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Fabrication of Titanium – Copper Alloy and Study the Antibacterial Activity

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Abstract

The composition of the phases and the microscopic structure of Ti-X% Cu (0, 0.5, 2.5, 5)% prepared alloys was investigated by scanning electron microscope (SEM), and the antibacterial activity was assessed to investigate the effect of the Cu content on the antibacterial activity with or without coating with sliver nanoparticles and graphene nanosheets. The alloys were coated using pulse laser deposition. The alloys of Ti-X%Cu were prepared by the metallurgy process. Finally, the thermal conductivity was studied to determine the effect of increasing the copper content and the coating with nanoparticles. Electron microscopy (SEM) of the alloys before coating showed that the pure titanium is homogeneously distributed and the (Ti-0.5%Cu, Ti-2.5%Cu, and Ti-5%Cu) there are precipitates of (200–800) nm while in scanning electron microscopy (SEM) images of the coated alloys the silver nanoparticles were distributed (20-26) nm, and graphene oxide was found to be less than (100) nm. The rate of inhibition of bacterial growth increases with increasing concentration copper and with the presence of coatings, and the best inhibition ratio for the sample was (Ti-5%Cu with AgNPs/rGONPs). On the other hand, thermal conductivity of the alloys after coating is less than it was in the uncoated alloys.

Keyword: Antibacterial, *Streptococcus mutans*, Thermal conductivity, Titanium alloys.

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تصنيع سبائك (تيتانيوم-نحاس) ودراسة فعاليتها للمضادات البكتيرية

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الخلاصة

في البحث الحالي, تم فحص تكوين الأطوار والهيكل المجهري لسبائك التيتانيوم المخلوط مع نسب مختلفة من النحاس (0, 0.5, 2.5, 5)% بواسطة المجهر الالكتروني الماسح (SEM) وتم تقييم النشاط المضاد للبكتيريا من أجل التحقيق في تأثير محتوى النحاس على نشاط مضاد للجراثيم مع او بدون الطلاء بجسيمات الفضة النانوية مع الواح الجرافين النانوية. تمت عملية الطلاء للسبائك بواسطة ترسيب الليزر النبضي (PLD), سبائك (Ti-X%Cu) تم تحضيرها بواسطة عملية التعدين. اخيراً تمت دراسة التوصيلية الحرارية للسبائك قبل وبعد الطلاء لتظهر تأثير زيادة محتوى النحاس وطلاء الجسيمات النانوية. اظهرت نتائج الفحص المجهري الالكتروني (SEM) للسبائك قبل الطلاء بأن سبيكة التيتانيوم النقي (Ti-0%Cu) موزع بشكل متجانس. وبالنسبة للسبائك (Ti-0.5%Cu)، (Ti-2.5%Cu) و (Ti-5%Cu) توجد فيها رواسب بعرض (200-800 nm) مشتتة بدقة في المصفوفة. اما بالنسبة للسبائك بعد الطلاء فأظهرت صور (SEM) بأن جزيئات الفضة النانوية موزعة بمدى (20-26 nm) مع أكسيد الجرافين بمدى اقل من (100 nm). يزداد معدل تثبيط نمو البكتيريا مع زيادة تركيز النحاس ووجود الطلاءات ، وكانت أفضل نسبة تثبيط للعينة هي (Ti-5٪Cu مع AgNPs / rGONPs). من ناحية أخرى ، الموصلية الحرارية للسبائك بعد الطلاء أقل مما كانت عليه في السبائك غير المطلية.

كلمات مفتاحية: مضاد للجراثيم, التوصيل الحراري ، المكورات العقدية الطافرة, سبائك التيتانيوم.

Introduction

Purified titanium (Ti) for commercial use is quickly becoming the most popular alternative for replacing lost teeth and currently holds the biggest market share in the oral metal materials and implants sector. Over 500 different species of bacteria may be found in an individual's oral cavity. These bacteria can rapidly colonize various surfaces, such as teeth, prosthetic devices, and dental implants [1,2]. Peri-implant diseases, such as peri-implant mucositis or peri-implantitis, can occur as a result of the formation and maturation of biofilms on the surfaces of dental implant materials. These diseases can have a negative impact on the long-term success of teeth implants [3,4]. Therefore, bacteria should be effectively killed, and the formation of biofilms on the surfaces of implants should be discouraged to avoid implants from failing due to bacterial infection and prevent further infections from occurring [5-7]. Despite the numerous preventative steps that have been taken, such as exhaustive disinfection and tight adherence to aseptic surgical protocols, patients can still get an infection days after surgical procedures. This phenomenon is often described as the colonization of microorganisms and production of biofilms on oral surfaces, including dental implants. The first bacteria to colonize a tooth surface recognize salivary pellicle receptors, which is the first step in the process that leads to bacterial attachment to the enamel and dentine of the tooth. Extracellular polymeric substance originates from bacterial surfaces, the salivary pellicle provides binding sites for microorganisms, and then the exopolysaccharide in the biofilm promotes biofilm stability and structural integrity. These processes occur on the surface of bacteria. Thus, biofilms can shield the bacteria from harm. *Streptococcus mutans* is one of the main species found in the biofilms that form on oral implants. This species has a marked influence on biofilm formation, inflammatory response, and any bone abnormalities that may exist [8-10]. On the basis of the above studies, an appropriate amount of Cu was immobilized into pure titanium to fabricate a Ti-Cu alloy to provide antibacterial properties for use as a dental material. In the present study, the antibacterial properties of the new implant material and the related antibacterial mechanism were investigated, and the alloy was prepared through characterization by scanning electron microscope (SEM) and thermal conductivity. Alloys were made with the powder metallurgy technique. Powder metallurgy (PM) is a production technique in which powdered metals are compacted with other materials and then heated without a melting step to solidify and harden . The microstructures and compositions that PM generates are ideal for making net-shaped goods . Mixing the powder, compacting the powder in the required mold, then sintering, is what is known as the standard technique or the "pressing and sintering process" [11,12]. Pulsed laser deposition (PLD) is a state-of-the-art thin film deposition technique. In order to create layers of different materials that may be processed into a pellet target, PLD is one of the most versatile methods. The ability to preserve the stoichiometry of the ablated target in the deposited layer is a key characteristic of this method [13,14].

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Material and Method

Material

99.995%, natural graphite rod, $KMnO_2$99%, $NaNO_3$ 99.5%, H_2O_2, 32%, HCl 37.5%, (H_2SO_4, 98%) from (LOBA Chemie), NaOH (99%) from Dae-Jung, titanium (99%) from Fluka chemika, copper (99.5%) from chem – lab, Silver Nanoparticles/Nano powder (Ag, 99.95%, 20~30nm), dark grey powder, spherical and true density (10.5 g/cm). sky spring nano materials and eucalyptus' leaves.

Experimental Work

1. **Preparation Ti – Cu Alloy by Metallurgy Method**

The materials were pressed at 600 Map. After the pressing process, we obtained alloys with a diameter of 1.5 cm and a thickness of 1 mm, and the alloys were burned in a heating furnace at (1250 °C) for (2 h).

2. **Coating of Ti – Cu Alloy by Graphene Oxide Nanosheet and Sliver Nanoparticles**

The target material was synthesized using 0.12 g of silver nanoparticles with 1.2 g of reduced graphene oxide by eucalyptus leaves liquid, and the target dimension after the pressing process was as follows: 0.5 cm in diameter and 1 mm in thickness. The experimental setup for the PLD of a solid metal target is shown in the figure. To achieve a high laser fluence, the Nd: YAG laser device system was used, and the HUAFEI type provides pulses of 1064 nm or a 532 nm Q-switched wavelength with an energy per pulse of 400 mJ, 200 pulses, a repetition rate of 1 Hz, a convex lens of 100 mm focal length.

3. **Antibacterial Activity AgNPs/rGONPs**

The antibacterial potential of the prepared samples (i.e., Ti-0%Cu with AgNPs/rGONPs (A1), Ti-0%Cu (B1), Ti-0.5%Cu with AgNPs/rGONPs (A2), Ti -0.5% Cu (B2), Ti-2.5%Cu with AgNPs/rGONPs (A3), Ti -2.5% Cu (B3), Ti-5%Cu with AgNPs/rGONPs (A4), and Ti -5% Cu B4) was investigated against streptococcus mutans bacterial strains using agar well diffusion assay [7,8]. About (20 mL) of Muller–Hinton agar was aseptically poured into sterile Petri dishes. The bacterial species were collected from their stock cultures using a sterile wire loop [9]. After culturing the organisms, 6 mm diameter wells were bored on the agar plates using of a sterile tip.Different concentrations of the samples were placed in the bored wells. The cultured plates containing the samples and the test organisms were incubated overnight at (37 °C) before measuring and recording the average inhibition diameter of the zones [10].

4. Statistical Analysis

The data were statically analyzed using the Graphpad prism program. The data are represented as the mean ± SD of three experiments, $p<0.05$ indicates a statistical significant difference [15].

5. Thermal Conductivity

Lee's disk method was used to measure the thermal conductivity. The device consists of three disks (i.e., A, B, and C), which are heated electrically by a (DC) power supply. The sample was installed between the disks (A and B),, and the two disks (B and C) were heated for 10 min to start the heat transfer from disk B to disk A through the sample. Then, the temperatures of the three disks (i.e., TA, TB, and TC) were recorded using a thermometer. The value of thermal conductivity k can be obtained by applying Equation (1):

K[(TB -TA/ds]= e[TA+2/r (dA+ds/4) TA+dsTB)/2r] …(1)

Results and Discussion

1. Morphology Properties of Titanium – Copper Alloy Coating by (AgNPs /rGONPs)

In the study of the microstructure evolutions and identification of phases, two typical microstructures of Ti-Cu alloy were observed by SEM. Figure (1-A) shows the SEM images of Ti-0%Cu, Ti-0.5%Cu, Ti-2.5%Cu, and Ti-5%Cu . For the Ti-0%Cu sample, the SEM photo shows that pure titanium is homogeneously distributed. Meanwhile, the Ti-0.5%Cu, Ti-2.5%Cu, and Ti-5%Cu in the sample are 200–800 nm wide precipitates that are finely dispersed in the matrix, which proved to be the Ti_2Cu phase by EDS analysis.

Furthermore, the SEM images mainly show the lamellar secondary pre-capitated α-Ti phase separated by aligned Ti2Cu particles [16-20]. Figure (1-B) shows the SEM images of Ti-0%Cu, Ti-0.5%Cu, Ti-2.5%Cu, and Ti-5%Cu with the PLD target (AgNPs/rGONPs). The SEM images show the distributed AgNPs ranging from 20 nm to 26 nm, with rGONPs less than 100 nm.

2. Anti- Bacterial Activity

The antibacterial of Ti-X%Cu (0, 0.5, 2.5, 5)% was analyzed using *streptococcus mutans*. The inhibition zones after the organism's exposure to different concentrations of Ti-X%Cu (0, 0.5, 2.5, 5)% were measured and are illustrated in Figures 2, 3, 4, and 5. The result shows that Ti-5%Cu more effective than Ti-X%Cu (0, 0.5, 2.5)%, and the results concentration dependent. The zone of inhibition produced has a diameter of approximately (19–27) mm streptococcus mutans, and the results indicate that the effect of the nanoparticles depend on the concentration. The micro-organisms demonstrated resistance to external agents because of their outer membrane that has a bacterial structure. The Ti-X%Cu (0, 0.5, 2.5)% effect on the growth of the organisms was time dependent, with more growth noticeable after 12 h of treatment as shown in Figures 2, 3, 4, and 5. Taken together, the results indicate that Ti-5%Cu has more inhibitory effect than Ti-X%Cu (0, 0.5, 2.5)%, as proven by the statistical analysis.

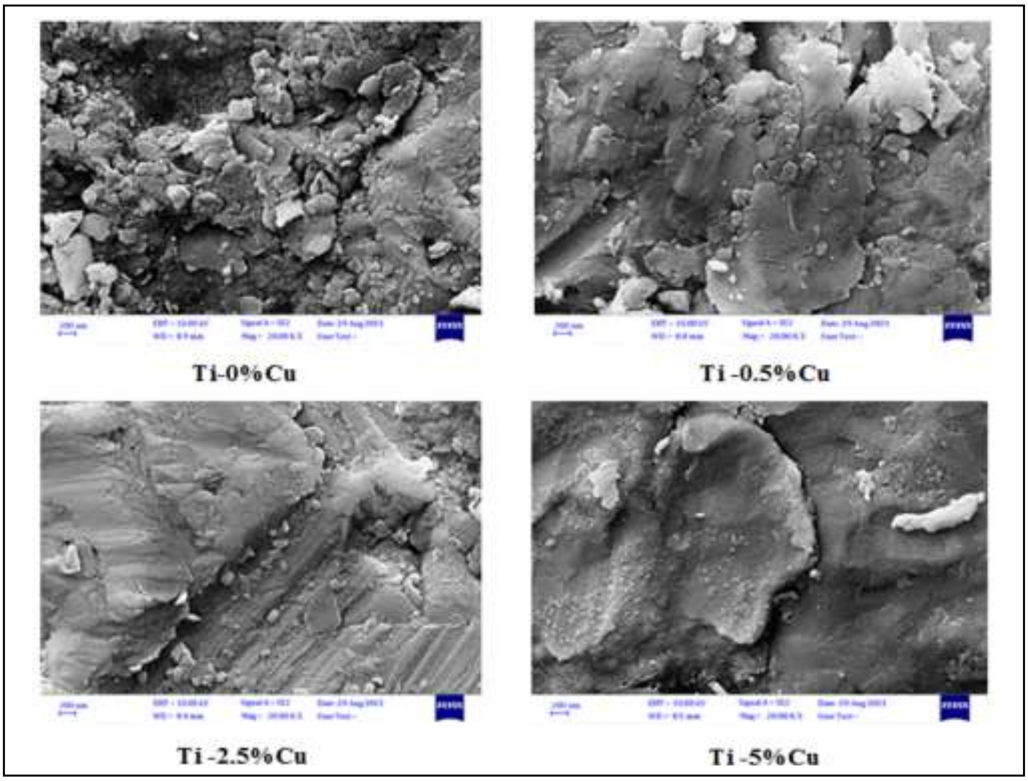

Figure 1-A: SEM images of prepared Alloy.

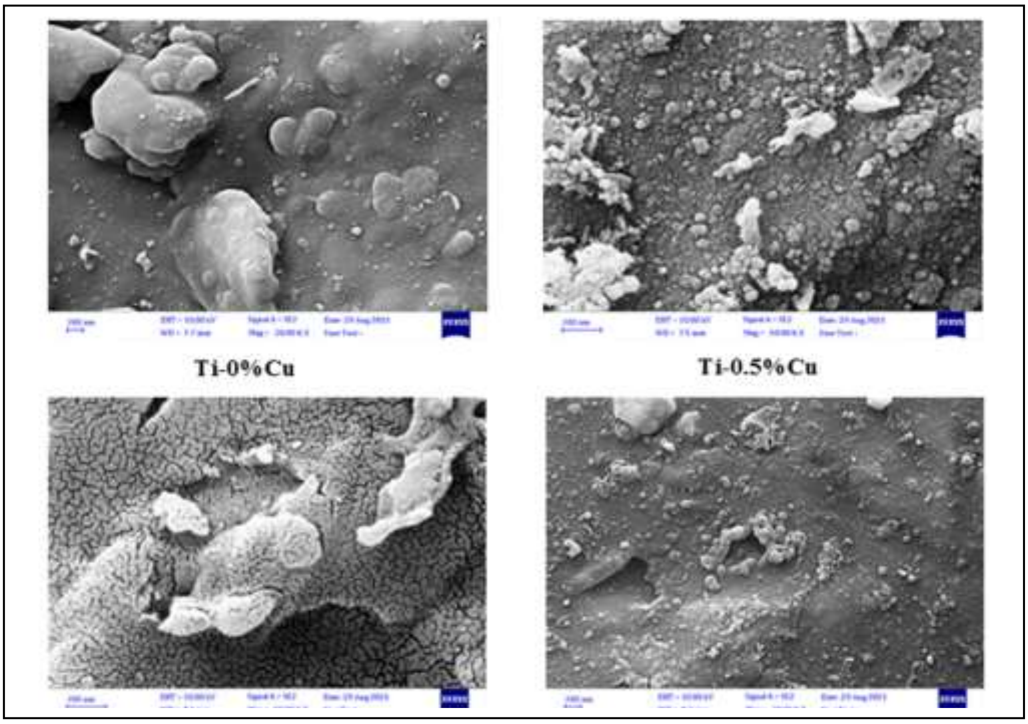

Figure 1-B: SEM image of Prepared Alloy After PLD.

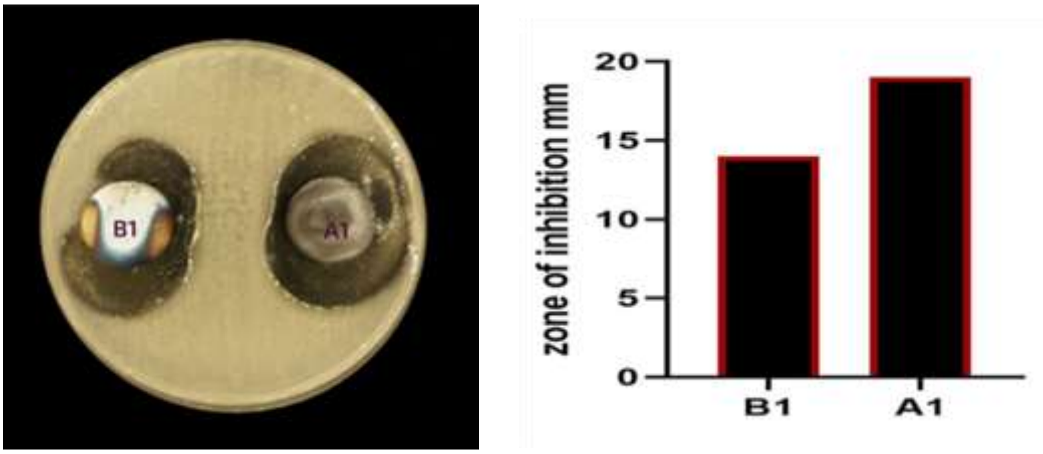

Figure 2: Antibacterial activity of (A1(Ti-0%Cu coated), B1 Ti-0%Cu) against *streptococcus mutans*

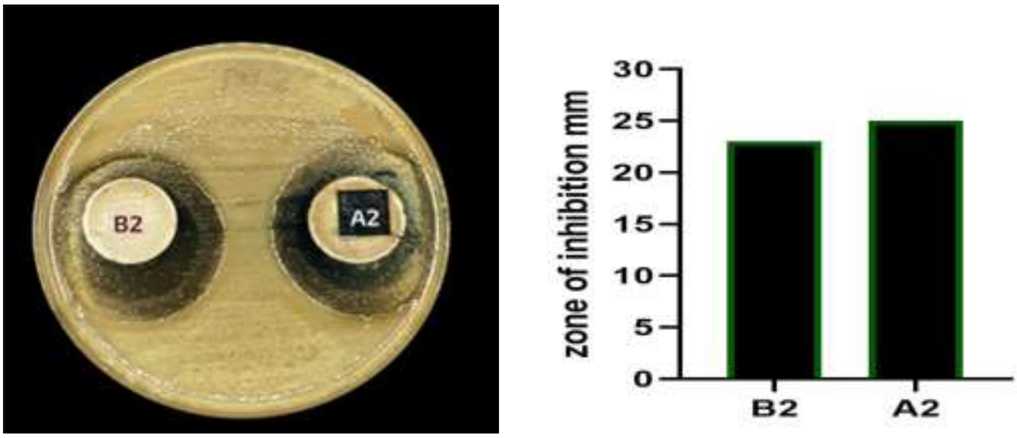

Figure 3: Antibacterial activity of (A2(Ti-0.5%Cu coated), B2 Ti-0.5%Cu) against *streptococcus mutans*.

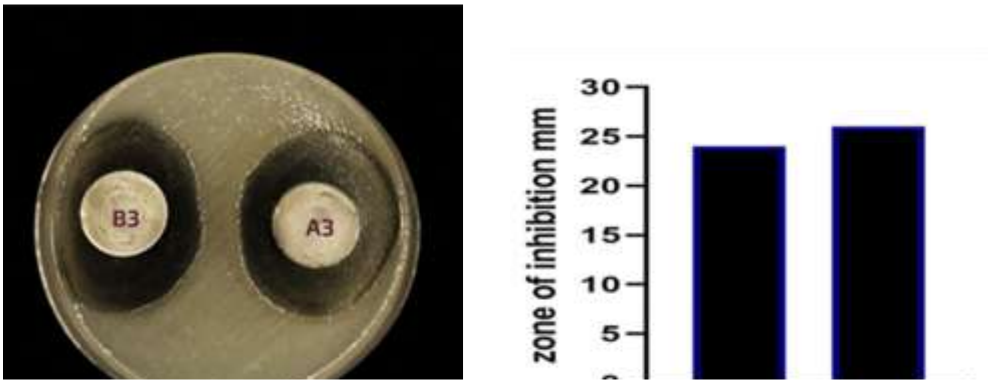

Figure 4: Antibacterial activity of (A3(Ti-2.5%Cu coated), B3 Ti-2.5%Cu) against *streptococcus mutans*.

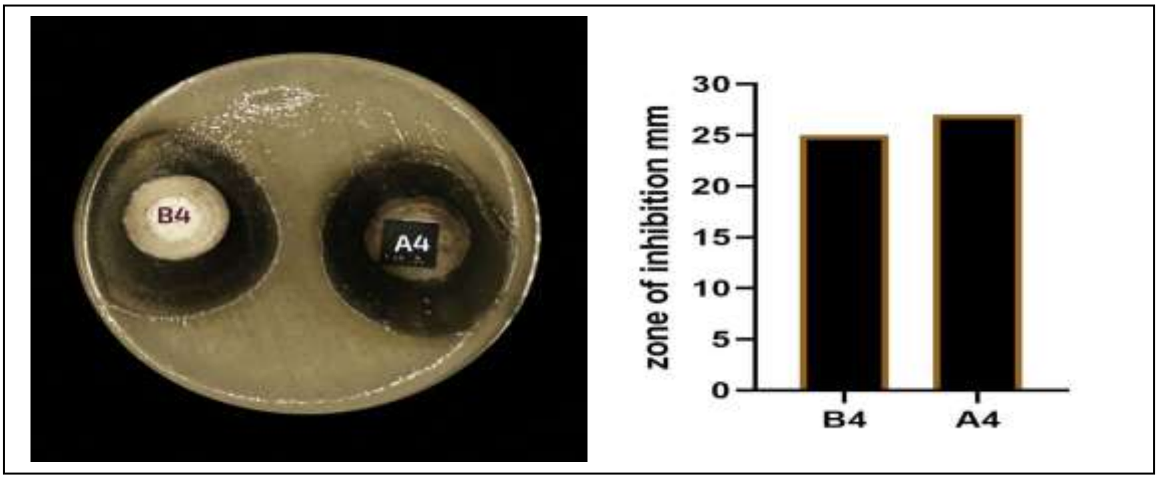

Figure 5: Antibacterial activity of (A4(Ti-5%Cu coated), B4 Ti-5%Cu) against *Streptococcus mutans*

3. Thermal Conductivity

Figure (6) shows that the thermal conductivity increased with the copper content, but the thermal conductivity decreased with AgNPs/GONPs coating.

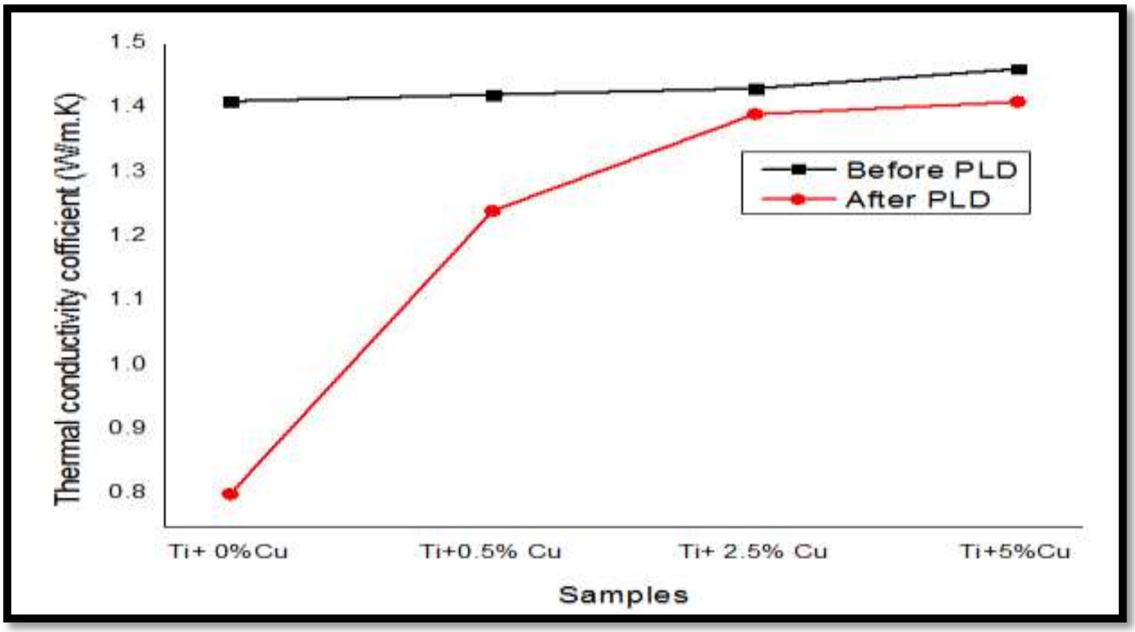

Figure 6: Thermal conductivity of prepared samples before and after PLD.

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Conclusions

The α-Ti, Ti2Cu and Ti2Ag phases are illustrated by the SEM images. After the PLD process, all the samples were coated with a thin layer of AgNPs/ rGONPs, as shown in the images. The anti-bacterial activity increased with the copper content, and the best results were recorded for the coated Ti-5%Cu alloy. The thermal conductivity also increased with the copper content but decreased with coating.

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