

The Influence of Urinary Catheter Materials on Forming Biofilms of Microorganisms

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Biofilms are commonly associated with an increased risk of catheter-associated infection. To study the efficacy of materials designed to reduce biofilm formation, microbial biofilms on clinically used urinary catheter were examined. We performed 2, 3-bis (2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay to determine of biofilm formation ability and observed with scanning electron microscopy (SEM) to analyze biofilm architecture. Additionally, we calculated relative cell surface hydrophobicity (CSH) to measure hydrophobicity of microorganisms. On SEM, catheter surfaces made of latex or anti-infective (IC)-latex were rough but those of silicone, hydrogel-coated silicone (HCS), or silver-alloy-coated silicone (SCS) were relatively smoother. According to XTT reduction assay, biofilm formation was reduced on the surface of smooth silicone-based catheters compared to rough latex-based catheters. The greatest to lowest formation of microbial biofilm were as follows for these material types: silicone-elastomer-coated (SEC) latex > latex > silicone > IC-latex > HCS > SCS. Catheter materials can affect the microbial biofilm formations. First, rougher surfaces on the catheter made the microbial attachment easier and a greater amount of biofilm was formed. Second, when chemicals that inhibit growth and attachment of microorganisms on the inner and outer surfaces of the catheters were applied, the biofilm formation was inhibited. SCS was found to be the most effective in reducing the microbial biofilm formation. These results indicate that microbial biofilm formation may be closely related to the surface roughness and microbial CSH.

Key Words: Biofilms, Urinary catheters, Hydrophobic interactions

INTRODUCTION

Among nosocomial infections, urinary tract infection (UTI) is the most common, and its major cause is the

urinary catheter, which is an implanted medical device (1). A number of hospitalized patients have urinary catheters inserted at least once during their stay; and patients receiving elective operations or who are in intensive care units are most likely to be using a urinary catheter (2, 3). Approxi-

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mately 5% of patients residing in long-term care facilities have urinary catheters inserted continuously. In cases where urinary catheters are inserted for a short period, most infections start with single microorganism and *Escherichia coli* (*E. coli*) is most frequently isolated. *Pseudomonas aeruginosa* (*P. aeruginosa*), *Enterococci*, *Klebsiella*, and *Candida* can also be involved (4, 5). Otherwise when catheters are inserted over a long term, an average of 3~5 species of bacteria are found at any time when urine samples are collected. These species include various *Enterobacteriaceae*, Gram-negative bacteria, Gram-positive bacteria, and fungi (6, 7).

Microorganisms may exist in a free-living or planktonic state, but they also have characteristics of being sessile to the surface of other organisms or non-living substances and forming biofilm (8). Biofilm formation is a critical problem in the development of catheter-associated UTI (CA-UTI). The microorganisms of the urinary catheter colonize in the form of a biofilm with single-layer cells or multi-layer cells surrounded by a matrix of extracellular polymeric substances (9). When biofilms of microorganisms form on a urinary catheter, encrustation and blockage may occur at the insertion site, and bacteria hidden in the biofilm or a portion of biofilm may break off and spread to other parts of the body (10). Compared to planktonic microorganisms, those existing within the biofilm have resistance against the host immune system, and they show drug resistance toward antibiotic treatment. Therefore, infections related to implanted medical devices are difficult to treat, and they eventually lead to removal of the device (11, 12).

In the past decades, researchers have focused on biofilm of microorganisms that form on implanted medical devices, and they have studied the micro-colonies forming on various solid surfaces. As the first step, it was found that the biofilm formation may differ based on chemical and structural differences in the surfaces where microorganisms attach (11). Therefore, in many studies attempts have been made to develop urinary catheter materials that reduce or prevent microorganism attachment and biofilm formation (13).

To assay an effective way to eliminate the biofilm formation, we, in this study, examined the biofilm formation of

UTI causative microorganisms according to the mechanical and physical properties of the catheter materials.

MATERIALS AND METHODS

Microorganisms and culture conditions

Clinical isolates of *Candida albicans* (*C. albicans*), *E. coli*, *Proteus vulgaris* (*P. vulgaris*), *Staphylococcus aureus* (*S. aureus*), and *Streptococcus salivarius* (*S. salivarius*) were obtained from the blood cultures of patients from Wonju Severance Christian Hospital at Yonsei University Wonju College of Medicine.

Prior to each experiments *C. albicans* isolates were cultured at 30°C for 18 h on Sabouraud's dextrose agar (SD, Difco™, BD diagnostics, Sparks, MD, USA), and a loopful of growth was inoculated into yeast nitrogen base (YNB, Difco™) medium supplemented with 50 mM glucose. *S. salivarius* were first subcultured at 37°C for two days on blood agar. A loopful of bacteria was inoculated into brain heart infusion (BHI, Difco™) medium and incubated at 37°C in a 5% CO₂ incubator for two days. *S. aureus*, *E. coli* and *P. vulgaris* were cultured at 37°C for 18 h on trypticase soy agar (Difco™) and then a loopful of bacteria was inoculated into trypticase soy broth (TSB, Difco™).

Urinary catheters

Six materials of commercially available urinary catheters (24 Fr) were used. The following latex-based catheters were used: latex (Unomedical, Kongevejen, Birkerød, Denmark), anti-infective latex (IC-latex, C. R. Bard, Inc., Covington, GA, USA), and silicone-elastomer-coated latex (SEC, Ah Sung international INC., Geumcheon, Seoul, Korea). For silicon-based catheters, catheters of 100% silicone (ONESP Co., Ltd., Bucheon, Gyeonggi, Korea), hydrogel-coated silicone (HCS), and silver-alloy-coated silicone (SCS) were purchased from C. R. Bard.

XTT reduction assay

Biofilm formation was quantified using the method developed by Ramage, *et al.* (14). *C. albicans* was inoculated in YNB supplemented with 50 mM glucose for 18 h at 30°C.

S. salivarius was inoculated in BHI medium and incubated at 37°C, 5% CO₂ incubator for two days. *S. aureus*, *E. coli* and *P. vulgaris* were inoculated in TSB medium and incubated at 37°C for 18 h. Catheter samples (diameter of 7 mm) of each material were prepared by using a hole-puncher and were placed in flat-bottom 96-well microtiter plate (Costar, Cambridge, MA, USA) and sterilized with ethylene oxide gas. *C. albicans* (OD₆₀₀=0.2) or each strain of bacteria (OD₆₀₀=0.2) were prepared and transferred into selected wells of a microtiter plate. The plate was incubated for 90 min at 37°C in orbital shaker at 75 rpm. After the initial adhesion phase, the cell suspensions were aspirated, and each well was washed twice with phosphate-buffered saline (PBS) to remove weakly adherent cells. A volume of 200 µl of medium was added to each well, and the plate was then incubated for 72 h. After biofilm formation, the medium was aspirated, and non-adherent cells were removed by washing the biofilm three times with PBS. A quantitative measure of biofilm formation was calculated using 2, 3-bis (2-methoxy-4-nitro-5-sulfo-phenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction assay. A 200 µl aliquot of XTT (1 mg/ml, Sigma, St. Louis, MO, USA) and menadione (0.4 mM, Sigma) solution was then added to each well. The plates were incubated in the dark for up to 18 h at 37°C. A colorimetric change resulting from XTT reduction was measured using a microtiter plate reader (Emax, Molecular Devices, Sunnyvale, CA, USA) at 490 nm.

Cell surface hydrophobicity (CSH) assay

The hydrophobicity of microorganisms was measured according to the protocol described by Rosenberg (15). Briefly, microorganisms grown overnight at 37°C, were harvest and washed twice with PBS. The microorganism suspension displaying an OD_{590nm} between 0.9 and 1 was prepared in PBS; 3 ml of this microorganism suspension was overlaid by 0.8 ml of the hydrophobic hydrocarbon, n-hexane (Sigma). After vigorous vortexing for 90 sec, phases were allowed to separate for 10 min and OD_{590nm} of the aqueous phase was measured. The relative CSH was calculated as follows.

$$\text{Relative CSH} = \frac{(\text{A590 of Controls}) - (\text{A590 of Treated cells})}{\text{A590 of Controls}} \times 100$$

Scanning electron microscopy (SEM)

For observation on SEM, the urinary catheter disks with the formed biofilm were washed twice with PBS and were fixed in 2.5% glutaraldehyde. They were processed with 1% osmium tetroxide for 1 h, and the fixed catheter disks were dehydrated by processing for 10 min in 70% ethanol, 10 min in 95% ethanol, and 20 min in 100% ethanol. The dehydrated catheter disks were observed under the SEM (TM-1000, Hitachi, Tokyo, Japan, 15 kV) after gold plating.

Statistical analysis

All experiments were repeated three times; and for all data, standard deviation corresponding to the mean value is shown. The statistical analysis was conducted using *t*-test and Mann-Whitney U tests, and a *p* value of less than 0.05 was considered statistically significant.

RESULTS

Surface roughness

The surfaces of six catheter materials were observed using SEM. The latex catheter surface was very coarse compared to the silicone catheter. IC-latex catheter also had wave-shaped creases, but they were less distributed than the latex. SEC had a porous surface with furrows in some parts; 100% silicone and HCS had very smooth surfaces; SCS had a smooth surface and there were wave-shapes in some parts (Fig. 1).

Effects of catheter materials

The five microorganisms biofilm formation based on six urinary catheter materials were examined by the XTT reduction assay. *C. albicans* had the greatest formation with 2.913±0.180 optical density (OD) on SEC; 2.09±0.039 on latex; 1.39±0.055 on IC-latex; 2.105±0.020 on 100% silicone; 1.3±0.139 on HCS; and 0.83±0.02 on SCS, which

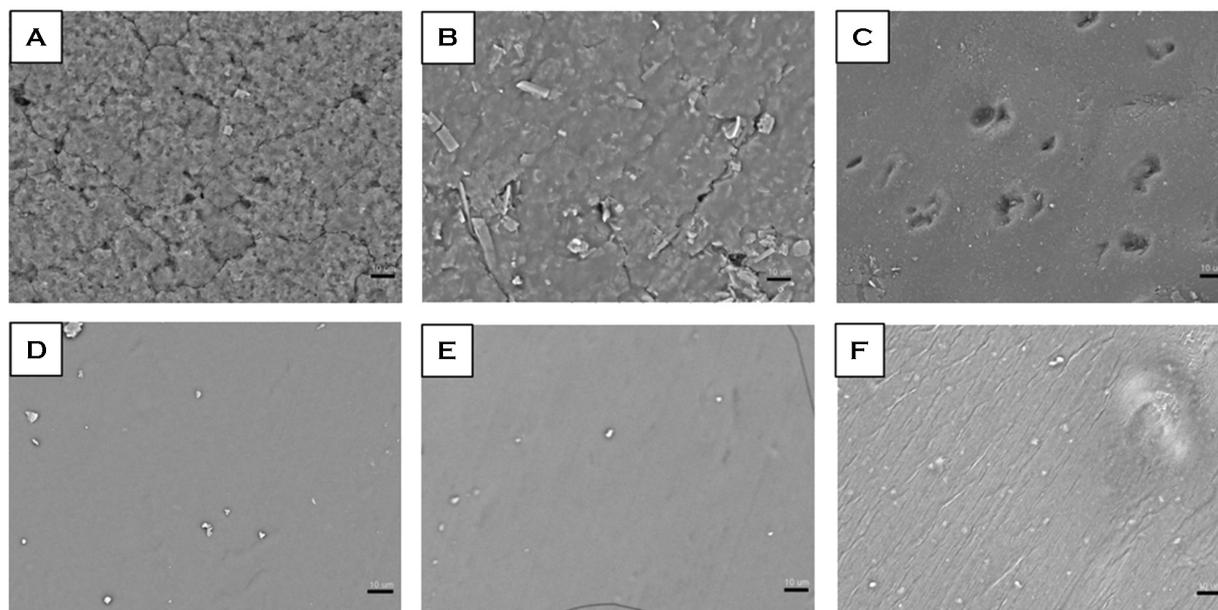


Figure 1. Scanning electron micrographs of the catheter surface. (A) Latex, (B) anti-infective latex (IC-Latex), (C) silicone-elastomer-coated latex (SEC), (D) 100% silicone, (E) hydrogel-coated silicone (HCS), (F) silver-alloy-coated silicone (SCS). (scale bar, 10 μ m)

Table 1. Biofilm formation ability of microorganisms on different catheter materials and cell surface hydrophobicity of microorganisms

| Species | Catheter materials | | | | | | CSH (%) |
|----------------------|-------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------------|
| | Latex | IC-Latex | SEC | Silicone | HCS | SCS | |
| <i>C. albicans</i> | 2.09 \pm 0.039 ^a | 1.39 \pm 0.055 | 2.913 \pm 0.180 | 2.105 \pm 0.020 | 1.3 \pm 0.139 | 0.83 \pm 0.02 | 58 \pm 1 ^b |
| <i>E. coli</i> | 0.462 \pm 0.067 | 0.35 \pm 0.03 | 0.617 \pm 0.085 | 0.416 \pm 0.014 | 0.221 \pm 0.023 | 0.163 \pm 0.051 | 86 \pm 0 |
| <i>P. vulgaris</i> | 0.59 \pm 0.162 | 0.387 \pm 0.094 | 0.612 \pm 0.005 | 0.486 \pm 0.08 | 0.379 \pm 0.02 | 0.235 \pm 0.053 | 76 \pm 0 |
| <i>S. aureus</i> | 0.783 \pm 0.155 | 0.647 \pm 0.057 | 0.858 \pm 0.013 | 0.699 \pm 0.080 | 0.579 \pm 0.049 | 0.390 \pm 0.081 | 99 \pm 3.7 |
| <i>S. salivarius</i> | 1.116 \pm 0.105 | 0.772 \pm 0.061 | 1.324 \pm 0.084 | 0.706 \pm 0.016 | 0.576 \pm 0.022 | 0.297 \pm 0.002 | 98 \pm 1.6 |

^a Values represent absorbance using XTT reduction assay. ^b Values represent percentage of CSH. Each value is the mean of three independent experiments carried out in triplicate. IC-latex, anti-infective latex; SEC, silicone-elastomer-coated latex; HCS, hydrogel-coated silicone; SCS, silver-alloy-coated silicone; CSH, cell surface hydrophobicity.

was the least formation. Other bacteria showed similar results to *C. albicans* (Table 1). The greatest to lowest formation of microbial biofilm were as follows for these material types: SEC > latex > silicone > IC-latex > HCS > SCS.

The CSH of microorganisms

The relative CSH value were as follows: *C. albicans*, 58%; *E. coli*, 86%; *P. vulgaris*, 76%; *S. aureus*, 99%; and *S.*

salivarius, 98% (Table 1). The relationship of CSH and microbial biofilm of different catheters were investigated. Latex, $r=0.708$; IC-latex, $r=0.878$; SEC, $r=0.742$; silicone, $r=0.788$; HCS, $r=0.719$; SCS, $r=0.702$ (data not shown). Significant correlation was found between the higher microbial CSH value and the more biofilm formation on catheter surfaces.

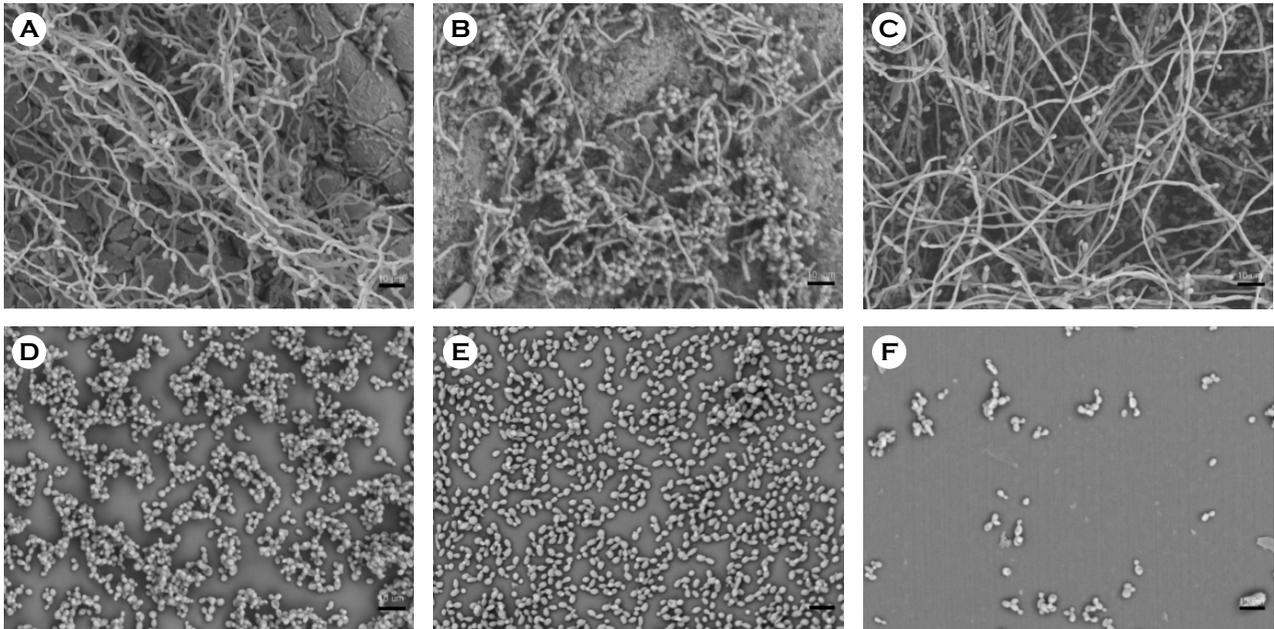


Figure 2. Scanning electron micrographs of biofilm formation by *C. albicans* on catheter discs. (A) Latex, (B) anti-infective latex (IC-Latex), (C) silicone-elastomer coated latex (SEC), (D) 100% silicone, (E) hydrogel-coated silicone (HCS), (F) silver-alloy-coated silicone (SCS). (scale bar, 10 μ m)

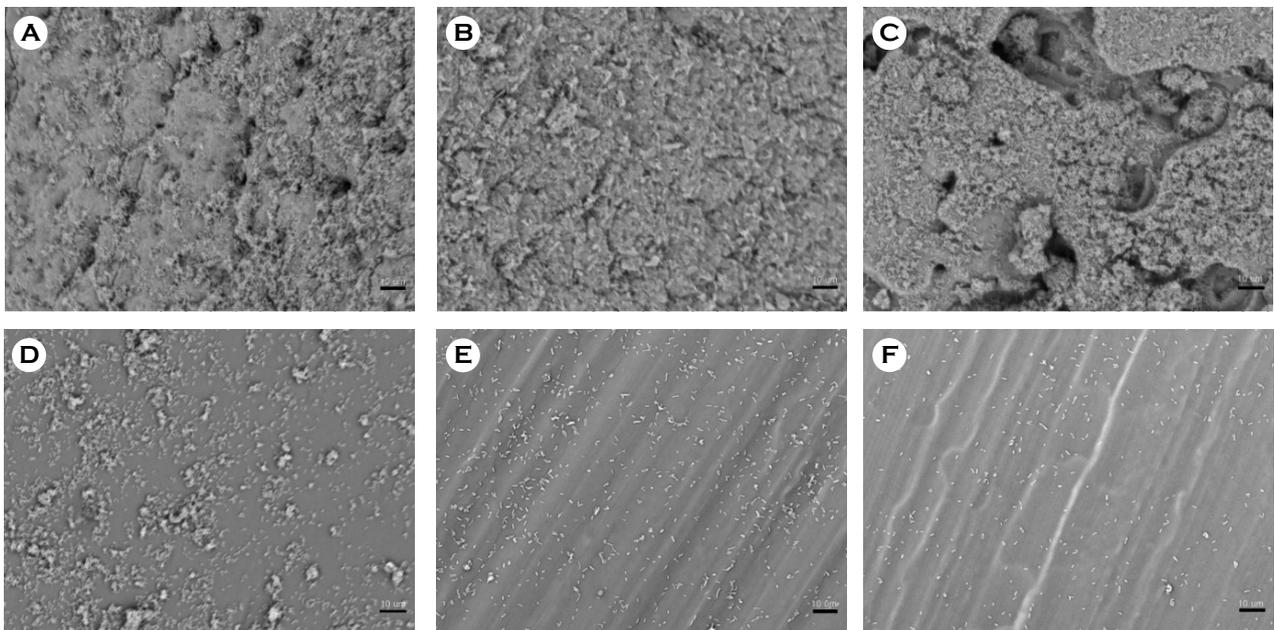


Figure 3. Scanning electron micrographs of biofilm formation by *E. coli* on catheter discs. (A) Latex, (B) anti-infective latex (IC-Latex), (C) silicone-elastomer coated latex (SEC), (D) 100% silicone, (E) hydrogel-coated silicone (HCS), (F) silver-alloy-coated (SCS). (scale bar, 10 μ m)

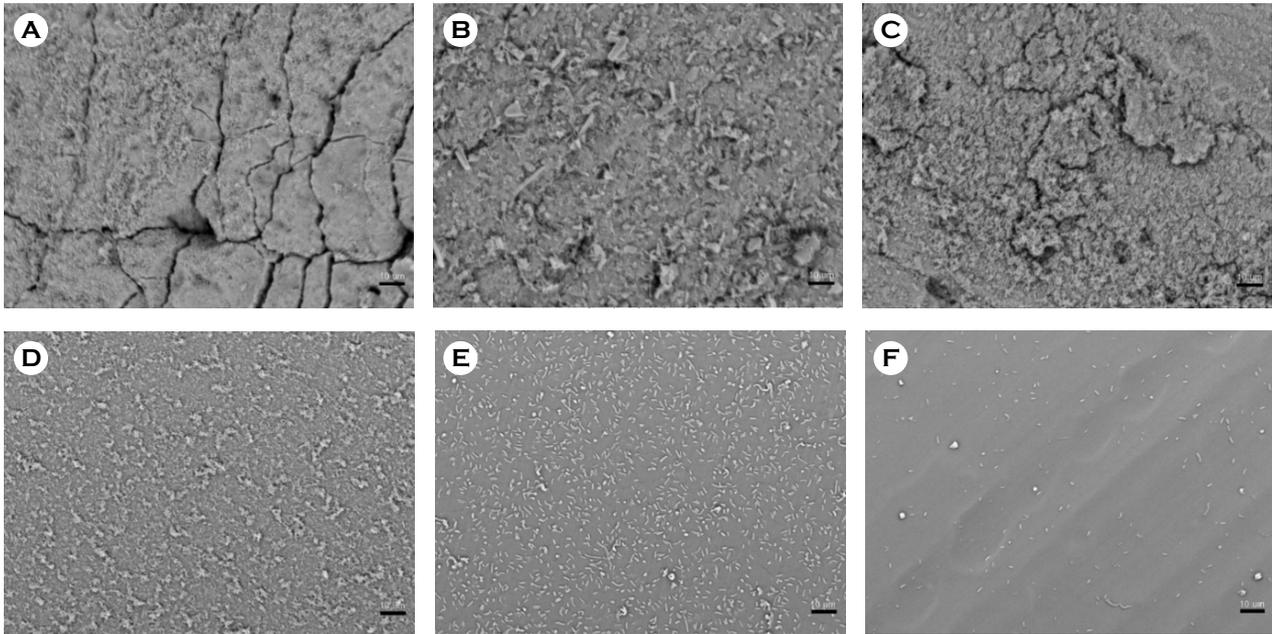


Figure 4. Scanning electron micrographs of biofilm formation by *P. vulgaris* on catheter discs. (A) Latex, (B) anti-infective latex (IC-Latex), (C) silicone-elastomer coated latex (SEC), (D) 100% silicone, (E) hydrogel-coated silicone (HCS), (F) silver-alloy-coated silicone (SCS). (scale bar, 10 μm)

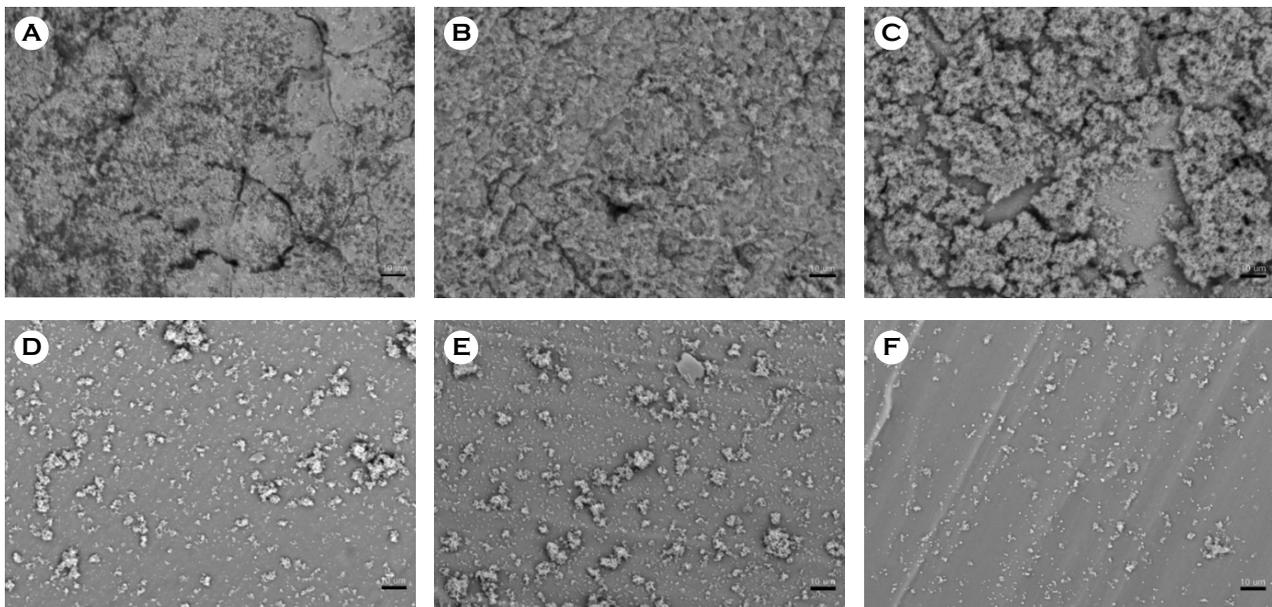


Figure 5. Scanning electron micrographs of biofilm formation by *S. aureus* on catheter discs. (A) Latex, (B) anti-infective latex (IC-Latex), (C) silicone-elastomer coated latex (SEC), (D) 100% silicone, (E) hydrogel-coated silicone (HCS), (F) silver-alloy-coated silicone (SCS). (scale bar, 10 μm)

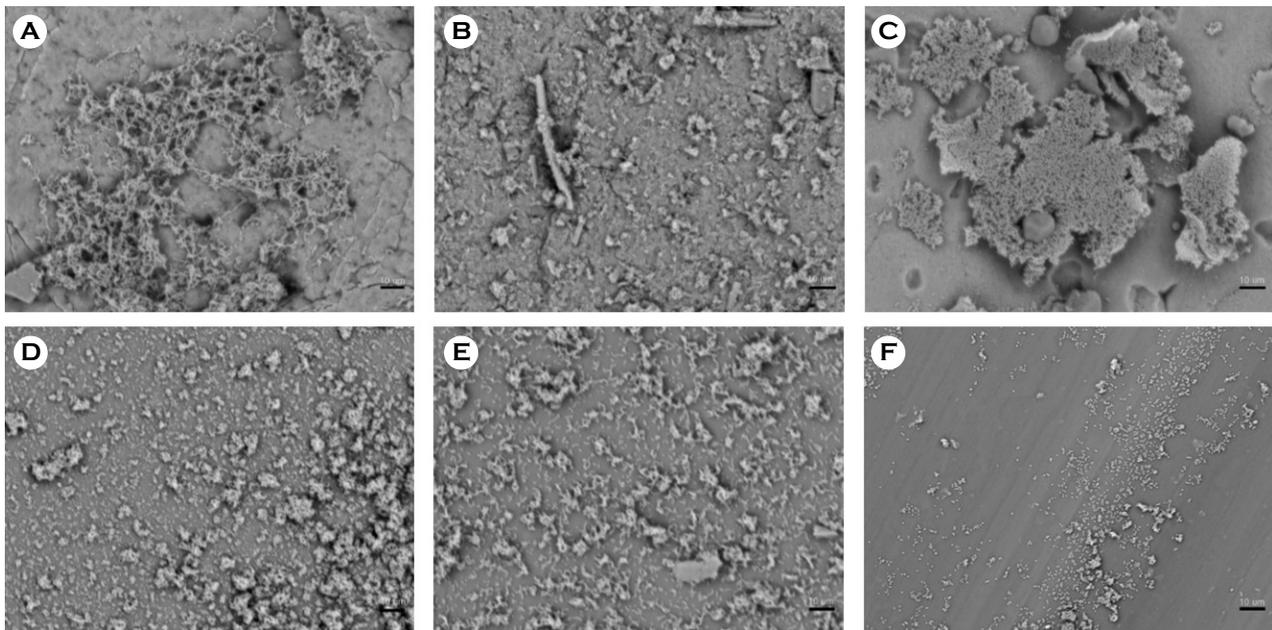


Figure 6. Scanning electron micrographs of biofilm formation by *S. salivarius* on catheter discs. (A) Latex, (B) anti-infective latex (IC-Latex), (C) silicone-elastomer coated latex (SEC), (D) 100% silicone, (E) hydrogel-coated silicone (HCS), (F) silver-alloy-coated silicone (SCS). (scale bar, 10 µm)

SEM photographs

The microbial biofilm that formed on each material type were observed under the SEM. When *C. albicans* was cultured on urinary catheter disk, the latex surface produced thin but generally spread-out biofilm. On the IC-latex, mycelium and yeast of *C. albicans* within the biofilm existed in a similar ratio. On the SEC, bacteria were tightly planted in the furrows of the surfaces, and a thick layer of biofilm was found. The mycelium was long and yeast form was only on the surface. Silicone had a smooth surface with locally observed thin biofilm layer, and more yeast forms were observed than mycelium (Fig. 2). In HCS and SCS, there were relatively very few mycelia in the *C. albicans* biofilm, and those that were found were short. SCS inhibited *C. albicans* biofilm formation more than the uncoated catheters. *E. coli* formed a thick biofilm on the entire surface of latex urinary catheter. However, on the silicone material, it partially made a thin biofilm; and on HCS and SCS, it mostly existed in single layers (Fig. 3). *P. vulgaris* also showed similar

biofilm as *E. coli*. However, unlike other bacteria, it formed crystalloids in latex and IC-latex materials (Fig. 4). *S. aureus* and *S. salivarius* showed similar appearances to *E. coli*, but they formed greater amounts of biofilm (Fig. 5 and 6). In the SEM, the amount of biofilm was not alike but the biofilm was similar in appearance.

DISCUSSION

For this study the formation of various microbial biofilms based on the different materials of urinary catheter were explored. CA-UTI are also known to involve biofilm formation of microorganisms. The microbial biofilm matures through many stages, and the first stage is attachment (12). There may be differences in the biofilm formation depending on the chemical and structural differences of the surfaces where microorganisms attach. Observation of the SEM images showed, that the latex-based catheter had a rough surface compared to the silicone-based catheter; it also had pores and the entire surface was not even.

When examining the quantity of biofilm formation according to urinary catheter materials, the latex-based urinary catheter had approximately 1.5 times the amount of biofilm formation compared to the silicone-based urinary catheter. Particularly, among the latex-based urinary catheters, SEC material had the most biofilm formation. The surface of SEC is porous and microorganisms attached to the pores, which facilitated the biofilm formation. *P. vulgaris* formed biofilms on latex and IC-latex, and formed diverse crystalloids, as observed under SEM. However, the crystalloids were not shown to have a great influence on the biofilm formation. According to previous studies, latex urinary catheters are more commonly used than the silicone ones because they are softer and cheaper (11). However, they can cause allergic reactions in the body; and when a latex catheter is used for a long time, it can easily cause UTI. Furthermore, encrustation phenomenon can cause blockage in the catheter lumen, urine retention, or trauma to the urethra and bladder mucosa at removal. Recently, latex with anti-infective materials has been developed for latex catheters. Microorganism biofilm formation was reduced in IC-latex compared to latex catheters.

Microbial adherence on the surface of biotic or abiotic is the initial stage of biofilm formation. CSH is considered an important property that contributes to adherence of microorganisms on different surfaces (16, 17). Our result showed that relative CSH value and biofilm formation ability of the Gram-positive bacteria was higher than Gram-negative bacteria. We observed a significant correlation between CSH and biofilm formation on different catheter materials. These results suggest that the hydrophobicity force of microorganisms might play an important role in biofilm formation (17).

When hydrogel is applied to the surface of silicone catheters, the hydrophobic surface of the catheter becomes hydrophilic. According to previous studies, microorganisms more easily attach to hydrophobic surfaces than to hydrophilic surfaces. The hydrophobic cells more strongly attach to hydrophobic surfaces, and hydrophilic cells more strongly attach to the hydrophilic surfaces (16~22). Therefore, microorganism attachment and biofilm formation can be reduced

by applying hydrogel on the catheter. Also, the biofilm formation was significantly reduced in SCS catheters compared to catheters with only hydrogel.

The silver ion has bactericidal activity by inactivating the thiol group in the vital enzymes of microorganism, markedly enhancing pyrimidine dimerization and causing structural changes in the cell envelope (23). When silver ions are coated on the catheter, they are slowly released and provide antimicrobial activity on the inside and outside of the catheter (24~26). The silver ions are commonly processed on the catheter in two ways: as silver-oxide or as silver-alloy. Among these two types, silver-alloy is more effective (27). In this study, SCS was used, and it inhibited the microbial attachment to the catheter and the formation of colonies and biofilms.

Urinary catheter materials can affect the biofilm formation of microorganisms. First, the rougher surfaces of the catheter made microbial attachment easier and a greater amount of biofilm was formed. Second, by applying chemicals that inhibit growth and attachment of microorganisms on the inner and outer surfaces of the catheters, biofilm formation was inhibited. This study utilized the different types of urinary catheter materials that are commercially available in Korea and in the U.S. Among them, SCS is the most effective one in reducing the microbial biofilm formation. In the future, *in vitro* and *in vivo* experiments should be conducted to serve as a further basis for techniques to prevent CA-UTI.

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