



## Nano-Composite Coating on Glasses for Biofilm Control

Hideyuki Kanematsu<sup>1</sup>, Takeshi Kogo<sup>1</sup>, Katsuhiko Sano<sup>2</sup>, Miwa Noda<sup>1</sup>, Noriyuki Wada<sup>1</sup> and Michiko Yoshitake<sup>3</sup>

<sup>1</sup>Dept. Materials Science and Engineering, Suzuka National College of Technology, Shiroko-cho, Suzuka, Mie 510-0294, Japan.

<sup>2</sup>D&D corporation, 7870-20, Sakura-cho, Yokkaichi, Mie 512-1211, Japan.

<sup>3</sup>Nano-Materials Field, MANA, National Institute for Materials Science (NIMS)3-13, Sakura, Tsukuba, Ibaraki, 305-0003, Japan.

### Article history

Received: 27-March-2014

Revised: 09-June-2014

Available online: 10<sup>th</sup> June 2014

### Keywords:

biofilm, biofouling, glass, nano-composite coating, organic tin, organic copper, organic titanium, organic silver.

### Abstract

The dirt and biofilm on materials was related in various meanings, even though the truth has not been well known so clearly. In this study, some nano-composite coating based on silane compound were proposed and shown to resist the formation of dirt on them. As substrate, the glass samples were chosen, since it does not corrode nor change chemically during the biofilm formation. In this experiment, biofilm was formed artificially, measured, analyzed and evaluated then. To make biofilm artificially, a special laboratory biofilm reactor (LBR) was used. And the biofilm formed on samples was analyzed by 3D light microscopy and UV-VIS qualitatively and quantitatively. The results indicate that organic compounds of silver, copper, titanium and tin dispersed in silane compound films could control biofilm formation and dirt effectively.

© 2013 JMSSE All rights reserved

### Introduction

Generally, the dirt on materials surfaces has some relations to biofilm, even though the important truth has not been well known so far. Biofilm is defined as a film-like inhomogeneous matter formed on various materials by bacterial activities. They tend to form on any materials due to a reason. That is obviously one of the dirt for materials in a broad meaning. However, there are many kinds of dirt in addition of that. And pretty much parts of it are composed of inorganic substances. So why does it have some relations to biofilm? The question should be answered from the viewpoint of biofilm formation mechanism. In most of the cases, fogging of glasses would cause negative effects on the original purposes. Basically, the dirt on materials surfaces is usually composed of inorganic contaminations or organic ones, such as silicate or calcium carbonate deriving from water in atmosphere or environments, or various organic matters. Those inorganic and organic matters are removed easily by natural rain shower, coincident contacts by other substances or by simple wiping. However, they are stuck to the substrate surface in a relatively very long time or eternally, and sometimes difficult to be removed, when bacteria form biofilms on the material surfaces<sup>1-6</sup>.

Bacteria generally like to attach to materials surfaces, since the carbon compounds as their nutrition exist there stably. After the detachment and reattachment process, the number of bacteria increases gradually. When it reaches a critical mass, bacteria simultaneously excrete a polysaccharide the result of which is a sticky matter, commonly known as biofilm. The sticky matter plays a role as adhesive agent between the contaminants and substrate to increase the dirt on materials in addition to that caused by the existence of itself<sup>7-9</sup>.

In this study, various composite films composed of dispersed metal organic compounds in silane compound films were proposed to control the biofilm formation. Biofilm was formed artificially

by a novel laboratory biofilm reactor (LBR) and evaluated by a 3D light microscopy and UV-VIS properly.

### Experimental

#### Specimens and Coating

Commercial glasses (general float glasses) were used as substrate. Before the coating, all glass samples were cleaned in an ultrasonic bath filled with tap water including surface active agents. Then they were rinsed by distilled water to remove tiny glass butt and/or oil contaminants. After the cleaning process, they were immersed into a solution composed of equivolume hydrogen peroxide solution and ammonium water at 110 degrees Celsius, which made the glass surface hydrophilic. Then the hydrophilic surface of glass specimen was painted and coated by various silane compound films. The thickness of film was about 7.5  $\mu\text{m}$  – 10  $\mu\text{m}$  which was confirmed by a light reflection microscope in advance. In the silane matrix, some organic metals and nano metal powders were dispersed finely. The nano metal powders were titanium, silver, copper, tin and nickel. And the organic compounds including those metals were also used as dispersed component, respectively. Regarding the organic compounds, organic titanium and copper were alkoxide basically. On the other hand, organic silver, tin and nickel were acetate. These silane films were hardened by the moisture in the air to produce good adhesive surface films<sup>10-12</sup>. The surface roughness by the coating was in advance adjusted to the thickness in submicron order (several hundred nanometers), so that the bacterial attachment would not affect the results. Previous studies show that the surface roughness would affect the biofilm formation<sup>5</sup>. Therefore, the surface roughness was checked by a surface roughness tester (Surfcoder SE-1700 $\alpha$ , Kosaka Laboratory). Other factors, for example, surface tension, contact angles etc. are generally involved into the biofilm formation<sup>6</sup>. However, they were not measured in

this current paper, so that the phenomenon should be simplified as much as possible.

### Laboratory Biofilm Reactor (LBR) and the Procedure for Biofilm Formation<sup>13-19</sup>

Figure 1 shows the schematic illustration for the biofilm formation<sup>13</sup>. It enables biofilm to be formed by planktonic germs in ambient air mainly. The apparatus is basically composed of a column, water tank, pump and pipes connecting those units by PVC. All of them constituted a cyclic water system. The column was made of transparent acrylic cylinder. The clean water was accumulated at the lower tank. It was pumped up through the pipe to the upper column. The water was flown into the column and out to the pipe at the other end. In the column, those glasses of 10 x 20mm (The thickness: 1.2mm-1.5mm) were placed with a supporter. The flow direction in the column was parallel to the specimen's surface and specimens could occupy the upper and lower positions. The water flowing out of the column was introduced into the tank. However, a plate was placed just above the tank and the water was designed to run down on it before it was poured down into the tank. A fan was also set above the plate and the ambient air was designed to blow down on it from the fan. On the intermediate plate, air including planktonic germs was mixed into the water and the mixed water was circulated. The cycle was repeated during the test. The test term was within 10 days. The specimen after the exposure was taken out of the system properly and served for the following some observations and analyses.

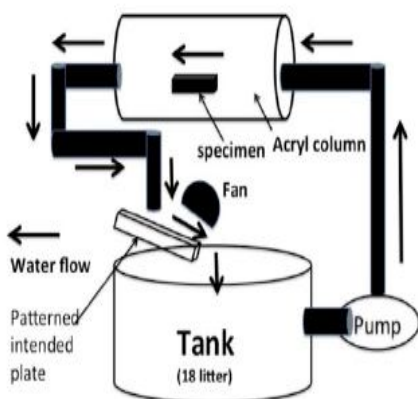


Figure 1: A laboratory biofilm reactor used in this experiment.

### Evaluations and Analyses for Biofilm

Biofilm was observed and confirmed by the two methods. One of them was the optical microscopic observation<sup>6</sup>. The contaminants attached to glasses were composed of silicate complexes, calcium carbonate, organic matters and biofilm itself usually. However, the contaminants were stuck to the glass surface persistently due to the existence of biofilm, since it produced sticky EPS (exopolymeric substances). Therefore, the existence of biofilm could be recognized easily as fouling and dirt on glasses after the test by the LBR shown in Fig.1. The light reflection microscopy used for the measurement was Keyence VH-Z100R). The image was obtained by bright field illumination basically. The light was irradiated from the right angle to the specimen put on the stage of light scope. Then the reflecting light was analyzed. Usually, the transparent glass was hard to be observed. However, the microscope could move the focus very slightly with its automation function. Therefore, it was easily detected and decided correctly which side of glass samples were observed. Particularly in this study, the specimen surface after the immersion into the LBR was stained by crystal violet and the

biofilm was dyed in blue color, since the crystal violet could stain particularly gram-positive bacteria as well as extracellular DNA. The stained surface of the specimen could be easily observed and told from contaminants without biofilm.

In addition to the microscopic observation, the biofilm was observed and evaluated by transmissivity of glasses after the immersion into the LBR. The evaluation was based on our previous experiments that biofilm growth observed by Giemsa stain had a certain correlation with the decrease of transmissivity for glasses<sup>13</sup>. The measurement for transmissivity of glasses was carried out by an ultraviolet-visible spectroscopy (UV-VIS, JEOL V-670). The wavelength used for the measurement ranged from 250 to 1000nm most of which belonged to the visible range. All of the specimens before the immersion into the LBR and after that were served for the measurement.

## Results and Discussion

### Design principle for nano composite films and the effects

Figure 2 shows the optical microscopic photos for the glasses coated only by silane compound film before (Fig.2-1) and after immersion (Fig.2-2). Before the immersion, biofilm was not observed on the surface, even though the surface's asperity was increased from the original glass one. Therefore, the transmissivity decreased, being compared than the original substrate glass. The high asperity gave the appearance as shown in Fig.2-2. As a result, only silane compound film could not control the biofilm, since high asperity accelerated to produce form very easily<sup>20-22</sup>. However, the silane compound is pretty stable under the ultraviolet light from the sun. In addition to that, the adhesive force of the film is relatively high, since the silane compound is condensed and hardened with the moisture in the air. To control biofilm formation, antibacterial metals were deposited on glasses by sputtering in our previous study<sup>23</sup>. Biofilm could be controlled well, while the original transmissivity decreased with sputtering drastically. And the dissolution of antibacterial metal films (Silver etc.) could not be controlled well and the film consumption due to the dissolution was too much to maintain the coating film in a very long time.

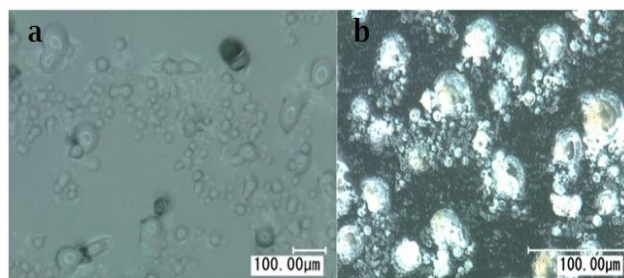
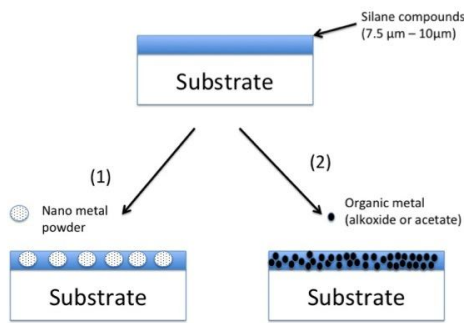


Figure 2: Optical microscopic observation of surfaces for the silane compound films on glasses (a) Before the immersion (b) After the immersion

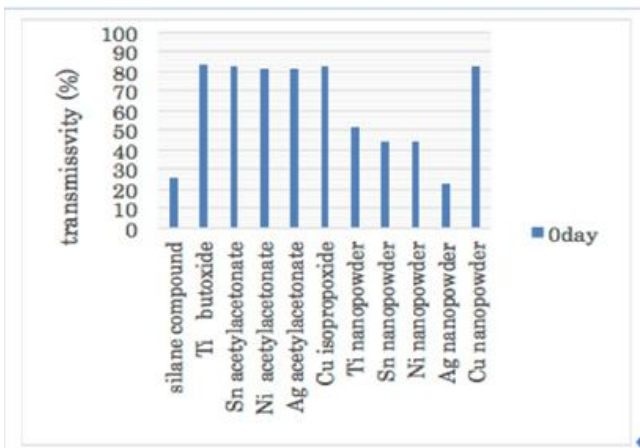
Therefore, As shown in Fig.3-1 schematically, the silane compound films with dispersed nano metallic powders were designed firstly. If the dispersed metals in the silane compound would dissolve on the wet glass surfaces as metallic ions properly, they might work well to suppress the biofilm growth with their antibacterial properties. However, high transmissivity, one of the requirements for the purpose of our current study, tended to decrease remarkably for the nano powder dispersion silane compound film. Then the antibacterial metals were incorporated as a part of organic compounds and scattered into the silane compound film, schematically shown in Fig.3-2. In this way, the antibacterial effect to control biofilm formation would be

maintained in the silane film, while the transmissivity was expected to be kept relatively high after the dispersion.



**Figure 3:** The design concept for nano-composite films in this study.

Figure 4 shows the transmissivity of all silane compound films with and without dispersed antibacterial metal powders or organic metals before the immersion test. Fortunately, the transmissivity was improved drastically, when the organic metals were dispersed in the silane films (more than 80% in all cases). On the other hand, the silane compound films with dispersed nano metal powders showed relatively low transmissivity, even though they were improved to some extent than that of the original silane compound film. Nano copper powder dispersed silane film was only an exception and it showed relatively higher transmissivity like organic metal dispersed ones. Even though the copper nano metal powder was an exception, the general tendency was that the dispersion of organic metals was more favorable than that of metals themselves from the viewpoint of transmissivity. It suggests that the dispersion was more uniform and homogeneous for organic metals. Therefore, we focused on the silane compound film specimens with dispersed organic metals and investigated the biofilm formation behaviors in the following section.

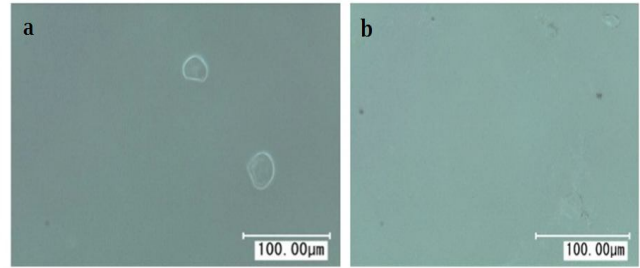


**Figure 4:** Initial transmissivity for silane compound films with dispersed nano metal powders and organic metals.

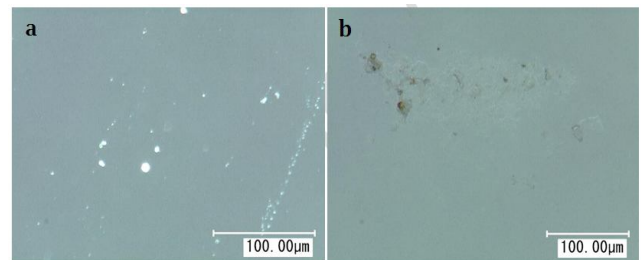
#### **Silane compound films with dispersed antibacterial organic metals**

Figure 5, Fig.6, Fig.7 and Fig.8 show the optical microscopic photos for glasses coated by silane film with dispersed organic titanium, silver, tin and copper. For all of those specimens, the biofilm formation was suppressed and well controlled even after the immersion in LBR, as shown in these photos. On the other hand, the silane compound film with dispersed organic nickel did not suppress biofilm formation on its surface at all, as shown in

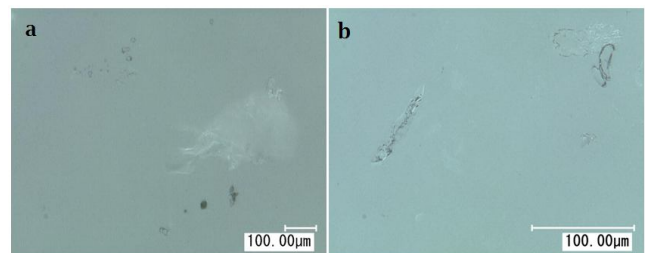
Fig.9. For all of those specimens, the transmissivity was measured by UV-VIS. Figure 10 – Fig.15 show these results. Each figure indicates respectively how the transmissivity (the vertical axis) changed with wavelength (the horizontal axis) almost in the visible light range (250 nanometers to 1000 nanometers).



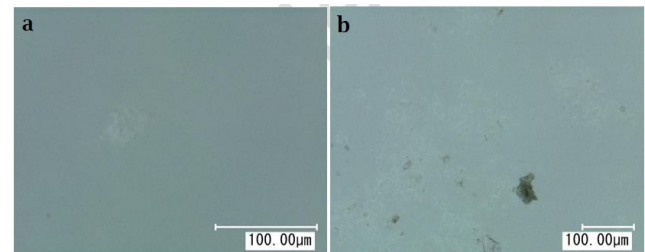
**Figure 5:** Optical microscopic observation for silane compound films with dispersed *organic titanium* before and after 14 days long immersion (a) before immersion (b) after immersion



**Figure 6:** Optical microscopic observation for silane compound films with dispersed *organic silver* before and after 14 days long immersion (a) before immersion (b) after immersion.



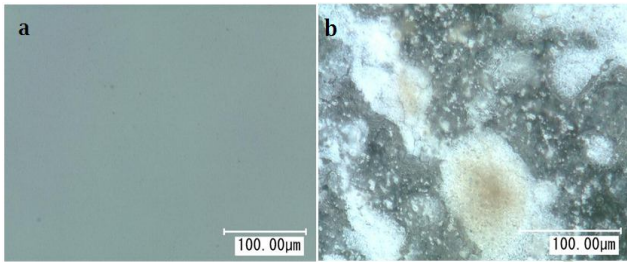
**Figure 7:** Optical microscopic observation for silane compound films with dispersed *organic tin* before and after 14 days long immersion (a) before immersion (b) after immersion.



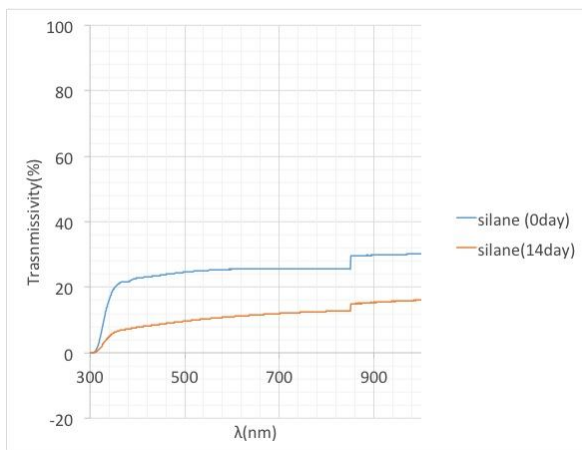
**Figure 8:** Optical microscopic observation for silane compound films with dispersed *organic copper* before and after 14 days long immersion (a) before immersion (b) after immersion.

Figure 10 shows the result for the silane compound film specimen without any dispersion components. As shown in Fig. 2 and Fig.4, the original transmissivity of silane compound film specimen was relatively low (35% at most even before the immersion). Correspondingly, the transmissivity for both

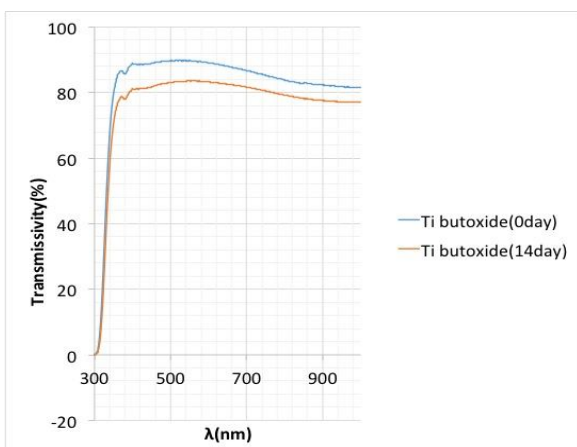
specimens in Fig.10 was kept low as a whole, being compared with other specimens, since the specimen after the immersion decreased the transmissivity further. It could be attributed to the promotion of biofilm formation and growth on silane compound films. Fig.11 shows the change of transmissivity with wavelength by UV-VIS for the silane compound film with dispersed organic titanium before and after the immersion into the LBR. For this specimen, the original transmissivity was relatively high and even after the immersion in LBR, the specimen's transmissivity did not decrease so much and was kept almost at 80%. The tendencies for those with dispersed organic silver, tin, and copper, even though the extents differed from specimen to specimen. However, the transmissivity decreased drastically after the immersion for the silane compound film with dispersed organic nickel (Fig.12).



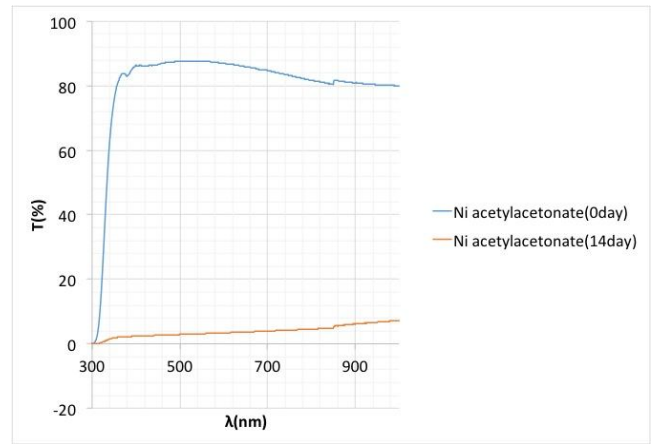
**Figure 9:** Optical microscopic observation for silane compound films with dispersed *organic nickel* before and after 14 days long immersion (a) before immersion (b) after immersion



**Figure 10:** Transmissivity of silane compound films before and after the immersion.

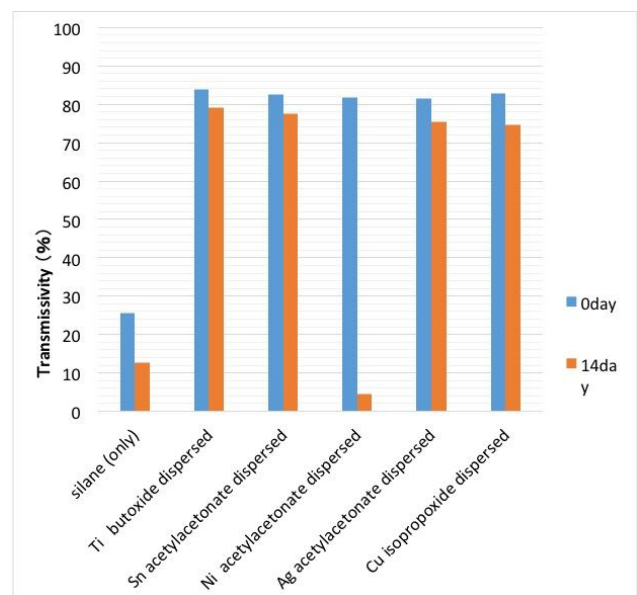


**Figure 11:** Transmissivity of silane compound films with dispersed organic *titanium* before and after the immersion.



**Figure 12:** Transmissivity of silane compound films with dispersed organic *nickel* before and after the immersion

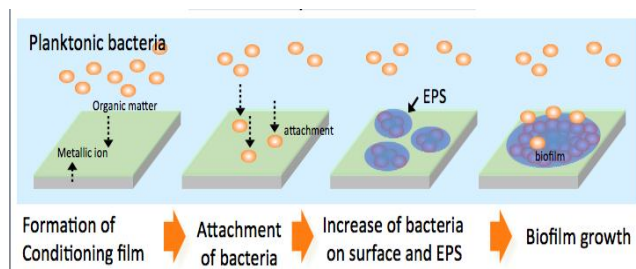
Fortunately, the transmissivity curve in the range was relatively stable and constant. Particularly, the tendency was remarkable in the relatively higher wave length range. Therefore, the transmissivity value at 800 nm was selected for all of these specimens and compared each other. The results were shown in Fig.13. The silane film without dispersed components showed pretty lower transmissivity and it decreased after the immersion in the LBR. For the specimens with dispersed organic titanium, tin, silver and copper, the transmissivity was relatively high and it did not decrease so much after the immersion. It suggests that the effective suppression of biofilm formation was remarkable for the silane film with dispersed organic nickel. As shown in Fig.9, the film did not show any positive effect to control biofilm formation.



**Figure 13:** Transmissivity changes for various silane compound films before and after the immersion.

For the silane film with dispersed organic titanium, tin, silver and copper, biofilm formation was well controlled, as shown in these results. Many possibilities for the reason could be mentioned, since biofouling (the process related to biofilm formation and growth) is composed of multi-steps and complicated. However, the antibacterial effect should be one of the main factors to control biofilm formation and growth<sup>23</sup>. Antifouling/antibacterial

materials surfaces should be the one where the increase of bacterial number would be inhibited and well controlled. For the silane film with dispersed silver and copper, antibacterial effect by the dissociation and dissolution of these metals worked well to control biofilm formation obviously. Usually, the metallic element dissolves too easily, when the metallic film forms on the substrate. However in this case, the metal existed in the compound dispersing in the substrate film. Therefore, the dissolution and dissociation process of metals did not affect the entire film so seriously. On the other hand, silver and copper ions need low concentration to control the bacterial growth generally<sup>24</sup>. Therefore, the silane films with dispersed organic silver and copper worked well to control biofilm formation. As for the film with dispersed organic titanium and tin, the mechanism for the mediation of biofilm formation should be still considered. Titanium have shown antibacterial effect so far<sup>25</sup>. However, in the most cases, the reason should be attributed to its photocatalytic effect and the ionic effect by its correlation with the outer membrane of bacterial cells has not been confirmed precisely. And also for the silane compound film with dispersed organic tin, the mechanism has not been clear yet. Since tin ion does not show antibacterial effect at all generally, we must seek for another reason for their anti-biofouling effect<sup>26</sup>. Biofilm formation is composed of multi-step processes, as shown in Fig.14 Generally, the conditioning film forms on a solid substrate initially, then bacteria approach to it, repeat attachment/detachment/reattachment processes, biofilm formation and grow after that. When only the antibacterial effect would be focused, the bacterial formation and grow on the coated film might be involved. However, organic tin might affect other steps due to some unknown reasons at this point. From the viewpoint, the ineffectiveness for the silane film with dispersed organic nickel should be also explained.



**Figure 14:** Schematic illustration for the mechanism of biofilm formation and growth on material surfaces.

The researchers have dealt with the same or similar problems relating to dirt on materials in the past. However, all of their investigations were carried out from the viewpoint of water repellency and hydrophobicity. In this study, we developed silane based composite film with some metals and their organic compounds and confirmed the effectiveness of the films against dirt on glasses. It was available by paying attention to the role of biofilm for dirt on materials<sup>27-31</sup>.

## Conclusions

Some nano composite films based on silane compound film with dispersed organic metals and metal powders were investigated to control and mediate biofilm formation and growth. The substrate material was glasses applicable to solar power generation. The biofilm formation was evaluated by an optical microscope observation as well as transmissivity.

The latter was evaluated by an UV- visible spectrophotometric analysis. Since the potential application for the composite films was the glass mirror of solar power generation apparatuses, the

transmissivity had the double importance and meanings in this study. The following results were obtained.

1. The silane compound film was vulnerable to biofilm formation and growth, even though it had high adhesiveness and stable under ultraviolet light. In addition, it decreased with remarkable biofilm formation and growth after immersion.
2. The silane film with dispersed nano metal powders such as copper, silver etc. might control biofilm formation. However, the initial transmissivity for the primary potential application was insufficient for all of specimens. Therefore, organic metals were used as dispersion agent.
3. The silane films with dispersed organic metals such as titanium, silver, tin and copper worked well to control or to mediate biofilm formation and growth.
4. The silane films with dispersed organic nickel could not suppress biofilm formation and growth at all.
5. The cause to control biofilm formation and growth by the silane compound films with dispersed organic metal would be attributed to the ions free from the compounds and films themselves and their correlation with proteins on bacterial cells. For organic silver and copper, the mechanism could be explained by their strong antibacterial effects. However, more detailed investigations will be needed further to clarify the reasons for organic titanium and tin which don't have any remarkable antibacterial effects.

## Acknowledgement

This experiment was carried out, supported financially by NEDO (New Energy and Industrial Technology Development Organization). The project title was "the development of minimum life cycle cost heliostat by the utilization of biotechnology and anti-fogged glass". And our study was also supported by Kakenhi(JSPS, grant number: 24656460) partly. We authors appreciate Hikari Kikai Seisakusho Co., LTD. (8-1 Isshinden Nakano, Tsu-city, Mie 514-0112) for their great contribution and advices.

## References

1. Characklis, W.G. and K.C. Marshall, *Biofilms*, ed. W.G. Characklis and K.C. Marshall 1990, New York: John Wiley & Sons, Inc., New York, the USA.
2. Flemming, H.-C., *Biofilme, Biofouling und mikrobielle Schädigung von Werkstoffen*. Stuttgarter Berichte zur Siedlungswasserwirtschaft 1994, München: Oldenbourg, München, Germany. Xvi.
3. Lappin-Scott, H.M. and J.W. Costerton, *Microbial biofilms*, Plant and microbial biotechnology research series. 1995, Cambridge, United Kingdom: Cambridge University Press, Cambridge; New York.
4. Costerton, J.W., *Introduction to biofilm*. International Journal of Antimicrobial Agents., 1999. **11**: p. 217-221; discussion 237-239.
5. Gharechahi, Maryam, Moosavi, Horieh, and Forghani, Maryam, Effect of Surface Roughness and Materials Composition on Biofilm Formation. *Journal of Biomaterials and Nanobiotechnology*, 2012. **3**: p. 541-546.
6. Lewandowski, Z. and H. Beyenal, *Fundamentals of Biofilm Research*. Second Edition 2014, Boca Raton, London, New York: CRC Press.
7. Characklis, W.G., *Fouling biofilm development: a process analysis*. *Biotechnology and Bioengineering*, 1981. **23**: p. 1923-1960.
8. Fletcher, M., *Bacterial biofilms and biofouling*. *Curr Opin Biotechnol*, 1994. **5**(3): p. 302-6.

9. Hall-Stoodley, L., Costerton, JW., and Stoodley, P., Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol*, 2004. **2**(2): p. 95-108.
10. Kanematsu, H., D.M. Barry, P. McGrath, and A. Ohmori, Corrosion Protection of a Metal Spray Coating by Using an Inorganic Sealing Agent for Its Micropores. *ITSC 2004*,
11. Kanematsu, H., S. Fujimoto, K. Nogi, and T. Oki, Application of Inorganic Silicon Sealer to Anodic Oxide Coating. *AESF Sur/Fin 2004 & Interfinish 2004 World Congress*, 2004: p. 478-485.
12. Kanematsu, H., K. Murakami, and K. Nakata, Surface Finishing of Concrete Structure by a Silane Series Solvent. *Sur/Fin 2009*,
13. Kanematsu, Hideyuki, Kogo, Takeshi, Itoh, Hideo, Wada, Noriyuki, and Yoshitake, Michiko, Fogged Glass by Biofilm Formation and Its Evaluation, in *Proceedings of MS & T' 13 2013: Montreal, Quebec, Canada*. p. 2427-2433.
14. Kanematsu, H., D. Kuroda, S. Koya, S. Shimada, H. Ikigai, and H. Itoh, Some New Evaluation Methods for Biofouling on Metallic Materials on Laboratory Scale and Their Results, in *The 16th Annual International Congress on Marine Corrosion and Fouling 2012: Seattle, Washington State, USA*. p. 71.
15. Kanematsu, H., D. Kuroda, H. Itoh, and H. Ikigai, Biofilm Formation of a Closed Loop System And Its Visualization. *CAMP-ISIJ*, 2012. **25**: p. 753-754.
16. Kanematsu, H., D. Kuroda, S. Koya, and H. Itoh, Development of Production Process on Labo Scale for Biofilm Formation by Immersion into Closed Circulation Water System. *Hyoumen Gijutsu (Journal of Surface Finishing Society of Japan)*, 2012. **63**(7): p. 459-461.
17. Kanematsu, H., T. Hihara, T. Ishihara, K. Imura, and T. Kogo, Evaluation for Corrosion Resistance of Nano-Cluster Layer and Biofilm Formation. *International Journal of Engineering Sciences & Research Technology*, 2013. **2**(9): p. 2424-2432.
18. Kanematsu, H., T. Kougo, D. Kuroda, Y. Ogino, and Y. Yamamoto, Biofilm Formation Derived from Ambient Air and the Characteristics of Apparatus. *Journal of Physics: Conference Series*, 2013. **433**: p. 012031: 1-6.
19. Kanematsu, H., T. Kogo, D. Kuroda, H. Itoh, and S. Kirihara, Biofilm Formation and Evaluation for Spray Coated Metal Films on Laboratory Scale, in *Thermal Spray 2013 - Innovative Coating Solutions for the Global Economy 2013*, ASM International: Busan, Republic of Korea. p. 520-525.
20. Pons, L., Delia, ML., and Bergel, A., Effect of surface roughness, biofilm coverage and biofilm structure on the electrochemical efficiency of microbial cathodes. *Journal of Biotechnology*, 2011. **102**(3): p. 2678-2683.
21. Percival, SL., Knapp, JS., Wales, DS., and Edyvean, RGJ, The effect of turbulent flow and surface roughness on biofilm formation in drinking water. *Journal of Industrial Microbiology and Biotechnology*, 1999. **22**: p. 152-159.
22. Schultz, M. P., Effects of coating roughness and biofouling on ship resistance and powering. *Biofouling*, 2007. **23**(5-6): p. 331-41.
23. Kougo, T., H. Kanematsu, N. Wada, T. Hihara, M. Minekawa, and Y. Fujita, Meta coated glasses by sputtering and their microfouling properties. *AIP Conference Proceedings*, 2014. 1585: p. 160-163.
24. Yotaro Murakami, Current Situation for Antibacterial Materials, in *MMC New 2005*. p. 7.
25. Kanematsu, H., H. Ikegai, and M. Yoshitake, Patents for Antibacterial Metallic Coating and Its Future Trend in Japan. *Research Inventory: International Journal of Engineering and Science*, 2013. **3**(6): p. 47-55.
26. Kanematsu, H., Alloy Plating by Heating Stacked Single Layers and the Possibility of its Application in the Future. *Products Finishing*, 2013. **78**(3): p. 1-14.
27. Hsu, C.-C., W.-L. Lan, N.-P. Chen, and C.-C. Wu, The hydrophobic omnidirectional antireflection coating of SiO<sub>2</sub> nanospheres with C18-TEOS. *Optics & Laser Technology*, 2014. **58**: p. 202-206.
28. Dave, V., H.O. Gupta, and R. Chandra, Nanostructured hydrophobic DC sputtered inorganic oxide coating for outdoor glass insulators. *Applied Surface Science*, 2014. **295**: p. 231-239.
29. Wang, C. and X. He, Preparation of hydrophobic coating on glass surface by dielectric barrier discharge using a 16kHz power supply. *Applied Surface Science*, 2014. **252**: p. 8348-8351.
30. Trespidi, F., G. Timo, F. Galeotti, and M. Pasini, PDMS antireflection nano-coating for glass substrates. *Microelectronic Engineering*, 2014. **126**: p. 13-18.
31. Ganbavle, V.V., U.K.H. Bangi, S.S. Latthe, S.A. Mahadik, and A.V. Rao, Self-cleaning silica coatings on glass by single step sol-gel route. *Surface & Coatings Technology*, 2011. **205**: p. 5338-5344.