Surface Modification of Silicone Rubber by Ion Beam Assisted Deposition (IBAD) for Improved Biocompatibility

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ABSTRACT: We studied the preparation of antimicrobial silicone rubbers of improved interfacial strength, which could be formed with the ion beam assisted deposition (IBAD) technique for coating metallic or inorganic materials (silver (Ag), Copper (Cu), and Hydroxyapatite(HAp)/TiO_2) on the silicone surface. Those coating materials provide high product safety as well as outstanding antimicrobial activity. The deposition methodology is composed of pre-etching with oxygen gas, vaporizing the coating materials, and post-treatment with Ar ion. With the evaporation of the coating materials, the Ar beam was focused on the substrate to assist deposition. It was found out that the ion assisting depositions in the IBAD process give a prominent enhancement in adhesion between silicone rubbers and coatings of Ag and Cu. The HAp/TiO_2 coating layer was easily dissolved in aqueous saline solution. All deposited layers display high antimicrobial activities against *Staphlococcus aureus* (ATCC 6538) and *Escherichia coil* (ATCC 25,922), showing 99.9% reduction of bacteria, respectively. In a cytotoxicity test, the Ag and HAp/TiO_2 coated silicone shows a decrease of cytotoxicity, while the Cu coating leads to a slight increase of cytotoxicity. The result on the surface modifications of silicone rubber will be employed in further study for applications of medical or rehabilitation devices. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 1095–1101, 2005

Key words: silicone rubber; ion beam assisted deposition (IBAD); biocompatibility; surface coating; antimicrobial activity

INTRODUCTION

Silicone rubber is one of the most widely used elastomers in rehabilitation devices as well as biomedical implants, because it has excellent mechanical properties and relatively good biocompatibility. However, it tends to be tacky on the surface and prone to biofouling, which is considered to be a major cause of discomfort and even clinical failure in many applications. The increase in use of silicone rubber has naturally revealed many related complications, one of which is primarily an infection problem. For wide applications, for example, from rehabilitation devices like socket liners to in-body implants including catheters, the associated infections can range from mild phlebitis at the contact site to septicemia and, in the worst case, fatality. A hypothesis regarding the pathogenesis of related infections is that bacterial colonization of the device first occurs at the skin infection,[1] and then the bacteria advance along the outer surface, thereby gaining access to the blood stream.[2–6]

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Many methods have been attempted to reduce the incidence of such infections, and have typically been aimed at disrupting the adhesion of skin commensals to the device at the cutaneous interface. One method is the use of antibiotics,[7] and several agents such as surfactants, antimicrobial agents, heparin, nonsteroidal anti-inflammatory agents, and silver formulations have been adopted for the purpose of preventing the adherence of bacteria.[2,3,7]

From the aspect of biomaterials, the previous studies on antibacterial silicone include a preparation of antibacterial silicone grafts dispersed with antimicrobial drugs such as rifampin, fusidic acid, and mupirocin.[8] Another is a synthesis of a strong cation exchanger based on a silicone polymer coated by silicas,[9] and a deodorant composition is prepared by blending antibacterial zeolite and silicones.[10] In this study, it was intended to prepare antimicrobial silicone rubber coated by silver (Ag), Copper (Cu), and Hydroxyapatite (HAp: $Ca_{10}(PO_4)_6(OH)_2$) / TiO_2. Those coated materials can provide high product safety as well as outstanding antimicrobial activity.

In the meantime, it has been considered to be quite difficult to make such a metal deposition on the surface of organic or inorganic materials. We introduced Ion Beam Assisted Deposition (IBAD) to deposit metallic or inorganic coatings on polymeric substrates and to improve the adhesion between the silicone substrate and coating layers. Woodyard et al. has reported the effect of silver-treated catheters treated by IBAD.[1] IBAD is one of the most efficient techniques to deposit thin films and coatings, in which a thin layer is deposited on a substrate simultaneously during ion beam bombardment. IBAD is able to prepare biocoatings with highly increased adhesive strength to the substrate compared to conventional coating methods, for example, plasma spraying at low temperature, ion beam sputtering deposition (IBSD), and physical vapor deposition (PVD). It stems from the effect of attractive interactions occurring between the coating and substrate molecules leading to mixing and compatibility at the interface. It also gives the advantages of low temperature operation, high reliability, and reproducibility.[11,12]

In this study it is attempted to improve the adhesive strength between the silicone substrate and various antibacterial coating layers. The effects of ion beam power during the deposition were also studied. We investigated the antibacterial ability[13,14] and cytotoxicity[14–17] of the surface modified silicone rubber. We mainly focused on the surface modification of silicone rubber by IBAD; the subsequent results in applications for medical and rehabilitation devices will be presented in later publications.

EXPERIMENTS

Materials

The deposition targets, silver (Ag) and Copper (Cu), were obtained from CERAC Incorporated (USA). And HAp (hydroxyapatite : $Ca_{10}(PO_4)_6(OH)_2$)/TiO_2 weight ratio 50 : 50)[18] was made by the ball mill method. Substrates were silicone rubber (Dow Corning Co.), slide glass (Matsunami Glass Ind., Japan), and silicon wafers (Metatec Co. Ltd., Korea).

Coating procedure

Thin silicone rubber layers were deposited on the metal substrate by utilizing an IBAD technique (Fig. 1). Using a Cryopump (OB-10, Helix Technology, Mansfield, MA, USA), the chamber was evacuated to a pressure of 10^{-7}Torr. Subsequently, Ar gas (P = 10^{-4}Torr) was introduced to the chamber. Before evaporating the materials, we treated the O_2, N_2, and Ar bombardment on the surface of the silicone rubber for 30min to increase bonding strength between the surface of the silicone rubber and the coating layer. While an electron beam (Telemark, Fremont, CA, USA) at 8.5kV and about 0.1A was evaporating the target, the end-hall type ion gun (Mark II, Commonwealth Scientific, Alexandria, VA, USA) was applied to the substrate surface to assist the deposition. The voltage was fixed at 120V, and the current level was gradually increased up to 0.6A. The evaporation rate was set to 1.0Å/s, and the substrate was rotated at 7.5 rpm during deposition to improve coating layer uniformity. After evaporating the materials, we treated an Ar ion bombardment for 10 min to make denser coating layers.

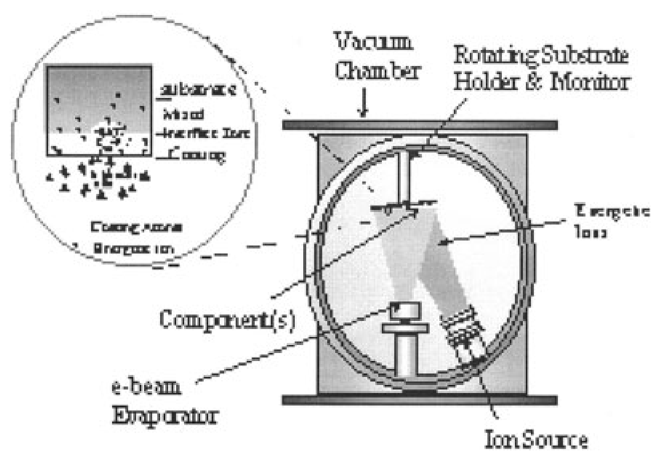

Figure 1 Schematic representation of Ion Beam Assisted Deposition (IBAD).

Characterization

The deposition layers were analyzed by X-ray diffractometer (XRD, RINI-D/MAX 2000, Rigaku Inc., Japan) and energy dispersion spectroscopy (EDS, Oxford Instruments, Bucks, England). The morphology of the sample was observed by scanning electron microscopy (SEM, JSM-5310, Jeol, Tokyo, Japan). We measured the solubility of the coating layers in distilled water by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, Vista-Pro AX, Varian Inc.). The water contact angle of the silicone surface pre-etched with several gases was measured using a contact angle meter (G-1, ERMA Inc., Tokyo, Japan). About 1 μL of distilled water was dropped on the silicone surface, and the averages were calculated from 5 measurements, respectively. The bond strength of the pre-etched silicone surface with epoxy was measured using an adhesion testing apparatus (Sebastian V, Quad Group, Spokane, WA, USA). A stud precoated by the manufacturer using an epoxy of a proprietary composition was adhered to the coating layer by curing the epoxy at 150°C for 1 h. The stud, with a diameter of 3.6 mm, was pulled with a loading rate of 4.5 mm/min until the failure of the coating layer, and the bond strength was determined from the maximum load recorded. Care was taken to minimize the effect of epoxy penetration and nonuniform failure of the coating layer. At least 10 measurements were made for each experiment. Antimicrobial activity of the coated silicone rubber was evaluated by testing methods for antimicrobial activity of polymeric materials (modified from KS M 0012). In this method, we used *Staph-*

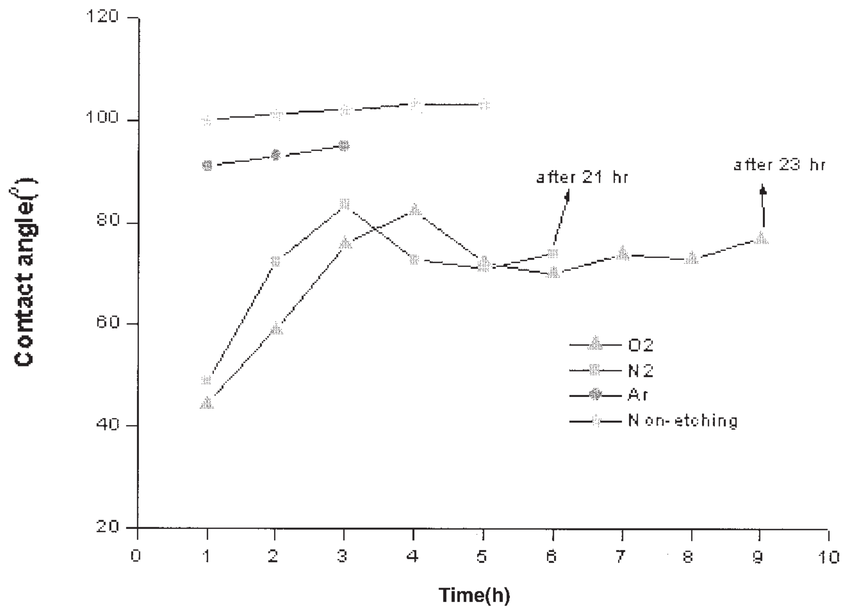

Figure 2 Change of water contact angle according to lapse period after the silicones were pre-etched with ion-gun assisting gases. The gases used are presented on the figure. (The pre-etching condition of IBAD is 2.0A/120V/30min.)

ylococcus aureus (*S. aureus*, ATCC 6538) and *Escherichia coli* (*E. coli*, ATCC 25,922) for test bacteria. The *S. aureus* and *E. coli* cultures were grown in the nutrient broth medium at 37 ± 1°C for 24 ~ 48 h. These were transferred to another flask containing 20 mL of fresh sterile medium and shaken at 37 ± 1°C for 18 ~ 24 h. The 0.4 mL of this suspension was adjusted to a concentration of $1 \sim 50 \pm 0.3 \times 10^5$ ea/mL and poured on the surfaces of the coated silicone rubber. After cultivating bacteria, we extracted them from the surfaces of the coated silicone rubber with saline solution. The results were expressed as reduction in bacteria (%) by the following formula:

$$Reduction\ in\ bacteria\ (\%) = [(M_b - M_c)/M_b] \times 100$$

where M_b is the number of bacteria recovered from the control and M_c is the number of bacteria recovered from the test specimen.

Cytotoxicity was analyzed using the SRB (Sulforhodamine B) assay. The specimen was prepared by cleaning with distilled water and ethanol. The NIH 3T3 fibroblasts were cultured in DMEM (Dulbecco's modified Eagle's medium, DMEM containing 100 units of penicillin and streptomycin, and 5% fetal bovine serum) at 37 ± 1°C for 24 h, and were cultured for 24 h after dipping the specimen in DMEM.

RESULTS AND DISCUSSION

Prior to depositing coating materials, the silicone surface was treated by pre-etching with different gases such as oxygen, nitrogen, and Ar, in order not only to clean the surface but also to increase the adhesive strength and hydrophilicity of the silicone surface. Figure 2 shows the changes of the water contact angle according to the lapse period after the silicones were pre-etched with ion-gun assisting gases for 30 min. For the nonetched surface, the contact angle is slightly above 100°, while the trend after pre-etching was that the contact angle after pre-etching decreases overall, depending on the ion-gun assisting gases.

The Ar pre-etching led to decrease of the contact angle to approximately 90°. The nonreactive Ar etching changes only the topology of the surface from ion bombardments. Subsequently, the contact angle does not change basically with the lapse time at least up to 3 h. The nitrogen and oxygen as ion-gun assisting gases lead to different effects, in which the silicate bonds existing primarily on the surface come to retain more hydrophilicity by the bond scissions and surface reactions between the bonds and bombarded gases. For both gases of oxygen and nitrogen, the contact angles were very low at the initial stage of exposition to the air, that is, just after the pre-etching was carried out. This reflects that chemical reactions occur between the substrates and the reactive gases and that they increase the hydrophilicity of the surface of the silicone rubber, which is considered to become unstable thermodynamically. Then, it regains some degree of hydrophobicity because hydrophilic polymeric chains migrate into the bulk of the silicone rubber for as many as 3 to 4 h. After that period, the contact angle does not change significantly over 20 h. The resulting contact angles are around 70° for both gases. Oxygen shows a slightly better etching effect than nitrogen.

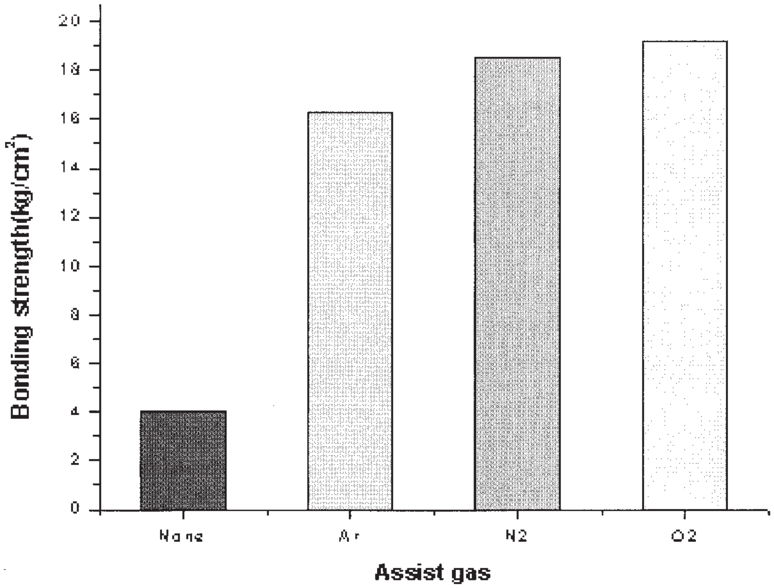

Figure 3 Bonding strength between epoxy and silicone substrate after pre-etching using different ion-gun assisting gases. (The pre-etching condition of IBAD is 2.0A/120V/30 min.)

The subsequent changes of bond strength with epoxy after pre-etching are shown in Figure 3. With a similar trend as shown in contact angle, the oxygen pre-etched silicone surface shows the greatest bond strength, since the improved hydrophilicity increases the adhesion strength of the interface between the silicone surface and epoxy glue. The oxygen was chosen hereafter as the ion-gun assisting gas for pre-etching prior to the physical deposition of coating materials.

Each Ag, Cu, and HAp+TiO_2 could be deposited on the surface of silicone rubber by using the IBAD technique, by which Ar was employed as an ion beam assisting gas because of its nonreactive character with coating materials as well as the substrate. From our previous study, it was known that the level of ion beam power during deposition had significant effects on the deposited layer composition. So in this study, we fixed the voltage at 120V, and the current level was gradually increased up to 0.6A during Ag, Cu, and HAp+TiO_2 deposition. And we also examined the effect of ion beam intensity on the layer dissolution rate in aqueous solution. The thickness of all the coating layers was controlled to about 10,000Å.

From the dissolution concentration of the deposited layer in distilled water by the ICP-AES analysis (Table I), the dissolution rates of the Ag and Cu coating layer in distilled water increase with the immersed time of the samples up to 48 h. And the concentration (mg/L) of Ag in the water is smaller than that of Cu either after 24 and 48 h. It is supposed that the silver coating is denser than the copper coated layer because the specific gravity of silver is 10.5g/cm^3 and that of copper is 8.9 g/cm^3. However, the dissolution rate of the HAp+TiO_2 coating layer did not show significant difference with various immersion times ranging from 24 to 48 h. This is due to the fact that the HAp+TiO_2 coating layers were almost dissolved before 24 h. Choi recently demonstrated the HAp coating with the IBAD technique.[18] The authors argue that before heat treatment, all HAp coating layers were amorphous with a thickness of approximately 700nm. Our results also correspond to that report.

Figure 4 illustrates the XRD spectra for Ag, Cu, and HAp+TiO_2 coating layers. The Ag and Cu coating layers represent maxima at 2θ value of 38.10°(111) and 43.35°(111) respectively, which are equivalent to the values assigned by the XRD index. This indicates that Ag and Cu coated layers prepared by an IBAD process practically form their intrinsic crystallization patterns. In the meantime, since the only HAp+TiO_2 coating layer was amorphous in this experiment, there was no peak in the XRD spectrum of the HAp+TiO_2 coating

TABLE I
The Results of ICP-AES Analysis for the Surface Layers of Modified Silicones

Element (voltage/current)		Wave len.	Submerged time (h)	Conc. (mg/l)
HAp+TiO_2 (120/0.6)	Ca	315.887	24	10.604
	Ti	4.1379		3.8908
	Ca	315.887	48	10.710
	Ti	4.1379		4.1379
Cu (120/0.6)		327.395	24	0.015950
			48	0.063711
Ag (120/0.6)		328.068	24	0.000645
			48	0.000822

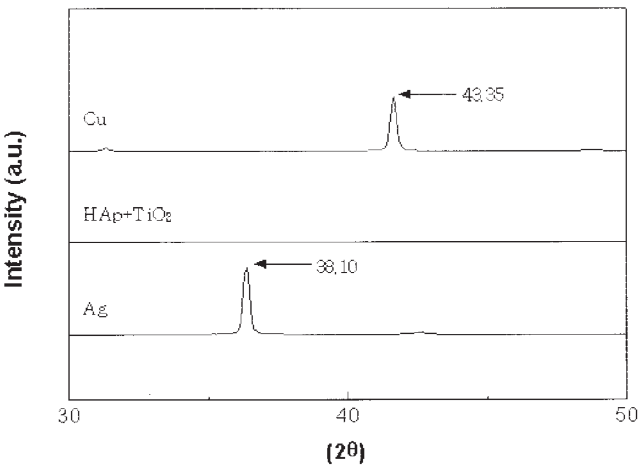

Figure 4 XRD patterns of different coating layers modified with Ag, Cu, and HAp+TiO_2, respectively, which were prepared by ion beam assisted deposition (IBAD) processes with Ar as assisting gas. The specimens were pre-etched with O_2 for 30 min, ion bombarding during deposition with beam power of 120V/0.6A, and post-treating with Ar for 10 min.

layer. The semicrystalline patterns of HAp could also be obtained by thermal annealing, which was explored in this study.

The ion bombardment during deposition is known to broaden the atomic intermixed zone, thereby increasing the adhesion strength between the coating layer and the substrate.[11,12] To confirm this, we adopted Ag as a deposition material by IBAD under various conditions for Ar ion bombardment. Figure 5 shows the dissolution rate of the Ag coating layer after 24 h. The Ag concentration (mg/L) in distilled water decreased with increasing Ar ion bombarding voltage. But the current level did not have a significant influ-

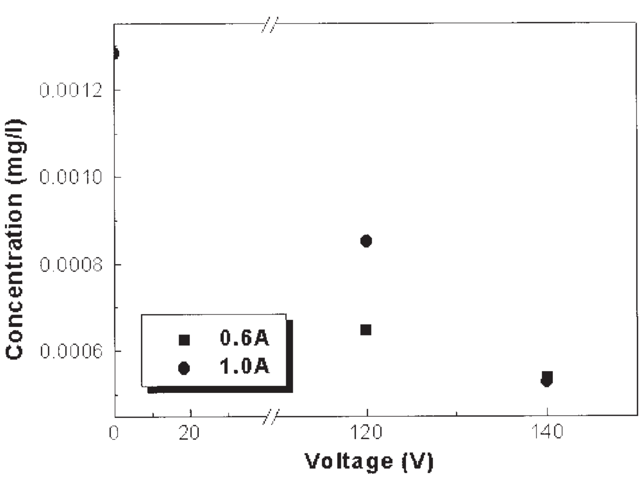

Figure 5 Concentration of Ag dissolved out from coating layer prepared at different current and voltage conditions. The Ag concentration was analyzed with ICP-AES after dissolving the coating layer in distilled water for 24 h.

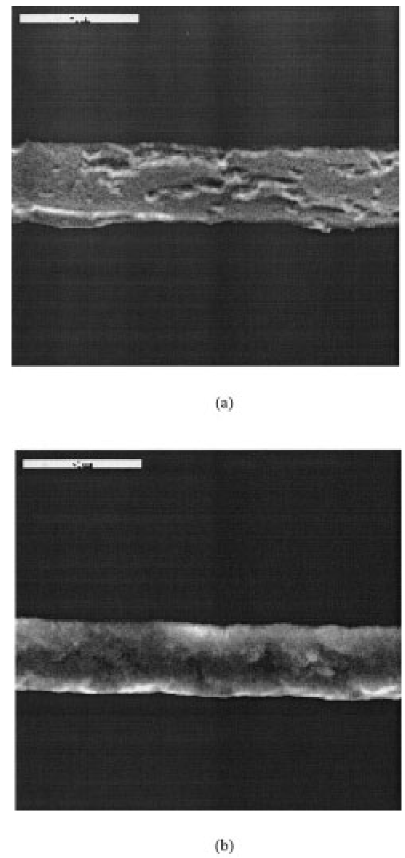

Figure 6 SEM photographs of Ag coating layer: (a) No bombardment during Ag evaporation, and (b) Ar ion bombardment during the Ag evaporation at 140V and 1.0A. White bar on the figure indicates 2 μm.

ence on the density of the coating layer. From this result, we could conclude that the level of ion beam power during deposition has significant effects on the compactness of the coating layer itself as well as the bond strength. The effect of Ar ion bombarding during Ag deposition was further confirmed by observing the morphology of the coating layers, as shown in Figure 6. Brittle and coarse layers and heterogeneous deposition spots are observed in the Ag coating without Ar ion bombardments (Fig. 6a). In contrast, the surface of the coating layer with Ar bombardment during Ag deposition is more uniform and retains higher compactness (Fig. 6b).

Figure 7 shows the SEM of the U-type bent surface of Ag coated silicone rubber prepared with and without Ar assisting bombardment. Without ion gun assistance (Fig. 7a), the interface between the substrate and the coating layer of the U bent surface shows discrete separations; while with ion gun assistance (Fig. 7b), interlocked particles of the coating material are dispersed in the bulk of the substrate. This indicates that the ion assisting deposition improves interfacial compatibility and the adhesive strength between the substrates and the coats, although it was not fea-

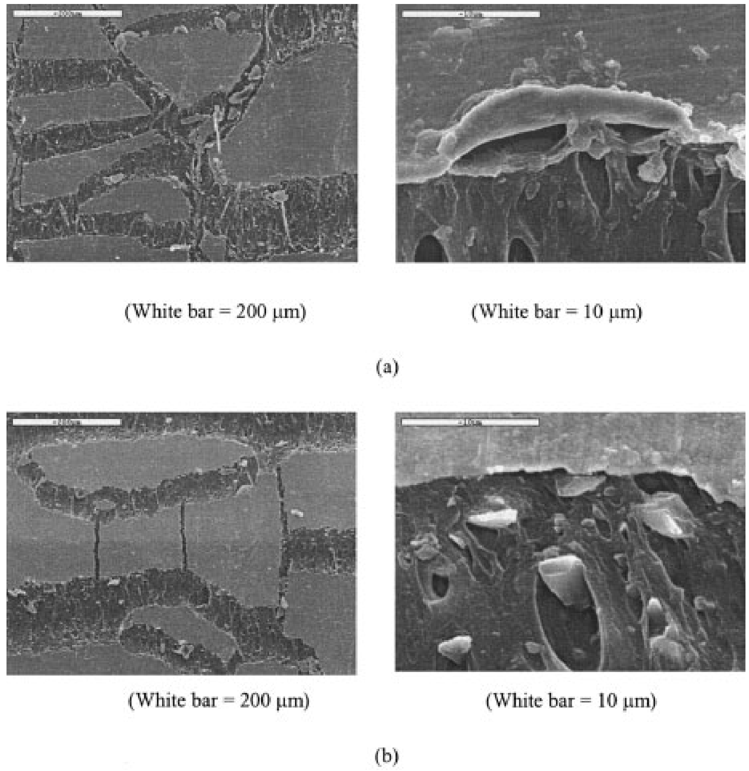

Figure 7 SEM of U-type bent surface of Ag coated silicone rubber prepared (a) without Ar assisting bombardment, and (b) with Ar bombardment.

sible for us to measure directly the adhesive strength of the interface in this study.

For the biocompatibility test of the modified surfaces, antimicrobial activity was examined. The effect of antimicrobes on *S. aures* and *E. coli* as bacteria used in this study are common and cause cross infections in the ambient environment. The *S. aureus* enterotoxin is well known to cause the quick onset of food poisoning, which can lead to cramps and severe vomiting. Infection can be traced to kinds of contaminated or rarely cooked meat, for example. These microbes also secrete leukocidin, a toxin that destroys white blood cells and leads to the formation of puss and acne. Particularly, *S. aureus* has been found to be a causative agent in ailments such as pneumonia, meningitis, boils, arthritis, and osteomyelitis (chronic bone infection). Most *S. aureus* are penicillin resistant, but vancomycin and nafcillin are known to be effective against strains. *E. coli* is facultatively anaerobic Gram-negative rods that live in the intestinal tracts of animals in health and disease. So, although most *E. coli* strains are harmless, some are endowed with the capacity to cause diarrhea or systemic illnesses. Particularly, *E. coli* is one of the most common causes of urinary tract infections. Besides, the strains have been linked to diseases in just about every other part of the body. Pneumonia, meningitis, and traveler's diarrheas are among the many illnesses that pathogenic strains of *E. coli* can cause.

Table II shows antimicrobial activity[13,14] of the samples treated with Ag, Cu, and HAp+TiO_2, respectively. The samples treated with Ag, Cu, and

TABLE II
Effect of the Coating Layers on the Antimicrobial Activity

		Control	Ag coated silicone rubber	Cu coated silicone rubber	HAp + TiO_2 coated silicone rubber
B-1	Start	$1.3 \times 10^{\circ}$	$1.3 \times 10^{\circ}$	$1.3 \times 10^{\circ}$	$1.3 \times 10^{\circ}$
	24Hours	$3.4 \times 10^{\circ}$	<10	<10	<10
	Reduction(%) of bacteria	13.3	99.9	99.9	99.9
B-2	Start	$1.6 \times 10^{\circ}$	$1.6 \times 10^{\circ}$	$1.6 \times 10^{\circ}$	$1.6 \times 10^{\circ}$
	24Hours	$3.8 \times 10^{\circ}$	<10	<10	<10
	Reduction(%) of bacteria	15.1	99.9	99.9	99.9

HAp+TiO_2 all display antimicrobial activity above 99.9% of reduction against both *S. aureus* and *E. coli*. Metal ions existing on the surface of the silicone rubber move into water around the rubber substrate with ions. And they either destroy the cell membrane or pass through the cell membrane, bonding with the –SH group of enzymes in the cell, thereby lowing enzyme activity, interfering with metabolism, and causing death or suppression of micro-organism growth.

Figure 8 shows the morphology of a cell group after culturing for 24 h (NIH 3T3 fibroblast cell). Dark spots indicate the cell nuclei, the optical density of which was measured at 570 nm. Results can be expressed as the mean optical density with ± 95% confidence limits. Figure 9 shows the cytotoxcity[15–17] result of the coated and noncoated silicone rubber. The % of control indicates the ratio of the cells surviving after the test. The specimen coated with Ag and Hap+TiO_2, respectively, showed slightly reduced cytotoxicity from the untreated silicone rubber, while the cytotoxicity of the specimen coated with Cu increased presumably due to the effect of the copper oxide made from the reaction between the Cu and ambient oxygen.

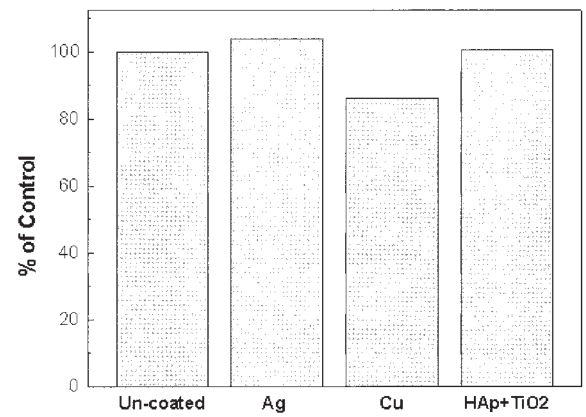

Figure 9 Cytotoxicity of uncoated silicone rubber and the silicone rubbers coated by Ag, Cu, and the HAp+TiO_2 mixture.

CONCLUSIONS

Different materials, such as Ag, Cu, and HAp/TiO_2, were coated on silicone rubber using IBAD to improve the biocompatibility. A dense coating layer was obtained on the silicone rubber, and its adhesive strength increased prominently when the coating layer was made using IBAD with Ar ion beam assisted deposition. Cu and Ag form crystalline coating layers on the surfaces, while HAp/TiO_2 was coated in an amorphous pattern. In this study, it is worthy of notice that we were able to acquire relatively stable and strong coating layers of metal or inorganic materials on the surface of polymeric materials with high efficiency. Antimicrobial activity of the coated silicone was greatly improved, showing bacteria reduction of 99.9%. Cytotoxicity was decreased for the Ag and HAp/TiO_2, but for Cu, the oxide obtained from the reactions from Cu and ambient oxygen may reduce the cytotoxicity slightly. These results will be adopted to develop enhanced biocompatible medical or rehabilitation devices, such as catheters, vasodilation devices, or socket liners for amputees in further study.

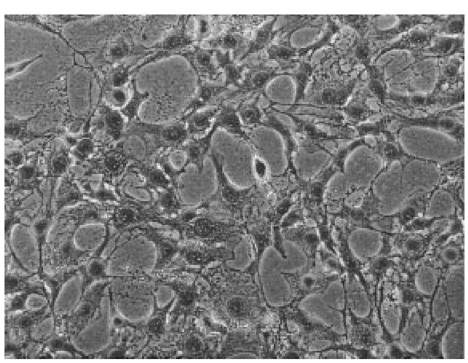

Figure 8 The morphology of a cell group after culturing for 24 h (NIH 3T3 fibroblast cell).

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