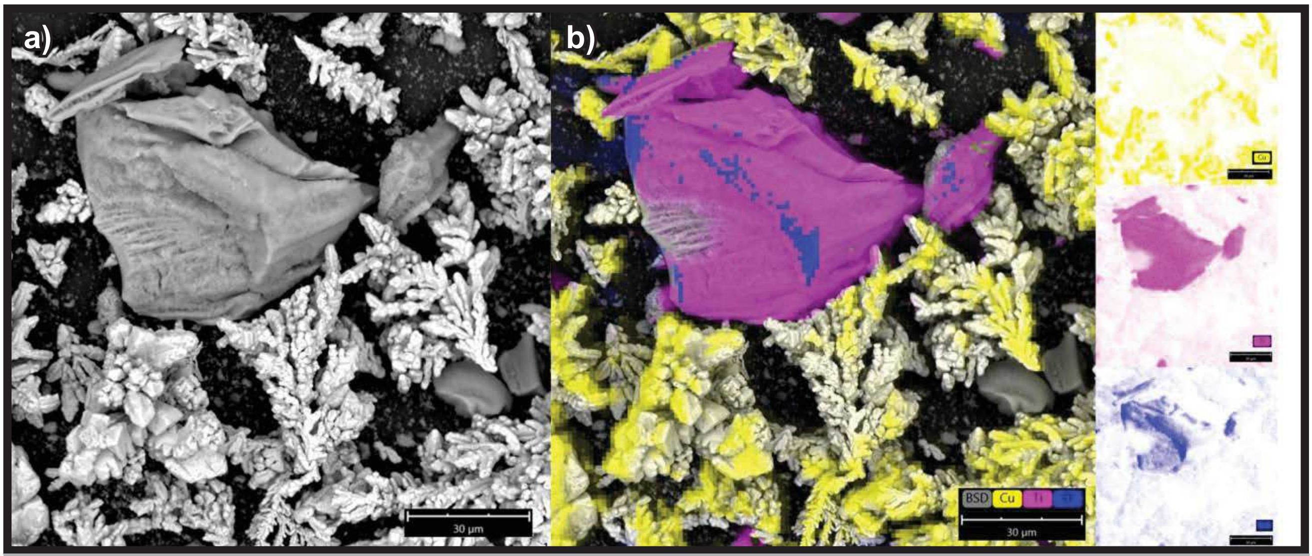

FIG. 3. SEM picture (a) and EDS elemental distribution (b) in composite powder with Cu-25TiO_2 composition.

The variety of structures has a positive effect on the formation of the coating during application using the Cold Spray method and coating deposition is much more efficient than the use of symmetrical spherical copper particles.

The powder of copper was mixed with submicron titanium dioxide powder produced by the sol-gel method at the Department of Mechanics and Materials Engineering at the Wroclaw University of Science and Technology [10]. TiO_2 powder of the amorphous type, which also possesses antibacterial activity properties, was used for the tests. The morphology of the TiO_2 powder has a diverse structure, both nanometric particle size and agglomerates with sizes of several dozen nanometres are visible in the transmission electron microscopy (TEM) pictures (FIG. 2 a,b). Diffraction analysis of TiO_2 powder confirmed the amorphous structure of the material (FIG. 2 b).

Four different concentrations of Cu and TiO_2 powders were used. The powders (Cu and TiO_2) were mixed in a mechanical stirrer for 4 h. Pure copper was used as a reference material, and further powders were additionally supplemented with 10, 25 and 50% mass concentrations of titanium dioxide. Analysis of the composition of the elements included in the powder showed that the titanium dioxide powder, despite the nanometric granulation, does not occupy the position between the branches of dendritic copper particles. The components of the composite powder form separate fractions despite intense mixing, which is visible on the map analysis of elements made by the electron dispersion spectroscopy (EDS) method (FIG. 3).

Zone inhibition assay is a qualitative method commonly used to measure antibiotic resistance, but also to test antimicrobial surfaces/substance properties. With this method, a pure bacterial culture is suspended in a buffer, standardized to a density (OD_{600}) and then spread over an agar plate. A filter-paper disk, impregnated with the compound (e.g. antibiotic), is placed on the surface of the agar. After required incubation time, which depends on a bacterial strain, the diameter of the zone of inhibition should be measured, including the diameter of the disc. If the bacterial strain is susceptible to the antimicrobial agent, then a zone of inhibition appears on the agar plate, if it is resistant to the antimicrobial agent, then no zone is observed.

Another method to determine the antibacterial effect is connected with MIC – minimal inhibition concentration. It can be done for aqueous samples using microplates or broth – 2-fold dilution are prepared for each concentration of the solution and then no microbial growth is observed. The lower concentration where no microbial growth is observed, the higher antimicrobial effect is. For plastic and non-porous surfaces, it can be also evaluated according to ISO Standard. Results are given as a difference in recovery of the bacterial inoculum after the incubation on the tested surface (shown in log10 or percentages).

The experiment included *E. coli* ATCC 11775 (Gram-negative bacteria) and *S. aureus* ATCC 6538P (Gram-positive bacteria), which were obtained from Polish Collection of Microorganisms (WFCC, No. 106). Strains were used after 24 h incubation at 37°C to prepare the inoculum to a concentration of 10^9 cfu/ml. A suspension of *S. aureus* and *E. coli* was sprayed over the total area of each Petri dish in triplicate. Then paper discs (7 mm diameter) soaked in a solution of a mixture of Cu-TiO_2 powders (10 mg/ml) were put on nutrient agar plates. After incubation at 35°C for 48 h, the diameter of the inhibition zone was measured by the calliper. The result equal to 7 mm is actually no zone of inhibition (no antimicrobial effect) as in the negative control.

The same bacterial inoculum was used to determine MIC (of an aqueous suspension of a mixture of Cu-TiO_2 powders (using broth suitable for bacteria growth). All solutions were prepared using sterile 96-well plate. 100 μl of nanoparticles suspension was added to each well according to the scheme shown in FIG. 4. The initial concentration of nanoparticles was 10 mg/ml and 2-fold dilutions were prepared from well number 1 to well number 7 (from 10 mg/ml to 156.25 μg/ml). Three types of control samples were prepared – broth without bacteria and nanoparticles (K-), broth with bacteria (K+) and nanoparticles suspended in broth (K_{Np}). The bacterial suspension (concentration – 10^9 cfu/ml) was added to each well (except control K-) and then incubated at 37°C for 24 h (with shaking). The optical density was measured using a Gen5 microplate reader at 600 nm. The results were given in MIC (mg/ml).

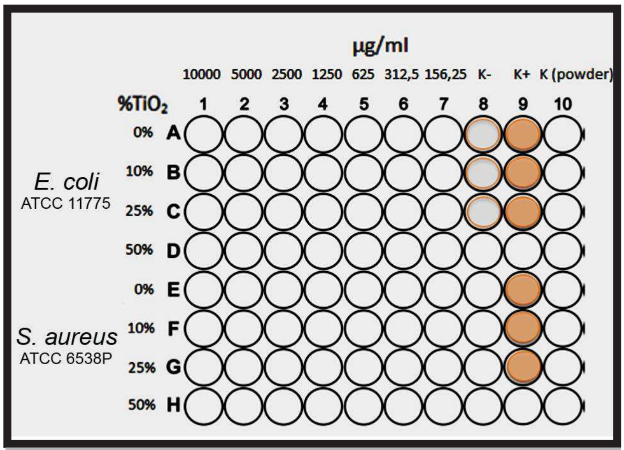

FIG. 4. Scheme of minimal inhibitory concentration test (MIC).

Results and Discussions

The analysis of the obtained results shows the beneficial effect of the addition of the titanium dioxide fraction on the microbiological activity of the copper powder against *S. aureus and E. coli*. The results of zone inhibition method are shown in TABLE 1 and TABLE 2.

Copper NPs and titanium dioxide at concentrations of titanium dioxide 10% and 25% show good inhibition zone against *E. coli and S. aureus.* There was no inhibition zone for titanium dioxide at a concentration 50% (FIG. 5). The best results were obtained for addition titanium dioxide at concentration 10% and 50% for both reference strains. The tests confirm the results obtained for TiO_2-Cu coatings produced by magnetron sputtering method where the authors found that addition of copper into titanium dioxide structure during sputtering process resulted in increasing antimicrobial activity for microorganisms (*E. coli, Bacillus subtilis, S. aureus, Enterococcus hirae* and *Candida albicans*) [11]. A significant effect on the test results and bactericidal activity could have been the oxidation state of the copper powder. The critical concentration of the modifying fraction seems to be very important in this method, it has a decisive influence on bactericidal properties.

In the microplate assay for determining MIC, there was no turbidity of broth in K- and K_{Np} control samples and noticeable turbidity in K+ control samples for *E. coli* and *S. aureus* strains, which confirm that the test was carried out properly. The results showed that a mixture of Cu-TiO_2 powders has higher antimicrobial efficacy against *S. aureus*. The MIC of mixtures of Cu-TiO_2 is varied, but the lowest was observed for Cu+10%TiO_2 samples for both reference bacteria strains (TABLE 3).

The lower concentration where no microbial growth is observed, the higher antimicrobial effect is. This test should be compared to the MBC (minimum bactericidal concentration), which is complementary to the MIC and also to the test according to ISO standard for antimicrobial efficiency of the surface.

TABLE 1. Zone of inhibition of Cu and TiO_2 powder against *E. coli* ATCC 11775.

		Diameter of inhibition zone [mm]	SD [mm]
Cu	*E. coli* ATCC 11775	10.47	0.32
Cu+10%TiO_2		10.27	0.99
Cu+25%TiO_2		11.87	1.86
Cu+50%TiO_2		0	-

TABLE 2. Zone of inhibition of Cu and TiO_2 powder against *S. aureus* ATCC 6538P.

		Diameter of inhibition zone [mm]	SD [mm]
Cu	*S. aureus* ATCC 6538P	10.34	0.37
Cu+10%TiO_2		11.37	0.85
Cu+25%TiO_2		12.28	0.56
Cu+50%TiO_2		0	-

TABLE 3. MIC (minimal inhibitory concentration) of Cu and TiO_2 powder (*E. coli* ATCC 11775, *S. aureus* ATCC 6538P).

	MIC, mg/ml	
Cu	*E. coli* ATCC 11775	2.5
Cu+10%TiO_2		0.625
Cu+25%TiO_2		10
Cu+50%TiO_2		>10
Cu	*S. aureus* ATCC 6538P	2.5
Cu+10%TiO_2		0.625
Cu+25%TiO_2		5
Cu+50%TiO_2		>10

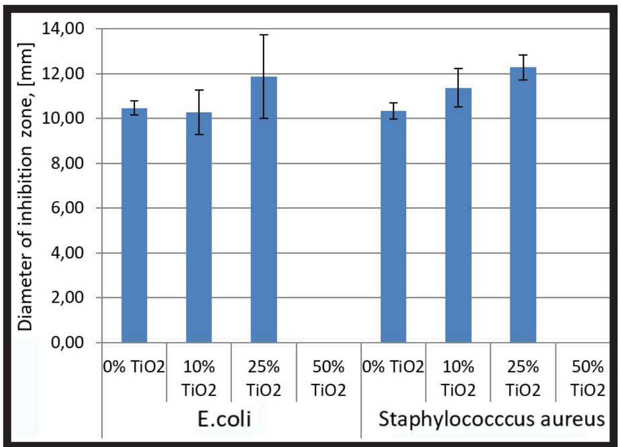

FIG. 5. Influence of the addition of titanium dioxide on bactericidal activity of copper powder.

Conclusions

The tests were carried out on copper powders modified with the amorphous fraction of titanium dioxide at various concentrations: 10, 25 and 50%. The bacterial strain *E. coli* ATCC 11775 and *S. aureus* ATCC 6538P were used for microbiological tests. The research involved the determination of the impact of the modification on the inhibition zone of bacterial proliferation after 48 h of exposure and also determination of MIC (minimal inhibitory concentration) of an aqueous suspension of a mixture of Cu-TiO_2 powders in conditions of 37°C for 24 h (with shaking). The studies showed a positive effect of the modification primarily against reference bacteria strains (*E. coli* and *S. aureus*). In both tests, the results clearly indicate that the addition of titanium dioxide at a concentration of 10% and 25% is beneficial. It seems reasonable to develop an optimal concentration of modifying TiO_2 fraction for individual bacterial strains, in order to obtain sufficiently high-performance antibacterial properties.

Acknowledgements

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