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Chlorhexidine and silver sulfadiazine coating on central venous catheters is not sufficient for protection against catheter-related infection: Simulation-based laboratory research with clinical validation

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Abstract

Objective: The efficacy of chlorhexidine- and silver sulfadiazine-coated central venous catheters (CSS-CVC) against catheter-related infection remains controversial. We hypothesized that the loss of silver nanoparticles may reduce the antibacterial efficacy of CSS-CVCs and that this loss could be due to the frictional force between the surface of the CVC and the bloodstream. The objective of this study was to investigate whether the antimicrobial effect of CSS-CVCs decreases with increasing exposure time in a bloodstream model and quantitatively assay the antimicrobial effect of CSS-CVCs compared with polyurethane and antiseptic-impregnated CVCs.

Methods: Each CVC was subjected to 120 hours of saline flow and analyzed at intervals over 24 hours. The analyses included energy-dispersive X-ray spectroscopy, scanning electron microscopy, and optical density after a *Staphylococcus aureus* incubation test.

Results: The weight percentage of silver in the CSS-CVCs significantly decreased to 56.18% (44.10% ± 3.32%) with 48-hour catheterization and to 18.88% (14.82% ± 1.33%) with 120-hour

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catheterization compared with the initial weight percentage (78.50% ± 6.32%). In the *S. aureus* incubation test, the antibacterial function of CSS-CVCs was lost after 48 hours [3 (N/D) of OD]. Similar results were observed in a pilot clinical study using 18 CSS-CVCs.

Conclusions: We found that the efficacy of CSS-CVCs decreased over time and that the antibacterial function was lost after 48 hours of simulated wear-out. Therefore, antibiotic-impregnated CVCs may be a better option when longer (>48 hours) indwelling is needed.

Keywords

Antibacterial activity, bloodstream model, central line infections

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Introduction

Nanomaterials, defined as functional particles of ≤100 nm,[1] are currently used as polymeric medical device coatings such as those in implanted devices containing silver nanoparticles (AgNPs). Silver (Ag) ions exert antimicrobial activity by preventing bacterial surface adherence, proliferation, and biofilm formation. AgNP coatings slowly release Ag ions, which are considered to have antimicrobial and disinfectant properties.[2,3] Free Ag ions form compounds with sulfhydryl groups found in enzymes in the cell wall of bacteria and fungi. These compounds affect electrolyte transport and transmembrane energy metabolism. Ag ions alter the NADH-succinate-dehydrogenase and cytochrome oxidase regions of the bacterial respiratory chain. Ag ions that bind to bacterial DNA inhibit proliferation caused by increased double helix stability and prevention of splicing.[4–6] Therefore, the use of AgNP-coated medical devices may decrease bacterial infections related to implanted devices.

AgNP-coated central venous catheters (CVCs), such as chlorhexidine- and silver sulfadiazine-coated CVCs (CSS-CVCs), are used as medical devices because of their antimicrobial effect.[2,7,8] AgNP-coated CVCs can reduce bacterial colonization and prevent the formation of biofilms.[9,10] Marik et al.[11] showed that CSS-CVCs are associated with a 5-fold lower risk of catheter-related infections than are standard polyurethane CVCs. Similarly, a meta-analysis revealed that the incidence of both colonization and CVC-related bloodstream infection was reduced in patients with CSS-CVC placement.[12] However, Kalfon et al.[13] reported that Ag-impregnated CVCs do not reduce the colonization rate compared with standard catheters in patients undergoing intensive care. In their study, similar colonization rates were found between standard CVCs and AgNP-impregnated CVCs (12.1% vs. 14.7%, respectively). In contrast, another Ag-impregnated medical device, the subcutaneous cuff, did not decrease the incidence of CVC-related bacteremia/fungemia or tunnel infection.[14] Thus, the efficacy of AgNP-coated CVCs against microbial infection remains controversial.

We hypothesized that the loss of the AgNP coating may reduce the antibacterial efficacy of CSS-CVCs and that this loss could be caused by the frictional force between the surface of the CVC and the bloodstream. The objective of this study was two-fold: (1) to determine whether the antimicrobial effect of CSS-CVCs decreases with the passage of time in a bloodstream model, and (2) to quantitatively assay the antimicrobial effect of CSS-CVCs compared with that of polyurethane and antiseptic-impregnated CVCs using energy-dispersive X-ray spectroscopy (EDS), scanning electron microscopy (SEM), and optical density measurements.

Materials and methods

An overview of the experimental study design is shown in Figure 1.

CVCs

In total, 90 CVCs were used in this study: 2-lumen radiopaque polyurethane CVCs (Blue FlexTip; Arrow International, Reading, PA), 2-lumen CSS-CVCs (Blue FlexTip ARROWg+ard Blue; Arrow International), and minocycline/rifampin-impregnated double-lumen polyurethane catheters (Spectrum; Cook Medical, Bloomington, IN).

Development of bloodstream model

The bloodstream model was designed (Figure 2) and developed using a circulating water pump (CW-05G; Lab Companion, Daejeon, Korea), silicone tubing (Korea Ace Scientific, Korea), and normal saline (isotonic sodium chloride (IR) injection; JW Pharmaceutical, Korea). The chamber of the water pump and the silicone tube were filled with saline. The water pump supported a temperature of 38°C and a flow of 5.6 L/min, which resembles human cardiac output.[15] The saline solution was continuously injected through the catheter at a rate of 50 ml/h. The length of the tube was 5 m, and the inner diameter was 17 mm. The saline solution was changed 10 times (once every 12 hours) to prevent contamination. It was exchanged through the machine without affecting the saline flow rate.

Each type of catheter was subjected to wear-out in the bloodstream model for 0, 24, 48, 72, 96, and 120 hours. The catheters (30 of each type) were partially inserted into the silicone tubing following clinical procedures; the length of catheter exposed to the flow was 15 cm. The distance between catheters was 50 cm, which was about twice the catheter length. The water pump was then

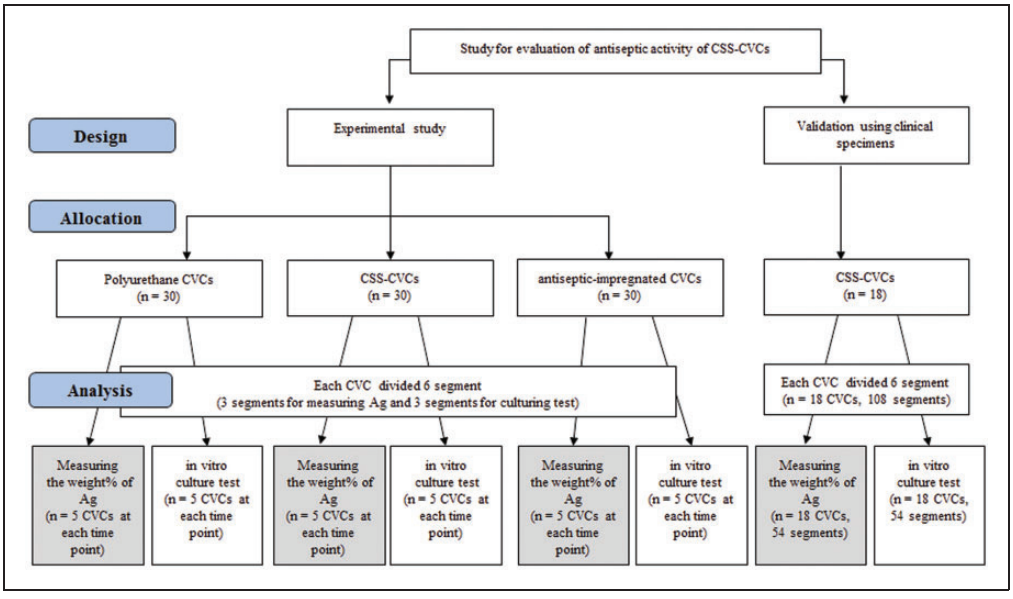

Figure 1. Experimental study design.
CSS-CVC, chlorhexidine- and silver sulfadiazine-coated central venous catheter; Ag, silver.

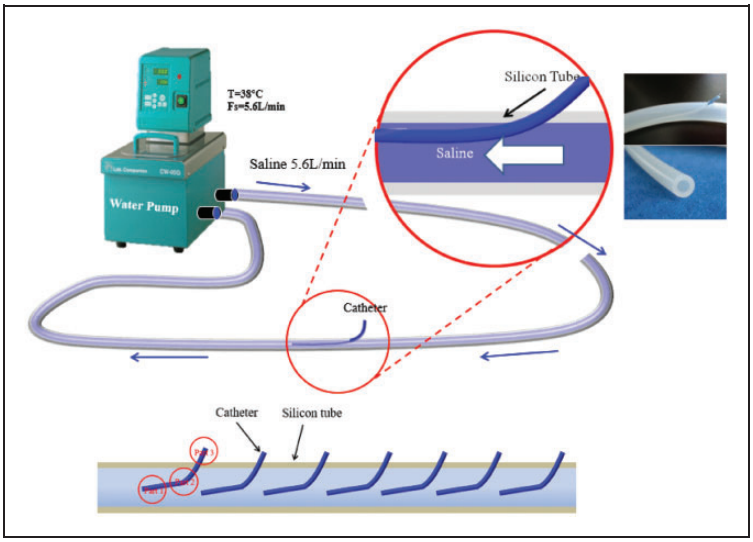

Figure 2. Central venous catheters and bloodstream model.

started to allow continuous saline flow for 120 hours, and the inserted catheters began to experience wear-out caused by the frictional force between the catheter surface and the fluid. The catheters were extracted at 0, 24, 48, 72, 96, and 120 hours and segmented into pieces starting at the tip. Each catheter was divided into six segments at 'part 1' zone. In total, 540 segments (1 cm) were prepared for analysis or testing, 270 segments (3 randomly selected segments from each catheter) were prepared for electron microscopic analysis to quantify the amount of Ag, and the remainder were prepared for *in vitro* culture testing to evaluate their antimicrobial activity.

EDS and SEM

Three segments of each catheter type were randomly arranged for the electron microscopic study. EDS was conducted for quantitative measurement of the Ag. The surface roughness worn by the fluid was evaluated using SEM. The catheter segments were subjected to a critical-temperature drying process in a platinum-coated chamber for specimen preparation. For scanning, the specimens were fixed to the stub using carbon tape.

In vitro culture for evaluation of antimicrobial activity

The *Staphylococcus aureus* colloid incubation test was performed in the following three steps.

(1) First culture: The worn CVC was cultured in *S. aureus* colloid for 3 hours.
(2) Physical cleaning: The cultured CVC was washed in phosphate-buffered saline (PBS) three times.
(3) Second culture: The washed CVC was cultured in pure lysogeny broth (LB).

The segments obtained after wear-out were sterilized in a low-temperature plasma chamber prior to the experiment. The sterilized segments were immersed in the prepared *S. aureus* colloid and incubated for 3 hours. They were then physically cleaned

using PBS. Prior to the third step, conical tubes were sterilized and filled with 15 ml of sterilized LB. Each PBS-cleaned segment was immersed in one of the prepared LB aliquots and incubated for a further 7 hours on a shaking incubator. During the incubation, the optical density (OD) of samples (1 ml) from each tube was measured at four different time points. The samples were removed by pipetting after 4, 5, 6, and 7 hours, and the OD was measured by an ultraviolet–visible spectrophotometry photometer (BioPhotometer; Eppendorf, Hamburg, Germany) with a range of 0 to 3 (N/D-Neutral Density).

Pilot study for clinical validation

With approval from the Korea University Hospital Institutional Review Board (no. MD13012), CVCs were removed from 18 patients after 1 month and subjected to a clinical validation study. The collected specimens were evaluated using the same testing protocols described above (wear-out bloodstream model and *S. aureus* incubation test).

Statistical analysis

Results are expressed as mean ± standard deviation. Statistical analysis was conducted with Student's t-test or one-way analysis of variance, followed by the Kruskal–Wallis test, using SigmaStat 3.5 for Windows (Systat Software, Chicago, IL). Linear regression was performed to develop a model for the predicted weight percentage (wt%) of Ag based on the wear-out duration (hours). A P value of <0.05 was considered statistically significant.

Results

The bloodstream model was built to create controlled experimental conditions representing wear-out of the CVCs, and the average flow speed of the saline was 2.47 m/min.

EDS and SEM

EDS was used to investigate the elements constituting the CVCs. EDS involves qualitative and quantitative analysis of materials by detection of scattered X-rays. Each element is characterized by a set of peaks on the energy spectrum, and its amount is assayed by the wt%, which is calculated by integrating the peak value.[16] The common elements detected in the three CVC types were silicone, sulfur, and barium. Cobalt was specific to the polyurethane and antiseptic-impregnated CVCs, chloride was specific to the CSS-coated and antiseptic-impregnated CVCs, and Ag was detected only on the CSS-CVCs.

The surface roughness and elements from each type of CVC as detected by SEM are shown in Figure 3. SEM is a microscopic technique that uses high-energy electron beams instead of the photons used in optical microscopy. It shows only topographical information of the specimen, and the maximum resolution is 1 nm.[17] The surface of the CSS-CVC was the roughest and the only one to show many crystal-shaped rods (second image in upper panel of Figure 3), which were identified as AgNPs by EDS. In addition, the Ag atom peak appeared only in segments of CSS-CVCs. Segments obtained from the polyurethane and antiseptic-impregnated CVCs did not exhibit Ag atom spectra by EDS.

Reduction of Ag on CSS-CVCs caused by wear-out in saline flow

The electron microscopy examination results of the CSS-CVCs analyzed using SEM and EDS during the 120-hour exposure are shown in Figure 4. Ag is shown as white spots in the SEM image, and the length of these spots decreased as time

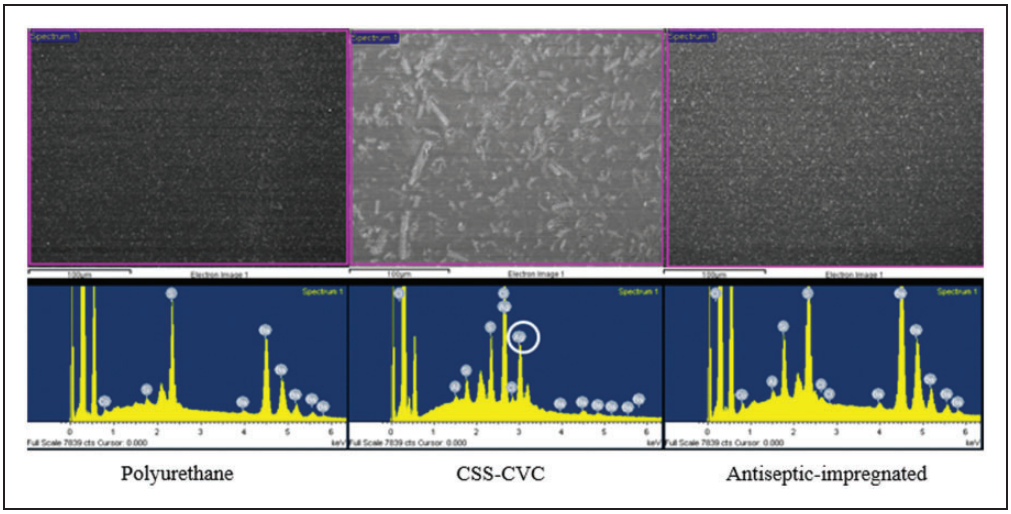

Figure 3. Electron microscopic analysis of three types of central venous catheters (CVCs).
Upper panels, scanning electron microscopy images; lower panels, energy-dispersive X-ray spectroscopy (EDS) results. Many crystal-shaped rods are visible in the second image of the upper panel and are identified as silver nanoparticles by EDS. As shown, the surface of the chlorhexidine- and silver sulfadiazine (CSS)-coated CVC is the roughest, and no uneven factors can be detected on the polyurethane and antiseptic-impregnated CVCs.

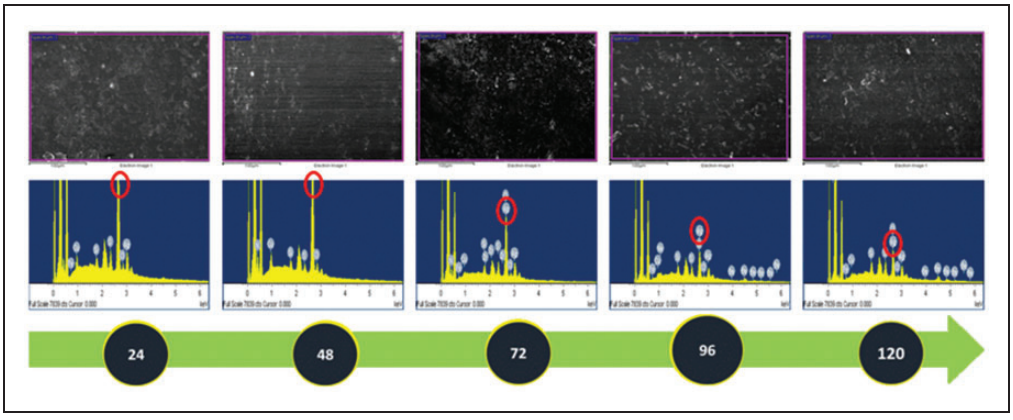

Figure 4. Scanning electron microscopy and energy-dispersive X-ray spectroscopy data for the chlorhexidine- and silver sulfadiazine-coated central venous catheters. The silver peak (red circle) gradually decreases as the wear-out duration increases.

progressed. These results suggest that the Ag wt% decreased as the wear-out duration increased. The wt% of Ag had significantly decreased to 56.18% (44.10% ± 3.30%) at 48 hours ($P < 0.001$); finally, only 18.88% (14.82% ± 1.33%) remained after 120 hours ($P < 0.001$) compared with the initial wt% of 78.50% ± 6.32% (Figure 5). Using linear

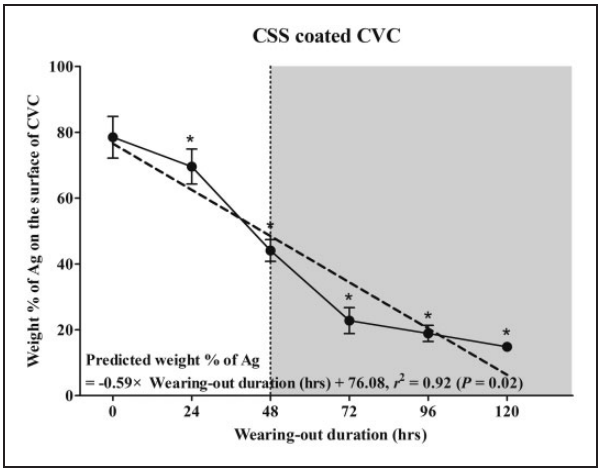

Figure 5. Changes in weight percentage of silver on the surface of chlorhexidine- and silver sulfadiazine (CSS)-coated central venous catheters (CVCs) with wear-out duration. *$P < 0.05$ in comparison with initial value. Ag, silver.

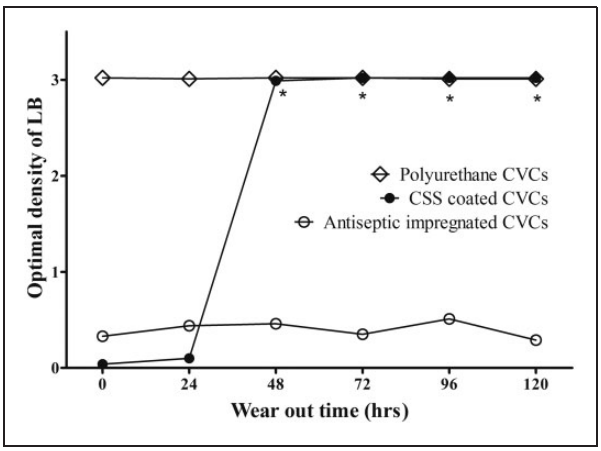

Figure 6. Optical density values of lysogeny broth (LB) cultures for three types of central venous catheters (CVCs) during a 7-hour incubation.
CSS: chlorhexidine and silver sulfadiazine. *$P < 0.001$ in comparison with initial value.

regression, the following formula was derived for the predicted wt% of Ag:

$$\text{Predicted wt\% of Ag} = -0.59 \times \text{wear} - \text{out duration(hours)} + 76.08,\ r^2 = 0.92(P = 0.02)$$

Dysfunction of CSS-CVCs caused by wear-out in saline flow

The antibacterial function of CVCs was evaluated using the *S. aureus* colloid incubation test (Figure 6). After 120 hours, the polyurethane CVCs were fully saturated to

3 (N/D), and the antiseptic-impregnated CVCs remained at <0.5 (N/D). The OD of the CSS-CVCs was <0.5 (N/D) after 24 hours but increased to 3 (N/D) after 48 hours.

Table 1. Characteristics of chlorhexidine- and silver sulfadiazine-coated central venous catheters.

Catheter	Catheterization time (hours)
1	1.00
2	2.00
3	3.00
4	4.00
5	4.00
6	4.00
7	53.00
8	53.00
9	55.00
10	73.00
11	96.00
12	99.00
13	120.00
14	121.00
15	124.00
16	130.00
17	144.00
18	238.00

Validation study using clinical specimens

Eighteen specimens were randomly gathered from patients who had been catheterized for a duration of up to 1 month. The characteristics of the collected CSS-CVCs are presented in Table 1.

The changes that occurred in the AG wt% with an increasing wear-out duration are shown in Figure 7. The Ag wt% decreased as the catheterization duration increased, which is consistent with the results of the simulation study conducted using the central vein model. The Ag wt% was $75.44\% \pm 0.39\%$ at 1 hour of wear-out time and $17.01\% \pm 19.01\%$ at 144 hours ($P < 0.001$) compared with the initial value (Figure 7).

Using linear regression, the following formula was derived to predict the Ag wt%:

$$\text{Predicted wt\% of Ag} = -0.56 \times \text{wear} - \text{out duration(hours)} + 60.06,\ r^2 = 0.75 (P < 0.0001)$$

The results of the *S. aureus* incubation test for the clinical specimens of the CSS-CVCs are shown in Figure 8. Although the mean optical density of the catheters with

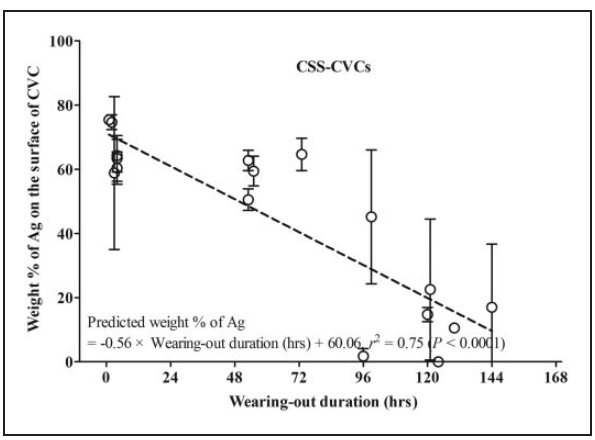

Figure 7. Changes in weight percentage of silver on the clinical specimens of chlorhexidine- and silver sulfadiazine (CSS)-coated central venous catheters (CVCs) with wear-out duration. *$P < 0.05$ in comparison with initial value.

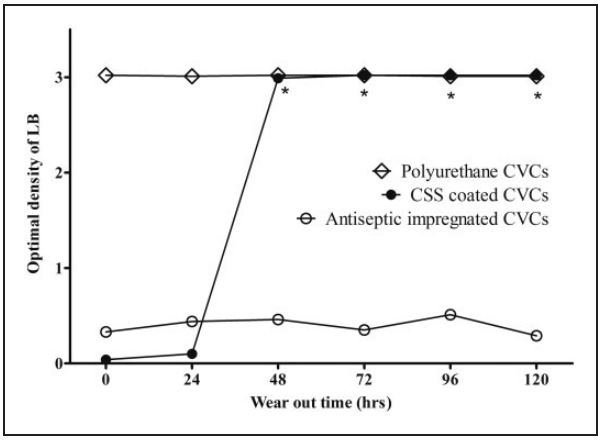

Figure 8. Optical density values of lysogeny broth (LB) cultures for three types of clinical specimens from central venous catheters (CVCs) during a 7-hour incubation.
CSS: chlorhexidine and silver sulfadiazine. *$P < 0.001$ in comparison with initial value.

a catheterization time of <48 hours was low [0.48–1.20 (N/D)], that of catheters with a catheterization time of >48 hours increased to 3.0 (N/D) after the 7-hour incubation period.

Discussion

In this study, we systematically investigated the changes in the antibacterial function of CSS-CVCs with catheterization time. As expected, the decrease in Ag on the surface of CSS-CVCs with the lapse of time was confirmed by electron microscopy. The antibacterial function of CSS-CVCs was lost after 48 hours of catheterization, as the OD values indicate. In addition, the results of the *in vitro* studies were reproduced by the *in vivo* study.

CVCs play an important role in the management of critically ill patients. However, the use of CVCs is associated with serious complications, especially bloodstream infection. A previous study revealed that device-related infection is a major cause of hospital-acquired bacteremia, particularly with CVCs.[18] These infections are caused by colonization of the CVCs, which occurs after migration of organisms from the patient's skin insertion site or from another part of the catheter itself (especially the hub of the CVC) into the intravascular part (distal tip) during catheter insertion. This can lead to local infection or complications such as endocarditis and metastatic abscess formation, commonly involving microorganisms such as *S. aureus* and coagulase-negative staphylococci.[19,20]

Various methods of reducing the risk of CVC-related bloodstream infection have been considered, including antibiotic or CSS coatings on CVCs.[7,9,21] Gilbert and Harden[19] suggested that the two most promising methods for prevention of catheter-related infection are the use of heparin-coated and antibiotic-coated/impregnated CVCs. Other studies have revealed that impregnation or coating with antiseptics or antibiotics can reduce catheter site colonization rates and even the incidence of catheter-related bloodstream infection.[22,23] Our results also showed that the antibacterial effect of antibiotic-impregnated CVCs lasts for 120 hours of wear-out time in a bloodstream model (Figure 6). However, antibiotic-impregnated CVCs are expensive and

may lead to increased antimicrobial resistance.[24]

Still, CSS-CVCs have been widely utilized as medical products because of their broad-spectrum antimicrobial activity.[8,23,25–27] However, the efficacy of CSS-CVCs is controversial. The chlorhexidine on the external surface of the CSS-CVC is an antibacterial agent generally used for wound irrigation or cutaneous disinfection, while silver sulfadiazine is typically used as a potent bactericidal and fungicidal agent in the management of patients with burns. Ag is nontoxic and has activity against gram-positive and gram-negative bacteria and *Candida* spp.[3] Materials containing AgNPs have greater antimicrobial activity and ion availability than Ag salts or metallic Ag coating. One study showed that around 15% of the coated Ag was released from CVCs over 10 days *in vivo*.[3] However, in our study, the Ag wt% of the CSS-CVCs subjected to wear-out using the bloodstream model decreased by around 56.18% at 48 hours of wear-out and further decreased to 18.88% at 120 hours of wear-out. In addition, the CSS-CVCs lost their antibacterial function after 48 hours. Several previous studies similarly reported that Ag-impregnated CVCs do not reduce colonization.[2,13,19,26]

Although the bloodstream model developed for verification of the antibacterial function of CVCs used saline for circulation and a non-pulsatile type water pump, the model could control the environment of the catheterized CVCs. Previous reports[2,8,13,19,25–28] on the antibacterial function of CSS-CVCs were based on the experimental environment or clinical specimens and therefore included various unknown and uncontrolled factors. In contrast, the condition of the CVCs was controlled in the present study by using a bloodstream model. Thus, we clearly showed that the CSS-CVCs underwent wear-out within 24 hours in the bloodstream.

A standard spectrophotometer to measure OD is a low-cost, rapid, and nondestructive method for assessing microbial growth and the cell or biomass concentration instead of measuring the cell concentration by hemocytometry or plating for colony forming units in microbiology laboratories. When light passes through a culture of known path length, both the light absorbance and scatter caused by transmission through the culture and the turbidity of the culture contribute to reduction of the light intensity.[29] The OD is directly correlated with the biomass.[29] In this study, an *in vitro* culture test using OD was also conducted to evaluate the antimicrobial activity of the CVCs (Figure 6). The growth curve of bacteria is not only well known and calculable but has already been evaluated in a mathematical model.[30] The number of bacteria at any time during culture is linearly proportional to the initial number of bacteria. For the present study, *S. aureus* was selected because it is one of the most frequently found microorganisms in infection sites in the clinical setting. Furthermore, it forms a colloid when it is cultured in LB, a nutritionally rich medium, as a badge for *S. aureus*. Because the number of particles in colloidal solution is linearly proportional to the optical absorption density, the OD at any time after culture compared with the initial value can be considered to represent the number of bacteria. Using OD values, our results showed that the amount of Ag was reduced to $<50\%$ after 48 hours of wear-out in saline flow (Figure 5), which is similar to that in clinical use, and the antibacterial function rapidly dropped in the CSS-CVCs.

This study has limitations. The flow rate (5.6 L/min) used to simulate cardiac output was more than twice that in normal human conditions and thus may have exaggerated the results. Therefore, it may necessary to adjust the flow speed in bloodstream models because the catheters are mainly in the

superior vena cava. However, the exact flow rate that causes wear-out on the surface of the CVC is unknown. Therefore, we chose the flow rate of 5.6 L/min considering that cardiac output increases by two to four times when a person exercises.[31]

Unfortunately, we did not monitor the central venous pressure during the experiment. If the central venous pressure had been measured and maintained at an appropriate level during the study, better results would have been obtained. However, the *in vitro* results were similar to those obtained in the clinical study.

We found that the efficacy of CSS-CVCs exhibits a decreasing pattern over time and that the antibacterial function can be lost within 48 hours of wear-out. In conclusion, CSS-CVCs may be safely used in veins for 48 hours. However, antibiotic-impregnated CVCs may be a better option when longer (>48 hours) indwelling is needed.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. Williams D. Defining nanotechnology. *Med Device Technol* 2008; 19: 8,10.
2. Furno F, Morley KS, Wong B, et al. Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection? *J Antimicrob Chemother* 2004; 54: 1019–1024.
3. Roe D, Karandikar B, Bonn-Savage N, et al. Antimicrobial surface functionalization of plastic catheters by silver nanoparticles. *J Antimicrob Chemother* 2008; 61: 869–876.
4. Guggenbichler JP, Boswald M, Lugauer S, et al. A new technology of microdispersed silver in polyurethane induces antimicrobial activity in central venous catheters. *Infection* 1999; 27(Suppl 1): S16–S23.
5. Chambers CW, Proctor CM and Kabler PW. Bactericidal effect of low concentrations of silver. *JA Water Works Assoc* 1962; 54: 208–214.
6. Thurman RB and Gerba C. The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses. *Crit Rev Environ Control* 1989; 18: 295–315.
7. McGee DC and Gould MK. Preventing complications of central venous catheterization. *N Engl J Med* 2003; 348: 1123–1133.
8. Samuel U and Guggenbichler JP. Prevention of catheter-related infections: the potential of a new nano-silver impregnated catheter. *Int J Antimicrob Agents* 2004; 23(Suppl 1)): S75–S78.
9. Bach A, Eberhardt H, Frick A, et al. Efficacy of silver-coating central venous catheters in reducing bacterial colonization. *Crit Care Med* 1999; 27: 515–521.
10. Stevens KN, Crespo-Biel O, van den Bosch EE, et al. The relationship between the antimicrobial effect of catheter coatings containing silver nanoparticles and the coagulation of contacting blood. *Biomaterials* 2009; 30: 3682–3690.
11. Marik PE, Abraham G, Careau P, et al. The ex vivo antimicrobial activity and colonization rate of two antimicrobial-bonded central venous catheters. *Crit Care Med* 1999; 27: 1128–1131.
12. Veenstra DL, Saint S, Saha S, et al. Efficacy of antiseptic-impregnated central venous catheters in preventing catheter-related bloodstream infection: a meta-analysis. *JAMA* 1999; 281: 261–267.
13. Kalfon P, de Vaumas C, Samba D, et al. Comparison of silver-impregnated with standard multi-lumen central venous

catheters in critically ill patients. *Crit Care Med* 2007; 35: 1032–1039.
14. Groeger JS, Lucas AB, Coit D, et al. A prospective, randomized evaluation of the effect of silver impregnated subcutaneous cuffs for preventing tunneled chronic venous access catheter infections in cancer patients. *Ann Surg* 1993; 218: 206–210.
15. Sullivan MJ, Knight JD, Higginbotham MB, et al. Relation between central and peripheral hemodynamics during exercise in patients with chronic heart failure. Muscle blood flow is reduced with maintenance of arterial perfusion pressure. *Circulation* 1989; 80: 769–781.
16. Goldstein J, Newbury D, Joy DC, et al. *Scanning electron microscopy and X-Ray microanalysis*. New York: Springer, 2017.
17. Egerton RF. The scanning electron microscope. In: Egerton RF (ed.) *Physical principles of electron microscopy*, 2nd ed. Cham, Switzerland: Springer International Publishing, 2016, pp.121–147.
18. Richards MJ, Edwards JR, Culver DH, et al. Nosocomial infections in medical intensive care units in the United States. National Nosocomial Infections Surveillance System. *Crit Care Med* 1999; 27: 887–892.
19. Gilbert RE and Harden M. Effectiveness of impregnated central venous catheters for catheter related blood stream infection: a systematic review. *Curr Opin Infect Dis* 2008; 21: 235–245.
20. Walz JM, Memtsoudis SG and Heard SO. Prevention of central venous catheter bloodstream infections. *J Intensive Care Med* 2010; 25: 131–138.
21. Darouiche RO, Raad II, Heard SO, et al. A comparison of two antimicrobial-impregnated central venous catheters. Catheter Study Group. *N Engl J Med* 1999; 340: 1–8.
22. George SJ, Vuddamalay P and Boscoe MJ. Antiseptic-impregnated central venous catheters reduce the incidence of bacterial colonization and associated infection in immunocompromised transplant patients. *Eur J Anaesthesiol* 1997; 14: 428–431.
23. Tennenberg S, Lieser M, McCurdy B, et al. A prospective randomized trial of an antibiotic- and antiseptic-coated central venous catheter in the prevention of catheter-related infections. *Archives of surgery (Chicago, Ill: 1960)* 1997; 132(12): 1348–1351.
24. Tambe SM, Sampath L and Modak SM. In vitro evaluation of the risk of developing bacterial resistance to antiseptics and antibiotics used in medical devices. *J Antimicrob Chemother* 2001; 47: 589–598.
25. Maynard AD, Aitken RJ, Butz T, et al. Safe handling of nanotechnology. *Nature* 2006; 444: 267–269.
26. Bayston R, Vera L, Mills A, et al. In vitro antimicrobial activity of silver-processed catheters for neurosurgery. *J Antimicrob Chemother* 2010; 65: 258–265.
27. Bambauer R, Latza R, Bambauer S, et al. Large bore catheters with surface treatments versus untreated catheters for vascular access in hemodialysis. *Artif Organs* 2004; 28: 604–610.
28. Navarro E, Piccapietra F, Wagner B, et al. Toxicity of silver nanoparticles to Chlamydomonas reinhardtii. *Environ Sci Technol* 2008; 42: 8959–8964.
29. Myers JA, Curtis BS and Curtis WR. Improving accuracy of cell and chromophore concentration measurements using optical density. *BMC Biophysics* 2013; 6: 4.
30. Fujikawa H and Morozumi S. Modeling Staphylococcus aureus growth and enterotoxin production in milk. *Food Microbiol* 2006; 23: 260–267.
31. Rodeheffer RJ, Gerstenblith G, Becker LC, et al. Exercise cardiac output is maintained with advancing age in healthy human subjects: cardiac dilatation and increased stroke volume compensate for a diminished heart rate. *Circulation* 1984; 69: 203–213.