

Antibacterial activity of porous anodized copper

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ABSTRACT

This study was carried out to synthesize 1D inorganic nanostructure using an electrochemical method without any template and additives. Copper foils were anodized in a KOH bath and were tested for their antibacterial performance. After anodizing, the obtained samples were characterized by scanning electron microscopy (SEM) and X-ray diffraction (XRD) to determine the corresponding morphology and crystal structure, respectively. Finally, the antibacterial activity of the samples against both *E. coli* and *S. aureus* was tested by agar diffusion test. The typical porous surfaces were realized in all samples. These micropores may be beneficial to cell attachment. The morphology of the anodized copper exhibited when the concentration of OH⁻ kept on going up, micropores and simultaneously nanoparticles were formed on the surface. By increasing the concentration of KOH, the water contact angle with anodized Cu foil varied within the range of 65.4 to 89.7°. Parent copper foil did not show antibiotic activity. The anodized copper exhibited acceptable antibacterial activities. The antibacterial action was the same for anodized copper at different concentration of OH⁻, which had nothing to do with the concentration of KOH electrolyte. The obtained results indicated that the porous copper could be employed to improve antibacterial activities of pure copper to meet the needs of bioactive surfaces.

Keywords: Antibacterial, Anodizing, Porous, Copper.

1. Introduction

In late years, one-dimensional nanomaterials have gained a lot of attention. These nanostructured materials not only have theoretical importance in physical phenomena related to quantum confinement, but also provide a strong potential application in e.g. electro-chromic devices, optical switching, solar cells, heterogeneous catalysis, gas sensing, field emission, and Li-ion batteries [1-6].

The nanoparticles of metals such as copper, silver and zinc are known for their excellent antibacterial activity [7-10]. Among these nanoparticles, Copper is widely known for its excellent antibacterial properties against bacteria, fungus, and algae. Besides, it is well established that Cu is one of the essential elements in living organisms and plays diverse roles in human health. Thus, different

coating technologies have been adopted to prepare Cu-containing antibacterial surfaces over the recent years[10-12].

Recently, the copper oxide nanostructure has attracted significant attention. The rising interest in copper nanostructures is due to various reasons such as abundance, chemical stability and non-toxicity [2]. Various synthesis methods have been researched for the preparation of copper nanostructures, such as hydrothermal, thermal oxidation, sol-gel, wet-chemical, electrochemical deposition, sputtering, thermal evaporation, thermal relaxation and electrochemical synthesis [13, 14]. Therefore, the synthesis of copper oxide nanostructures demands complex process control, high reaction temperatures, long reaction times, expensive chemicals, and a specific method for

specific nanostructures. Among these methods, electrochemical synthesis is favored by many researchers due to its simplicity, low-temperature operation process, viability of commercial production, flexibility and relatively low cost [15-17].

The photocatalytic activity of anodized copper has been widely studied and reported [2, 3]. The antibacterial application of Cu-contained materials has also been investigated in detail [8, 11, 14]. As far as this research is concerned, this is the first attempt to synthesize 1D antibacterial nanostructures using an electrochemical method without any template and additives. Wu et al. prepared TiO₂ coatings with different concentrations of Cu by microaroxidation on pre-sputtered Cu-Ti films and studied their antibacterial effect. The porous Cu-TiO₂ coatings show excellent antibacterial activities to *E. coli* and *S. aureus* [18].

2. Materials and methods

2.1. Sample preparation

A copper foil (0.1 mm thick and purity >99.9%, Dirgodaz azar Co., Tabriz, Iran) was used as the working electrode (surface area 0.78 cm²). The counter electrode was a stainless steel rod (AISI 304, surface area 1.6 cm²). Before anodizing, the copper sheet was rinsed in distilled water and ethanol. The distance between the cathode and the anode was kept at 1 cm. The electrolysis cell was made up of Teflon. The electrolyte was an aqueous solution of 0.1, 0.3 and 0.5 M KOH (Merck Co.). The electrolysis was performed at 10 V without any potentiostatic control, for a period of 1 h, which was determined as the optimum anodizing time.

2.2. Characterization

The morphology of samples was observed with a scanning electron microscope (LEO 1430VP, Germany). X-ray diffraction (XRD) was carried out by Philips PW 1050 diffractometer (The Netherlands).

Static contact angles were measured with a handmade contact angle meter at room temperature. Water droplets of approximately 5 μL were dropped gently onto the surface of a sample. Three points of each sample were tested and the average value of the three left and right contact angles was calculated as the specified static contact angle [19, 20].

2.3. Antibacterial activity

The antibacterial activity of the samples against both *E. coli* (gram-negative) and *S. aureus* (gram-positive) was tested by agar diffusion test. Samples were exposed to bacteria in solid media (nutrient agar), and the inhibition zone around each sample was measured and recorded as the antibacterial effect. Agar plates were inoculated with 100 μL suspensions of bacteria. Then, anodized samples were placed on agar plates and incubated at 37 °C for 24 h. The inhibition zone for bacterial growth was detected visually.

3. Results and Discussion

3.1. Formation mechanism

First, the possible electrochemical reaction for the synthesis of CuOH will be presented under electrolysis conditions on the copper anode. The formation mechanism of CuO on Cu foil by anodizing is as follows: Cu was Electro-oxidized to Cu²⁺ (Eq. (1)), which compounds with OH⁻ in the electrolyte solution to form Cu(OH)₂ (Eq. (2)).



According to the reactions, at the initial phase, exterior copper lost electrons to form Cu²⁺ ions adsorbed on the copper surface. At the same time, OH⁻ ions in the solution moved to the anode side under the electric field force. Then the Cu²⁺ combined with OH⁻ to form Cu(OH)₂ nanoparticles on the substrate surface. During this process, highly crystalline Cu(OH)₂ nanoparticles was first deposited onto the copper substrate [21].

3.2. Morphology

Using the SEM, the morphology and microstructures of the particles obtained at different concentration of KOH have been investigated. Fig. 1 shows the typical SEMs of the film formed by anodizing a copper foil at a constant voltage of 10 V for 1 hr. It is observed that the concentration of KOH in the anodizing cell during the electrolysis process influenced on the surface structure and the amount and size of holes. The appearance of the anodized sample indicated that the surface of Cu foil was covered with a kind of blue substance. The top SEM images of such as-prepared Cu foil after anodizing confirmed that this blue substance was well separated and homogeneous nano and micropores [22]. The typical porous surfaces can

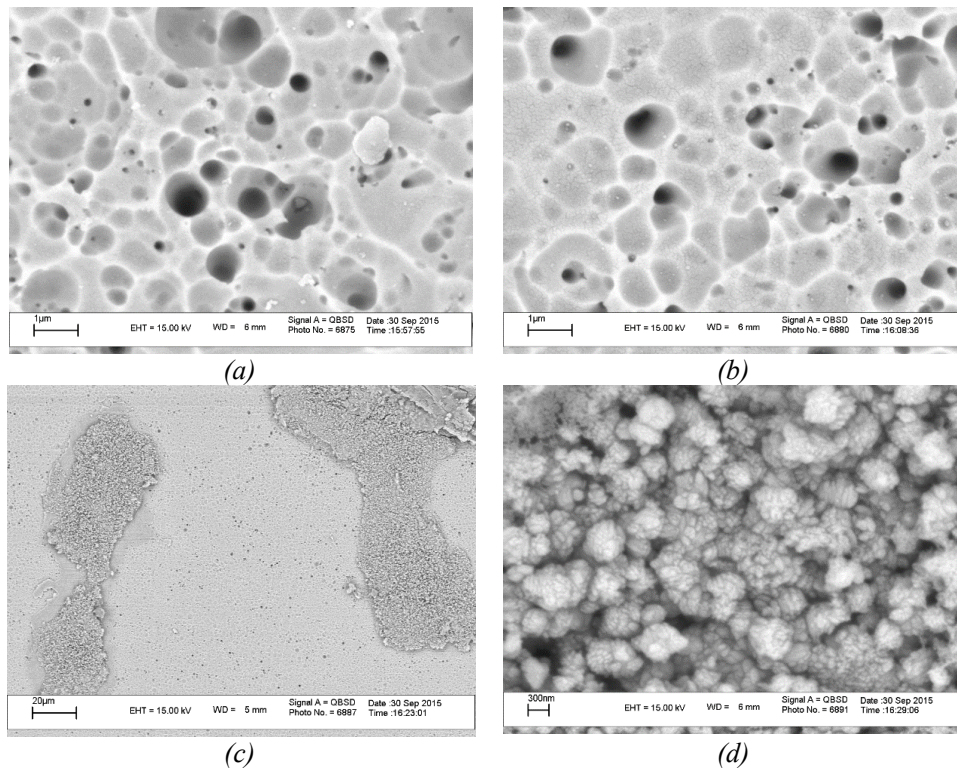


Fig. 1- SEMs of anodized copper foil by using 0.1 (a), 0.3 (b) and 0.5 M (c) and (d) KOH as electrolyte at a constant voltage of 10 V for 1 hr.

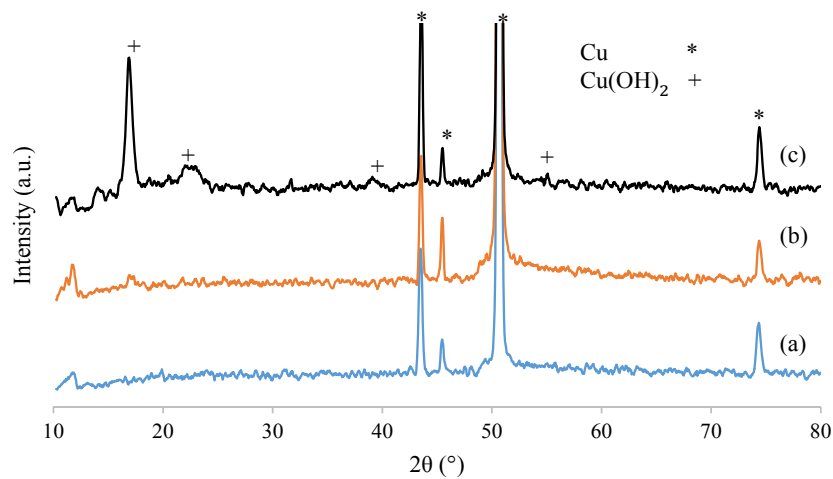


Fig. 2- XRD patterns of anodized copper foil by using 0.1 (a), 0.3 (b) and 0.5 M (c) and KOH as electrolyte at a constant voltage of 10 V for 1 hr.

Table 1- Water contact angle of anodized copper

Concentration of KOH (M)	Contact angle (°)
0	87.3 ± 2.3
0.1	65.4 ± 1.7
0.3	75.4 ± 4.5
0.5	89.7 ± 2.3

be realized in all samples. These micropores may be beneficial to cell attachment [18]. When the concentrate of OH⁻ kept on going up, more pores and simultaneously nanoparticles form on the surface (Fig. 1-d).

3.3. XRD patterns of CuO

Figure 2 illustrates the XRD patterns of films grown on Cu substrate and made in different KOH concentration at 25 °C and under constant voltage. The presence of peaks of bulk Cu indicates that the structure of bulk Cu is preserved in these structures [12]. Diffraction peaks were observed at 16.6°, 23.8°, 39.8° and 53.2°, and could be assigned to the (020), (021), (002), (130) and (150) planes of orthorhombic Cu(OH)₂, which confirmed a high degree of crystallinity, similar to the previous literature [14, 17, 23]. Except for the diffraction peak of Cu substrate, no other characteristic peaks of impurities were detected in the XRD pattern, indicating that the grown films only contain pure Cu(OH)₂. Anodizing Cu in dilute KOH solutions resulted in continuous dissolution of the metal with nothing formed at the copper foil surface[17].

3.4. Contact angle

Water contact angle test result confirmed that the surface of pure Cu foil was hydrophilic with an angle of 87.3 ± 2.3° (Table 1). By increasing the KOH concentration, the water contact angle with anodized Cu foil varied from 65.4 to 89.7°, which supported the change in the anodized Cu foil surface structure and composition [22].

The more hydrophilicity of anodized sample in low-concentration KOH electrolyte may be due to the strong cohesive force between the water droplet and hydroxide present in the copper oxide compound. The increased contact angle are higher concentration of OH⁻ may be due to increasing the roughness of the surface [5-6]. Hydrophilicity is attributed to nanocrystalline nature that is expected to possess a very high surface energy. Nanocrystalline material with hydrophilic nature is one of the prime requirements for antibacterial material [7].

3.5. Antibacterial properties

The antimicrobial properties of samples were investigated with Gram-negative *E. coli* and Gram-

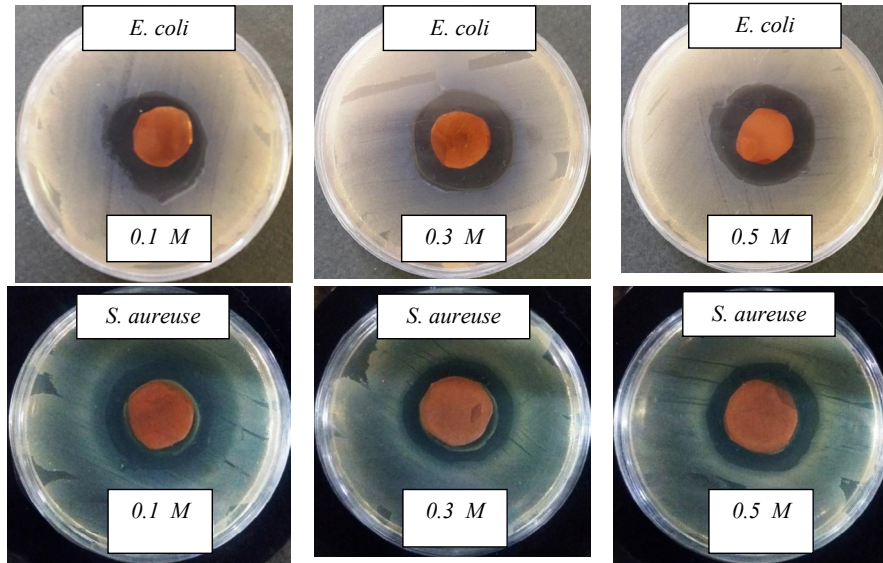


Fig. 3- Antibacterial properties of anodized copper foil by using 0.1 (a), 0.3 (b) and 0.5 M (c) KOH against *E. coli* and 0.1 (d), 0.3 (e) and 0.5 M (f) against *S. aureus*.

Table 2- Antibacterial activity of anodized copper

Concentration of KOH (M)	Inhibition zones (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
0 (Copper foil)	0	0
0.1	15.1	13.6
0.3	14.9	13.4
0.5	14.8	13.0

positive *S. aureus* at 37°C for 24 h (Figure 3). Parent copper foil did not show the antibiotic activity. Interestingly, all of the anodized samples presented antibacterial activities (Table 2). A number of studies have already dealt with the kinetics of contact killing upon bacteria exposure to copper and copper alloy surfaces. The mechanism of contact killing cannot yet be answered clearly, but a number of factors contributing to contact killing have been identified: (A) Copper dissolves from the copper surface and causes cell damage. (B) The cell membrane ruptures because of copper and other stress phenomena, leading to loss of membrane potential and cytoplasmic content. (C) Copper ions induce the generation of reactive oxygen species, which causes further cell damage. (D) Genomic and plasmid DNA becomes degraded.^[25] Based on the SEM images, it was confirmed that the surface of anodized copper was covered with separated and homogeneous nano and micropores. This porous structure provides more surface area for each of the mentioned mechanisms of contact killing. Thus, after anodizing of the copper foils, their antibacterial activities were enhanced. According to Fig. 3, the antibacterial activities of samples against *S. aureus* seems less efficient than *E. coli*. CuO nanostructures exhibit improved antibacterial activity for gram-negative bacteria when compared to gram-positive bacteria. The difference in activity against these two types of bacteria could be attributed to the cell wall structure, cell physiology, metabolism or degree of contact. The cell wall of the gram negative bacteria is composed of a thin layer of peptidoglycan, whereas gram-positive bacteria are composed of a thick layer of peptidoglycan, consisting of linear polysaccharide chains cross-linked by short peptides thus forming more rigid structures [18, 24].

Conclusions

In this paper, the possibility of using an anodized copper as an antibacterial surface was studied. Accordingly, three anodized samples in different concentration of KOH electrolyte (0.1, 0.3 and 0.5 M) were prepared. The morphology of the anodized copper exhibited that when the concentration of KOH electrolyte kept on going up, micropores and nanoparticles form on the surface. Furthermore, by increasing the concentration of KOH, the water contact angle of anodized Cu foil varied within the range of 65.4 to 89.7°. According to antibacterial test results, parent copper foil did not show the

antibiotic activity. While, all of the anodized copper foils show antibacterial activities against both *E. coli* and *S. aureus*. Therefore, the obtained results indicated that the anodized copper could be employed to improve antibacterial activities of pure copper to meet the needs of bioactive surfaces.

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