to hand their characteristics down to their posterity in its long history. We wrote "materials" in the description above. However, we might as well include metallic materials or minerals here. Metals and bacteria have been correlated with each other in these 4 billion years or more.

Some metals have high affinities with bacteria. And the others do not at all. Some metals are incorporated into the inside of bacteria. And the others kill them. From this viewpoint, the correlation varies so much with the combination and case. In this chapter, we would like to focus on the antibacterial effect of metals among those various correlations. In many advanced countries such as the United States, some European countries, and Japan, people are surrounded by high-function materials, devices, and apparatuses and enjoy amenities of life. In those many countries, the increase of population hit the ceiling. The birth rate is declining and the society is being aged, usually. In such a mature society, materials based on the infrastructure must have not only high function but also user-friendliness. The antibacterial effect by metallic materials is also one of the characteristics. Technically, it will be needed in food industries, medical field, plumbing products in households, etc. Since it relates to our daily lives directly, the antibacterial effect provided by materials would bring us the safety and secure feeling. There are some bulk matters showing antibacterial effects directly. However, the commercial products usually need versatile properties such as mechanical, chemical, and physical ones. Therefore, materials could not be designed only from the viewpoint of antibacterial effect, since it would be very hard for a material to have all of those beneficial properties at the same time. To make a material get those beneficial properties simultaneously, surface coating is the most attractive, useful, accessible, and reasonable process. While the surface could show antibacterial effect, the bulk could keep other characteristics for the application. From this point of view, we would say that surface coating would be generally a kind of composite material.

In this chapter, the antibacterial effects of metals will be described first, and it will be compared with photocatalytic oxides and organics. Then, antibacterial coatings that have been proposed in practical industries will be introduced. The merits and demerits will be discussed, and the concept of composite materials for antibacterial effect will be proposed followed by a detail discussion. Some possibilities will be mentioned. And finally, the two concrete examples by the authors will be introduced and explained.

2. ANTIBACTERIAL EFFECTS AND METALS

2.1 What is Antibacterial Effect?

We knew that some materials would have antibacterial effects so far [1–4], even though we still do not know the mechanism correctly. Particularly, some metallic materials, such as silver and copper, have had been well known for the characteristics, and they had been considered as magic or miracles. Conventionally, silver has been utilized for eating utensils. It means that people have known the antibacterial effect of the metal since the ancient time. So why and how do some materials including metals show antibacterial effect?

Before the explanation of antibacterial effect shown by materials, we have to clarify the definition of antibacterial effect of materials more clearly. In English, almost all cases to suppress the growth of bacteria could be expressed as antibacterial effect. However, Japanese generally subdivide the concept into smaller ones, depending on the extent and the effect.

Usually, we have five technical terms for antibacterial effects [5,6]. The first one is microbiocidal effect. In this case, all bacteria are killed perfectly. However, the concept does not contain any objects nor extents. For example, if only several bacteria would be killed and the rest would be still alive, one could say that the microbiocidal effect would still work in that case. The second is the sterilization that has broader meaning. Not only to kill but also to remove bacteria or viruses from the target objectives could be included, even though they might be harmful or harmless. From the viewpoint of probability, it is impossible to make the bacterial number completely zero. Therefore, SAL (sterility assurance level) is often used to judge the extent of sterilization. Internationally, sterilization is defined as the situation where SAL is smaller than 10^{-6}. The third is disinfection by which the number of pathogenic bacteria would decrease down to the nonharmful extent. This could be achieved by microbiocidal effect, the first concept. In this point, one may say that disinfection would be almost the same with microbiocidal effect. However, disinfection could be achieved only by causing the pathogenicity to disappear. Therefore, the two concepts are different with each other, strictly speaking. The fourth is Jokin. This Japanese technical term should be translated into "decreasing the bacterial number." The concept does not include targets nor extents. It means that the concept doesn't matter; the target would be how much bacteria might be removed. And the fifth is "Kokin." Kokin can be defined as an action that would suppress the growth of bacteria in the target object. Usually, we need to confirm the decrease of bacteria in the order of 10^2. As for these five antibacterial effects, the authors used some technical terms in English tentatively. However, they are not correct, strictly speaking. All of them in Japanese might be included in the concept of antibacterial effect used in the western world. Kokin is the most desirable among these five categories, when we come to think about the environmental friendliness, since it doesn't require any harmful or strong chemicals to kill them completely. Regarding the onset of antibacterial effects by materials, they are not so strong to kill bacteria completely in usual cases. Therefore, we deal with Kokin as antibacterial effect in this chapter.

2.2 Metallic Antibacterial Groups

The materials are classified into three groups from the viewpoint of antibacterial effect on the target. These classifications include metallic materials, some photocatalytic materials such as titanium oxide, and organic materials [5,7]. And each has its own characteristic mechanism of action for the antibacterial effect. The first group is metallic materials. Some metals having obvious antibacterial effects are dissolved to bind with some proteins, leading to changes of the higher-order structure. The 3-D conformation of proteins [8] is generally composed of relatively weak bonding among amino acids, such as hydrogen bonds, ionic bonds, hydrophobic bonds, and S–S bonds. The bond between metallic ions and proteins could weaken the bonds between amino acids and sometimes break them. The high-order structure generally has a close relation to the function of protein. Thus, the following high-order structural change would disturb the microbial activity and the metabolism to develop antibacterial effects. Usually, there are many pores (channels) on membrane cells, where ions and various matters on cell membranes are moved into the inside cells. Ikegai et al. [9] pointed out in the past that several divalent metallic ions tend to bind with the "channel" to decrease its function as the moving passage of matters. They also confirmed that zinc ion, nickel ion, and copper ion inhibited the function of channel proteins on the cell membranes of *Thiobacillus ferrooxidans*. The mechanism is shown in Figure 1 schematically.

Conventionally, silver, as well as some heavy metals, has also been known for its antibacterial effect. Regarding the antibacterial effect of silver, there have been lots of discussions about the mechanism. Generally, silver ion tends to react with function groups containing S, N, O, etc. very easily whose electron densities are generally high. Particularly, the silver ion binds

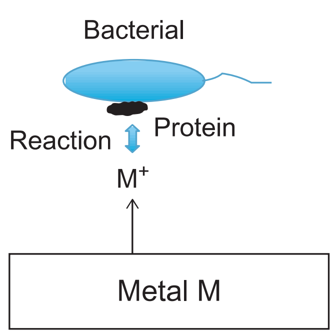

Figure 1 The mechanism of antibacterial effect by metals (hypothesis).

with sulfhydryl group (–SH). As a result, the enzyme's function is inhibited. And in addition, silver ion can react with amino acid residue, DNA, RNA, and NH group to form insoluble salts, which would lead to inactivation of enzymes on the cell membranes.

We presume that antibacterial metals have the same effects with those described above. Therefore, the antibacterial effects by metallic ions should be considered from the viewpoint of inherent affinity with metallic ions and degree of dissociation for them.

As for some commercial coating metals, the authors et al. also confirmed the extent of antibacterial effect in the past [10]. Those metals were zinc, manganese, nickel, copper, cobalt, chromium, titanium, aluminum, silver, iron, and magnesium. These metallic powders were added into a usual agar media. Then, the 0.1 ml solution containing 10^6 ml^{-1} bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*) was added into the agar media. After the culture in 18 h at 37 °C, the formed colonies were measured. The relation between the colony number and metal content evaluated the antibacterial effect, shown in Figure 2 schematically. The colony formation units for these metals could be shown with the metal contents, respectively. Generally, the colony formation unit decreased with the increase of content. The extent of decrease and the behavior could show their antibacterial effects. As a result, these commercially available metals were classified into three types. The first group was composed of metals that show strong antibacterial effects. Zinc, manganese, nickel, copper, and cobalt constitute this strong antibacterial group. On the other hand, the second group was composed of chromium, titanium, aluminum, and silver whose antibacterial effect was medium. And the third group was composed of iron and magnesium. Since this evaluation method did not take the specimen's shape, diameter of particles, and the inherent dissolution characteristics into consideration, the results might not be appropriate for detailed quantitative

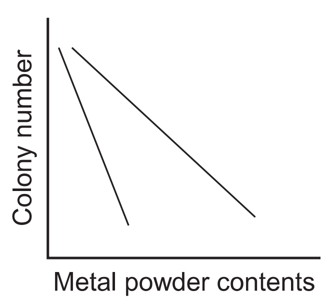

Figure 2 Evaluation for tentative antibacterial effect of metal powder.

analysis. However, the differences among these elements were convictive for us, when we compared the results with those in the past.

2.3 Antibacterial Test and the Standard

As quantitative evaluation method of antibacterial effect, an international standard is already established as ISO 22196 [11]. The test is usually called the "film covering method." The standard could provide us a useful way to evaluate the antibacterial effect for practical materials and products. The procedure can be described as follows.

The specimens were put in a plastic Petri dish, while the bacterial solution was prepared as follows: the bacteria were incubated in 10 mL of a nutrient broth for 24 h at 35 °C and then diluted 2000-fold with sterilized water and established as a bacterial solution. The diluted bacterial solution was applied to the specimen (16 μL/cm), and then, a polymer film was laid over the solution. The sample was kept in an incubator for 24 h at 35 °C. After the incubation, a solution of 10 mL of sterilized water containing 200 μL of Tween® 80 (a nonionic surfactant and emulsifier) was introduced into the plastic Petri dish, and the bacteria attached to the specimen and polyethylene film were washed into the aqueous solution. To determine the number of viable cells, serial decimal dilutions of the cell suspension were made, a 0.1 mL portion of which was uniformly spread on an agar medium. The plate was incubated at 35 °C for 24 h and the colonies formed were counted. The viable cell count was represented as colony-forming unit per milliliter (CFU/mL). The final colony formation number was measured to evaluate the antibacterial properties.

This method is very useful for us to check the antibacterial effect not only for metallic materials but also for most industrial solid materials. However, the concept for the antibacterial effect might be too static. It means that the antibacterial effect evaluated by the method would not show us any time-dependent characteristics. Figure 3 shows a problem for an example, which

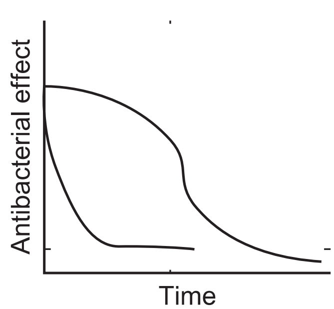

Figure 3 A problem of film covering method for antibacterial effect.

has been often discussed as personal communication between authors. The vertical axes correspond to an indication. The vertical solid lines in the figures indicate for antibacterial effect such as colony formation unit or bacterial numbers, while the horizontal ones to time. In any case for antibacterial effects, they would decrease with the increase of time, more or less. The dotted lines would correspond to the points when the film covering method fixed antibacterial effects. However, who could tell which case would have higher antibacterial effect, when the final values would be compared. At this point, there are no alternative standards to show the time dependence of antibacterial effect. To predict the effect more properly, such a test method is needed.

2.4 Other Antibacterial Group [5]

Other than metallic materials, there are still two groups of materials showing antibacterial effect. The second group is composed of photocatalytic substances, such as titanium oxide. As shown in Figure 4 schematically, photocatalytic oxides such as titanium oxide have the energy gap between valency and conduction band, since they are basically semiconductors. When they are irradiated by ultraviolet light, electrons in valency band become energized to conduction band. Then, positive holes form in valency band. Generally, the positive hole has the strong oxidation power to compensate the lack of electrons and to disappear themselves by depriving of electrons. As a result, hydroxyl ions near the surface of oxide are changed to hydroxyl radical. They could kill bacteria and inhibit the growth. Titanium oxide is the most well known, even though there are some photocatalytic substances such as tungsten oxide.

The third group for antibacterial substances is organic matters. As shown in Figure 5 schematically, organic antibacterial agents generally damage cell membranes. When they would be damaged, denaturation of membrane

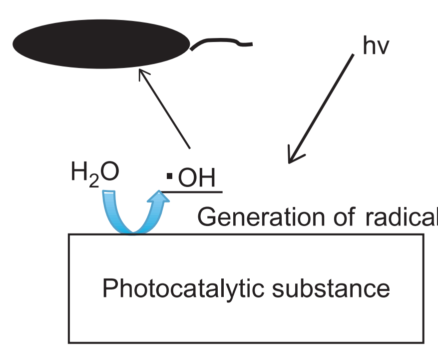

Figure 4 The schematic principle of antibacterial effect by photocatalytic substance.

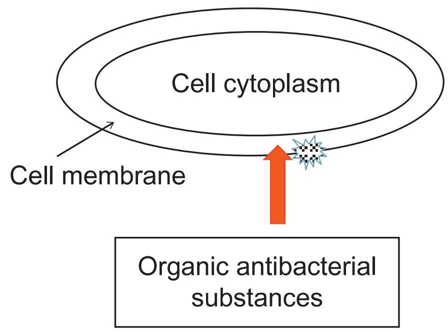

Figure 5 The schematic principle of antibacterial effect by organic substance.

proteins, the change of membranous structures, etc. might occur. Antibacterial effect by organic agents would appear in such a way.

3. ANTIBACTERIAL SURFACE COATINGS

3.1 Application to Surface Coating

There are many types of antibacterial agents. They are usually classified and organized systematically in the authors' country (Japan) and other countries. In Japan, antibacterial matters have been generally classified into six types from the viewpoint of patent application—photocatalyst, silver, heterocyclic compound, copper, vegetable matter, and amine/quaternized ammonium salts [12]. And all of them are applied and used in the following classified fields—water treatment, domestic housewares, basic goods (fibers, leather, paper encasement, container, clothes, etc.), machinery/appliances, construction/pain, separation, and others. The most utilized substances for antibacterial purposes have been photocatalyst (titanium oxides) and silver in Japan, followed by heterocyclic compounds and copper. Therefore, we should think that antibacterial surface coatings would be the proper application of these antibacterial substances to surface coating. Of course, only surface coatings could realize the effective outcomes from the viewpoint of some inevitable restrictions.

3.2 Polymer Coating: Antifouling Coating

Most of polymer coating aims to make the surface hydrophilic, since the hydrophilic surface very often could repel proteins, bacteria themselves, etc. From the viewpoint, the coating does not correspond to antibacterial effect based on killing or controlling bacteria. The basic concept for such a polymer coating would be antibiofouling effect. Biofouling can be defined as the whole process composed of attaching of organisms on materials and the following phenomena. The biofouling brought about by bacteria is called "microfouling." The antibacterial effect is related to the microfouling. Figure 6 [13] shows the general case for microfouling occurring on materials surfaces. Generally, the biofilm formation process is explained in the following way [14–18].

Carbon compounds form on materials surface in advance. It is called "conditioning film." That should be nutrition for bacterial. Floating bacteria called "planktonic bacteria" move toward the materials surface to get the nutrition. Through some complicated phenomena, bacteria attach to the surface and the number of bacteria attached to the materials surface increases. When the number exceeds a certain threshold value, polysaccharides are excreted from bacteria and the sticky matters surround materials surface including bacteria on the surface and also water in the vicinity of surface. Then, the sticky and inhomogeneous filmlike matters form on the materials surface. It is called biofilm. However, it is not the homogenous film, strictly speaking. The film is composed of consecutive towerlike thin matters covering materials surfaces. Biofilm is composed of more than 80% water, bacteria, and extracellular polymeric substance (EPS). In the past, researchers believed that EPS is almost equal to polysaccharide. However, we got to know recently that EPS contains a lot of proteins and polysaccharides [18]. Hydrophilic polymer coatings could repel the proteins and bacteria mainly. Usually, such a coating is called "brush polymer coating." The outline of the mechanism is shown in Figure 7 [19]. The nonhydrated polymers

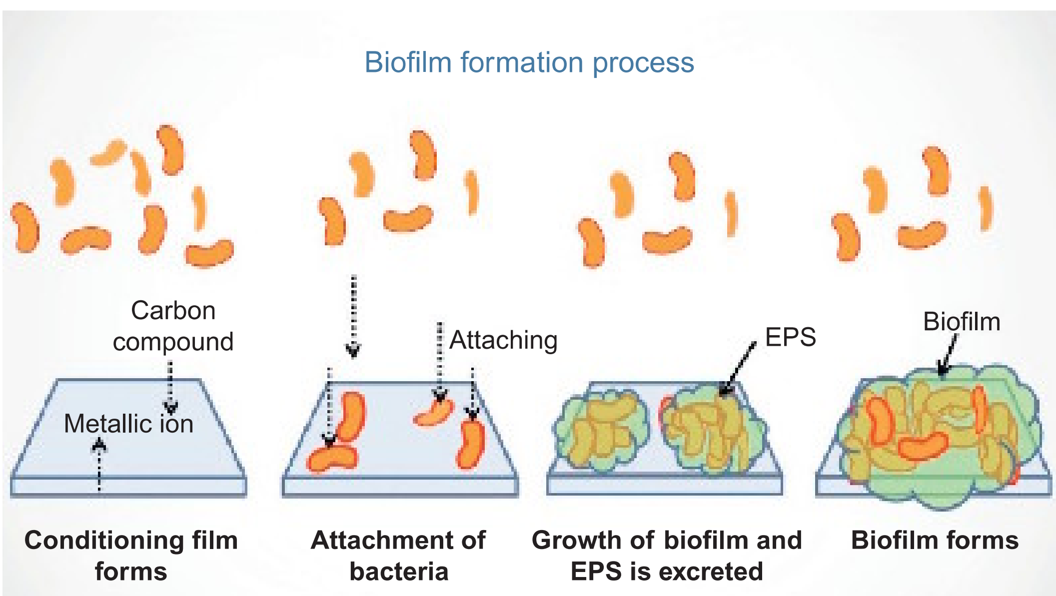

Figure 6 Mechanism of microfouling process on material surface.

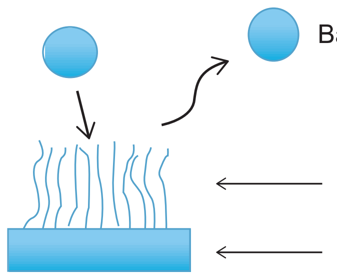

Figure 7 Polymer brush surfaces repelling bacteria and protein.

are randomly coated on the surface. When the coating is too difficult, some pretreatment may be required to enhance the bonding of polymers with the substrate. Then, tightly packed highly hydrated brush is created in an aqueous environment. As shown in the figure, proteins and bacteria would be repelled by the action of bound water in the brush and the elasticity of the polymer chains. According to this concept, Khoo and coresearchers achieved polymer coating through a peptide-linked PEG. Since the polymer film would have longer polymer chains, the repellent effect would be stronger. This is just an example for medical applications, and the reader can refer to many good reviews about this topic. However, such a polymer coating does not relate to "Kokin" where the bacterial growth and metabolism are controlled by the direct reaction of antibacterial substances. It controls biofilm formation and biofouling, the wider and more comprehensive concepts relating to bacterial activity.

3.3 Metallic Coating

As described above, silver and copper have been antibacterial metals. Figure 8 shows the percentage of dominated application by silver and tin in Japanese patents. When the cases would be restricted to coating technique, 177 cases for silver-related coating and 167 cases for copper-related coating were applied to "Japan Patent Office" as antibacterial coating from 1993 to 2013 [20]. Even though the mechanism would be not fixed, many circumstances, phenomena, and results show clearly that dissolved metallic ions must play an important role to reveal antibacterial effects. As described above, there have been so many intentions also for metallic coatings; we are afraid that we could not mention all of them. For the detailed information, the reader could refer to one of our papers in the past [20]. We have to mention their application to surface coating first of all as well as the dominated patents for bulk materials.

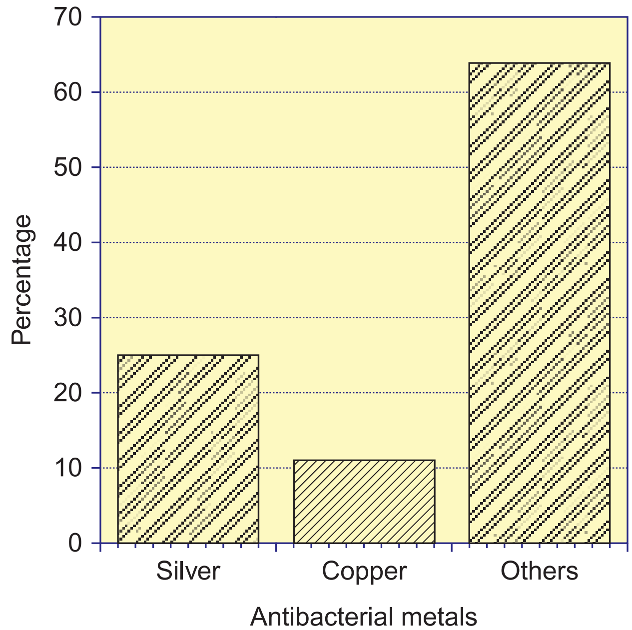

Figure 8 Application percentage of antibacterial metals, silver and copper, in Japan.

Koura and Sakado proposed silver-zeolite coating [21]. Zeolite could contain silver ion in a stable condition and control the reactivity of silver ion well. They devised a solution composed of aluminum alkoxide, alkoxysilane, alkoxide of alkali metal or alkaline earth metal, amines, amino-based organoalkoxysilane, and a silver salt. The specimen was coated with silver-zeolite film by dipping and spraying after calcined at 200-800 °C.

Miyasaka et al. devised the stacked films composed of n-type semiconductor oxide and silver compounds [22]. N-type semiconductor film would show photocatalytic antibacterial effect, while silver compounds would produce silver ion. They aimed to achieve combined effects by photocatalytic and silver ion effects simultaneously.

Otani et al. devised laminated silicate carrying some antibacterial metals such as silver, copper, and zinc [23]. They achieved the coating by spray coating, dipping or bushing, etc. Hirose et al [24] devised dissolutive glass containing silver and copper ions. The antibacterial metal ions gradually dissolve to work as antibacterial agent. They crushed the glass down to tiny particles in micrometer orders and successfully attached on clothes, etc.

Toda et al. devised a new agent to achieve antibacterial and antifungal ceramic coating [25]. The coating films are composed of titanium oxides and one of the antibacterial metals. They also aimed to show combined effects for antibacterial effects deriving from different groups.

Mase et al. devised the coating method for some antibacterial metals by plating, sputtering, vacuum coating, etc [26].

Miyauchi et al. also devised sputtering mask composed of silver, zinc, copper, titanium oxide, and zinc oxide [27]. These are concrete examples that physical vapor deposition processes were applied to antibacterial coating concretely.

Recently, nanoparticles of antibacterial metals have come under the spotlight. And the nanopowder has been applied to various coatings, particularly for medical fields [19]. Kowsett and Strom-Versloot et al. found that dressings could be impregnated with silver sulfadiazine and other silver compounds [28,29]. The antibacterial effect of the wound dressing with silver and silver salt for medical applications has been still discussed [30–33], since the experimental conditions were versatile and complicated. External ventricular drains (EVDs) that have been used for hydrocephalus treatment are also one of the medical tools where silver coating was applied. Fichtner et al. applied the nanosilver coating to EVD and confirmed that the colonization of drains decreased by a factor of 2-4 [34]. However, the negative results were also obtained [35]. The nanopowder coating was also applied to catheters. However, the positive result was still obtained only for animals [36,37]. The results for human bodies are still investigated.

Medical applications always have such difficulty. Generally, it takes a long time to pass the safety regulations and to get approved from government and authorities. And in addition, the environments are too complicated to simulate and analyze on laboratory scales. The more accurate and appropriate evaluation techniques should be improved and highly developed.

4. NEW POSSIBILITIES AND PREDICTIONS FOR COMPOSITE COATING

4.1 The Basic Principle for Antibacterial Composite Coating

As described in previous section, metallic coating of silver, copper, etc. might kill bacteria. However, metals on material surfaces must dissolve to exhibit antibacterial effects. It might lead to the two defects, as shown in Figure 9. One of them is the change and deterioration of surfaces. Since the dissolution means corrosion, the surface would be lost more or less. When the extent would be serious, the surface would be damaged and deteriorated. They might not be able to keep the other purposes such as wear resistance and color tone. And the coating thickness would be decreased due to the dissolution. That

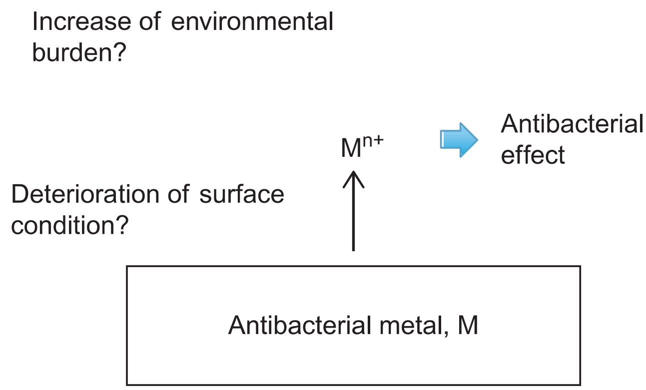

Figure 9 Problems for antibacterial metal coating.

might lead to the decrease of life cycles for the coating. Secondly, the dissolution might lead to the infringement of some environmental regulations such as "REACH" in European Community. Even if some metallic components would not be regulated at this point, they might be listed as hazardous components in the future. Due to those reasons and some possible others, the direct coating of antibacterial heavy metals would not be always good ideas to solve the solution for antibacterial effects. So what type of coating would be desirable? The answer should be composite coating. Concretely, the problem solution should contain the following points:

1. Antibacterial metals should be contained in the coating more or less.
2. The antibacterial effect should appear by the action of metallic ions.
3. The dissolution rate should be controlled properly.

To achieve those points simultaneously, composite coatings are the most beneficial. The problem is how to combine antibacterial metals into the other basic component(s). According to the concept, there are already some investigations and proposals as follows in these recent years, even though there are still not so many investigations at this point.

4.2 Recent Research and Proposals

Voccia et al. proposed a new method for the electrochemical deposition of a metal/polymer composite layer on a conducting substrate [38]. The electrochemical solution was a mixture of an acrylate (ethyl acrylate, EA; 2-phenyl-2-(2,2,6,6-tetramethylpiperidin-1-yloxy) ethyl acrylate, PTEA; and 8-quinolinyl acrylate, 8QA), a metallic salt (silver (I) acetate), and a conducting salt in dimethylformamide.

Sunkara et al. investigated and showed a distinct enhancement of antimicrobial activity of $W4^{+}$-doped titania that was coated on nickel ferrite nanoparticles in comparison with undoped titania [39]. The composite nanoparticles were synthesized by uniquely combining reverse micelle and chemical hydrolysis synthesis methods.

Ahn et al. proposed experimental composite adhesives (ECAs) containing silica nanofillers and silver nanoparticles and compared with two conventional adhesives (composite and resin-modified glass ionomer, RMGI) to analyze surface characteristics, physical properties, and antibacterial activities against cariogenic *streptococci* [40]. And they confirmed that their new ECAs were effective to control the bacterial growth.

Necula et al. carried out the synthesis of a porous TiO (2)-Ag composite coating and assessment of its *in vitro* bactericidal activity against methicillin-resistant *S. aureus* [41]. The coating was produced by plasma electrolytic oxidation of Ti-6Al-7Nb medical alloy in a calcium acetate/calcium glycerophosphate electrolyte bearing Ag nanoparticles. Following oxidation, the surface of the titanium substrate was converted into the corresponding oxide (TiO_2) bearing Ca and P species from the electrolyte. In addition, Ag was detected to be associated with particles present in the oxide layers. They confirmed successfully that the coating could kill *S. aureu*s effectively.

Shirokova et al. proposed a new polymer composite film based on carboxymethyl chitin and silver nanoparticles [42]. The composite films revealed a pronounced concentration-dependent antibacterial activity toward strains *Salmonella typhimurium* and *S. aureus*.

Dastjerdi et al. proposed a novel method to overcome the problem that the treatment of textiles with Ag/TiO_2 nanoparticles caused a brownish color and limited the application of this otherwise good composite [43]. To achieve the purpose, they investigated the effect of various concentrations of cross-linkable polysiloxane (XPs) and Ag mixed with XPs on TiO_2-treated fabrics.

Qu et al. proposed a new hydroxyapatite (HA) coating loaded with nanosilver particles [44]. They produced porous Ti scaffolds with high porosity and interconnected structures by polymer impregnating method. And then, they successfully made uniform Ag/HA composite coatings on the surfaces of porous Ti substrates by a sol-gel process. They used Ca $(NO_3)_2{\cdot}4H_2O$ and P_2O_5 in an ethyl alcohol-based system to prepare the sol, which ensured the homogeneous distribution of Ag in the sol. They successfully achieved the good balance between the biocompatibility and antibacterial properties of the coatings.

Yu et al. prepared neat TiO_2 and Ag-TiO_2 composite nanofilms on silicon wafer via the sol-gel method by the spin-coating technique successfully [45].

The synthesized Ag-TiO_2 thin films showed enhanced bactericidal activities compared to the neat TiO_2 nanofilm both in the dark and under UV illumination. They achieved antibacterial effects by both metallic ion and photocatalytic oxides.

Basheer et al. synthesized W-TiO_2 composite and incorporated them into the zinc bath during the hot-dipping process [46]. They found that the composite was effective in controlling the growth of bacteria and formation of biofilm thereafter.

Apart from all of the composite films, the authors proposed some composite films for the antibacterial effect. In the next section, some of them are introduced.

5. EXAMPLES OF COMPOSITE COATINGS FOR ANTIBACTERIAL PURPOSES BY THE AUTHORS

5.1 Heating Stacked Single Layers Process [47–49]

We, the authors, proposed intermetallic compounds between an antibacterial element and non-antibacterial element to show antibacterial effect, while the dissolution of antibacterial metallic ion could be controlled properly. Figure 10 shows the concept of this method. In the conventional alloy plating from an aqueous solution, alloying process takes the path shown in Figure 10a. However, this process would take the double steps path shown in Figure 10b. The latter requires a more complicated process clearly. However, the degree of freedom is larger in the latter than that in the former. We usually call it HSSL process standing for *h*eating *s*tacked *s*ingle *l*ayers process. Both for copper and silver systems, the intermetallic compound films could be produced by the processes. The concrete example cases were introduced as follows.

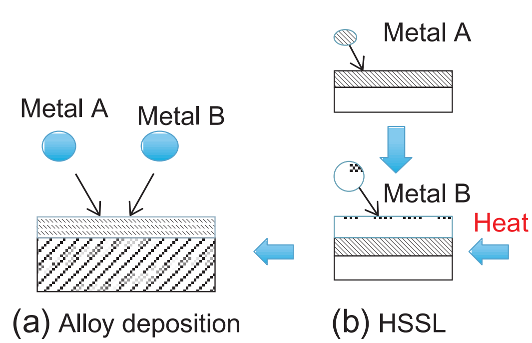

Figure 10 Conventional alloy plating (a) and HSSL process (b).

5.1.1 Tin-Copper Alloy Film [50,51]

A carbon steel (JIS SS400) was immersed in copper sulfate solution composed of 150 g/L copper sulfate and 40 g/L sulfuric acid at 25 °C. The steel was used as cathode and a platinum wire was used as anode. The constant current source provided the cathode 1 A/dm^2 for three minutes to form 10 μm copper layer on the cathode specimen. Then, the steel was immersed into tin sulfate (30 g/L)/sulfuric acid (180 g/L) and electrolyzed with 1 A/dm^2 for a certain time to form tin layer as top layer. The stacked single layer specimens were heat-treated at around melting point of tin. Table 1 shows the produced phases by HSSL process, being identified by X-ray diffraction analyses. When the specimens were heated to the temperatures below the melting point of tin (200 °C), the produced phase was only tin and the alloying did not occur. When the specimens were heated to the temperatures above the melting point (250 °C), various intermetallic compounds formed. For an hour treatment, Cu_6Sn formed, while Cu_3Sn also formed for 2 h treatment. It suggests that the alloying process made progress with time. When the time was prolonged to 3 h and more, tin oxide, as well as Cu_3Sn, was found. It suggests that the specimen should be heat treated for an appropriate heat treatment time.

Table 2 shows the results by Film Covering Method (ISO 22196). The bacteria used were *E. coli* (ATCC25032). Table 2a shows the result for the stacked

Table 1 Surface phases produced by HSSL process for tin-copper system

Heat treatment temperature	1 h	3 h	5 h
200 °C	Sn, Cu	Sn, Cu	Sn, Cu
250 °C	Cu, Sn, Cu_6Sn_5	Cu_6Sn_5, Cu_3Sn, SnO_2	Cu_3Sn, SnO_2

Table 2 Evaluation of specimens' antibacterial effects for tin-copper by film covering method

Time (h)	Specimen	*S. aureus*
(a) Before heat treatment of stacked single layers specimens		
0	Control	9.5×10^4/a plate
24	Control	2.04×10^6/a plate
	Plating specimen	7.51×10^5/a plate
(b) After heat treatment of stacked single layers specimens		
0	Control	9.5×10^4/a plate
24	Control	2.04×10^6/a plate
	Plating specimen	0/a plate

single layers without heat treatment. On the other hand, Table 2b shows the result for the heat-treated specimen at 250 °C for 5 h. For non-heat-treated specimen, the number of colonies increased with time, and it indicates that the specimen did not show any antibacterial effects. For heat-treated specimen, the numbers of colonies were zero in any cases. All of these results suggest that intermetallic compounds between copper and tin showed antibacterial effects clearly. According to our hypothesis that metallic ions would be the main cause for antibacterial effect, the dissolution of intermetallic copper compounds on materials surfaces would work to control the bacterial growth. Since the intermetallic compounds are generally very stable, the infinitesimal quantity of copper ion on materials surfaces seems to work as antibacterial substance. In this point, three principles for antibacterial composites are satisfied by the intermetallic compounds. The schematic principle is shown in Figure 10.

5.1.2 Tin-Silver Alloy Film [52,53]

In the same way, silver intermetallic compounds could be produced. For example, the authors prepared the stacked single layers as follows. The tin under layer and silver top layer were prepared by high-frequency magnetron sputtering to produce stacked single layers of tin and silver. Then, the specimens were heated at various temperatures and for various times. Table 3 shows some of the experimental results. The evaluation of their antibacterial effect was carried out also by film covering method and the bacteria used for the evaluation were *E. coli*. The produced phase after heat treatments was Ag_3Sn. As the table shows clearly, the colony number decreased down to zero with the existence of the intermetallic compound. As in the case of

Table 3 The results of tin-silver surface coating (Sn-Ag alloy)

Heat treatment temperature	Treatment time	Intermetallic compounds?	Ag thickness (μm)	Colony number after heat treatment
200	60	Yes	0.01	0
200	60	Yes	0.1	0
200	60	Yes	1	0
200	120	Yes	0.01	0
200	120	Yes	0.1	0
200	120	Yes	1	0
300	120	Yes	0.01	0
300	120	Yes	0.1	0
300	120	Yes	1	0

copper, the stable intermetallic compound could control the bacterial growth due to their infinitesimal dissolution. However, the existence of intermetallic phase is very important for antibacterial effect. The formation depends on the time and temperature for the heat treatment. The authors proposed the following experimental equation to get the intermetallic compounds of silver by HSSL process:

$$Y = 64\exp\left(\frac{-X}{46}\right) + 1681\left(\frac{-X}{1.4}\right) \tag{1}$$

where Y is the heat treatment temperature and X is the heat treatment time kept at a certain temperature.

5.2 Theory of Segregation Prediction and Its Application [54–59]

The authors proposed other possibility by HSSL process. We have not carried out the experiments concretely. Instead, we predicted a certain type of antibacterial composite films, using simulation software. It is called Surf-Seg, and anyone can use the simulation software on the Internet. The software on a web is based on a theory of segregation prediction by one of the authors. Yoshitake established the theory to predict if the substrate atom might move through the surface film to reach the top of the surface or not, when metallic atom B would exist on the other metallic substrate A. The theory made it possible to evaluate the energy of atoms, when the excited atom by heating would adsorb onto grain boundaries of surface materials with a probability. If the energy would be small (stable), then diffusion process would continue, while it might stop for higher energy conditions. Concretely speaking, the calculation and evaluation require the following equations:

$$\begin{aligned}\Delta H_{\mathrm{ad}}(\mathrm{A\,on\,B}) = & -F\times\gamma_{\mathrm{B}}\times S_{\mathrm{A}} \\ & + (1-F)\times\gamma_{\mathrm{A}}\times S_{\mathrm{A}} + F\times\Delta H_{\mathrm{sol}}(\mathrm{A\,in\,B}) - \Delta H_{\mathrm{vap}}(\mathrm{A})\end{aligned} \tag{2}$$

where $\Delta H_{\mathrm{vap}}(\mathrm{A})$ is the sublimation enthalpy of metal A, $\Delta H_{\mathrm{sol}}(\mathrm{A\,in\,B})$ is the heat of dissolution when A dissolves into B, γ_{A} and γ_{B} are the surface free energy of metals A and B at absolute zero point, S_{A} is the average surface area per mole of metal A, and F is the ratio of surface area for metal A setting in touch with metal B. (When the cover ration of the film is low, the ratio is usually from 0.3 to 0.4. In this case, 0.4 was used.)

ΔH_{sol}(A in B) of Equation (2) is calculated by Equation (3):

$$\Delta H_{\mathrm{sol}}(\mathrm{A\,in\,B}) = \left(\frac{2V(\mathrm{A})^{2/3}}{n(\mathrm{A})^{-1/3}+n(\mathrm{B})^{-1/3}}\right)\times N_0 \times P\left\{-\exp\left(\Delta\phi^2\right)+\frac{Q}{P\left(\Delta n^{1/3}\right)^2}-\frac{R}{P}\right\} \quad (3)$$

where V(A) is the molar volume of metal A, n(A) and n(B) are the electron densities of metals A and B at the boundary of Wigner-Seitz cell, and $\Delta\phi$ is the difference between the work functions of metals A and B.

When $\Delta H_{\mathrm{ad}}(\mathrm{A\,on\,B})$ would be compared with $\Delta H_{\mathrm{ad}}(\mathrm{A\,on\,A})$ from the viewpoint of energy value, one can estimate the possibility of segregation.

Using the theory, we predicted that the following case might be realized. Figure 11 shows the schematic illustration for the new proposal. The upper layer should be the base metal, such as nickel and zinc chromium. Antibacterial metals such as silver and copper are set as under layer. When the heat would be applied to the system, we could predict if the under layer nickel could diffuse into the upper layer or not. If the antibacterial atom could reach the top of the surface by diffusion, the surface would have antibacterial effect. When the top layer would be nickel and antibacterial element would be silver, $\Delta H_{\mathrm{ad}}(\mathrm{Ag\,on\,Ni})$ was calculated as 288 kJ/mol and $\Delta H_{\mathrm{ad}}(\mathrm{Ag\,on\,Ag})$ was done as 222 kJ. In this case, we could predict that the silver atoms would appear gradually, while the surface would be heated. This case could be applied to antibacterial composite coating. If some devices to heat the surface area would be set to the system, the infinitesimal amount of antibacterial silver could continue to appear on the top of the surface gradually and consecutively. Then, the three principles would be satisfied.

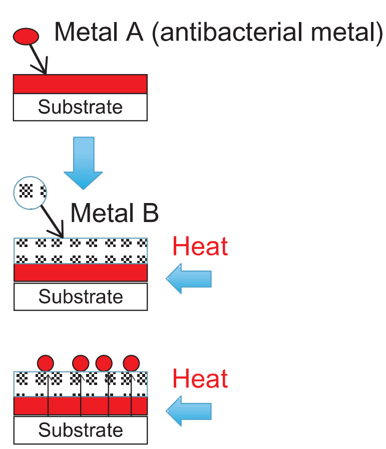

Figure 11 New proposal by segregation prediction theory.

5.3 Dispersion Film of Inorganic Metals [60]

The authors have devised the new composite coating composed of silane base coating and the dispersed nanopowders of antibacterial metal elements (Ag, Cu, and Ti) or those organic substances. The concept for the composite coating was shown in Figure 12 [13].

Three kinds of raw materials for the coating were used. (A) Alkoxysilane oligomer including methyl and phenyl (Permeate, D&D Cooperation, Japan), (B) N-2-(aminoethyl)-3-aminopropyltrimethoxysilane (KBM-603, Shin-Etsu Chemical Co., Ltd.), (C) tetra-*n*-butoxytitanium (B-1, Nippon Soda Co., Ltd.), tin acetylacetonato (Sigma-Aldrich Co.), or nickel (II) acetylacetonato dehydrate (Wako Pure Chemical Industries, Ltd.). The following process was an example, when float glasses were used as substrate. When the other substrates would be chosen, some pretreatment processes could be slightly changed to other appropriate ones. Float glasses were cut down to the tiny sheets whose sizes were 10×5 cm. And they were cleaned in an ultrasonic bath filled with clean water containing various surfactants to remove tiny glass residues and oil components. Then, the specimen surfaces were cleaned by distilled water to remove tiny glass residues, oil, etc. Then, the surfactant was removed by pure water completely. After cleaning, glass specimens were immersed into a solution composed of

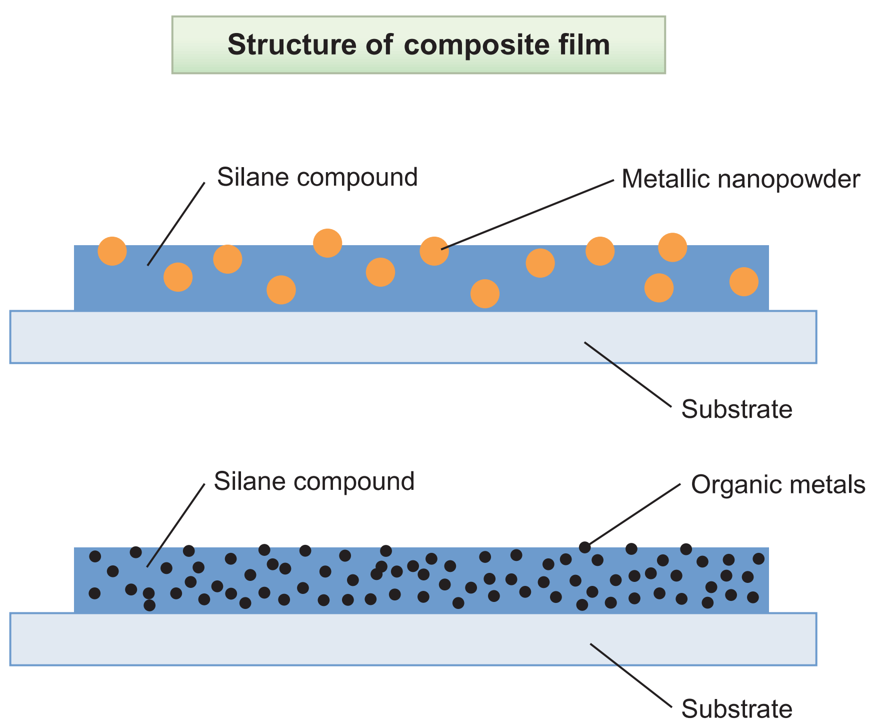

Figure 12 The schematic illustration of composite film's structure.

hydrogen peroxide solution and ammonia water (equivalent volume ratio) until ammonia water was vaporized and the pH could reach neutral values. During this process, the temperature was kept constant at 110 °C. This process made the glass specimens surfaces hydrophilic.

The raw materials, (A), (B), and (C), were mixed for an hour by a disperser (Paint Shaker, Toyo Seiki Seisaku-sho, Ltd.). And the solution as product was used as coating material. The material was coated on those hydrophilic glasses by an air spray. The thickness of coating was 10 μm. All of coated glasses were cured for a week.

The structure of the silane-based antibacterial coating is shown in Figure 13 [13]. At this point, the nanopowder dispersion coating can't achieve the initial goal except for copper nanopowder, since their coatings have high roughness. The rough surface generally attracts lots of bacteria, and the negative effect seemed to be dominant over all of other factors. However, inorganic chemical dispersion coatings worked well to control or inhibit bacterial growth. Since the coating has high transparency, we could evaluate the bacterial growth and the following biofilm formation as the decrease of transparency for the specimens quantitatively, using UV-VIS. Specimens were immersed into a newly develop laboratory biofilm reactor (LBR) for 14 days, and the decrease of transparency for each specimen was measured. According to the experimental results, organic titanium, organic silver, and organic copper specimens showed only 2-4%

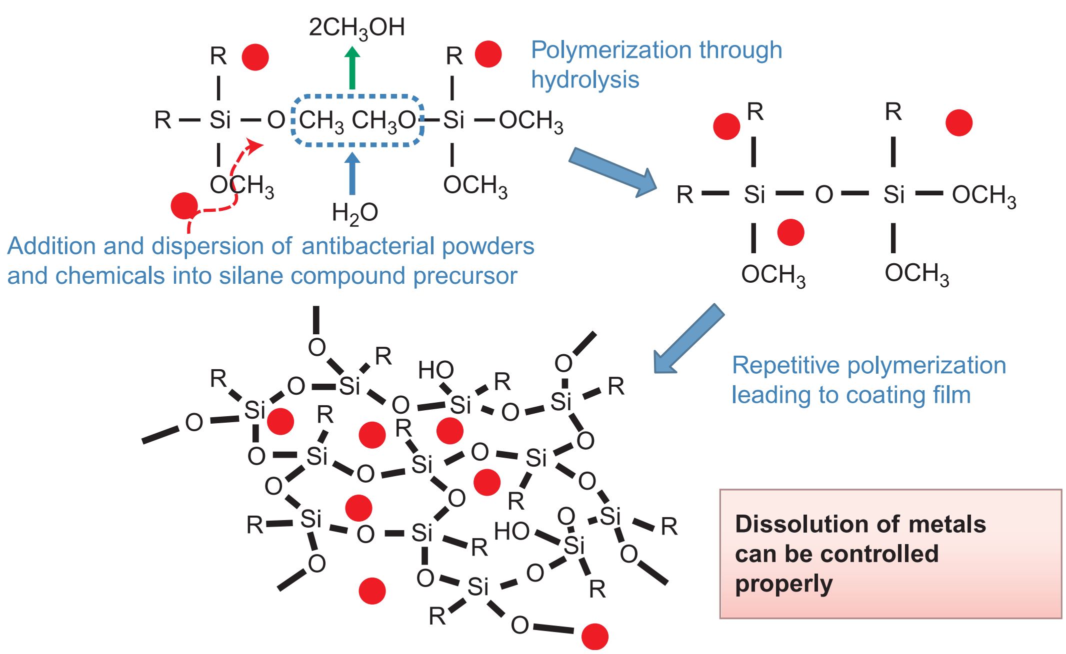

Figure 13 The silane-based composite film and its structure.