In Vitro Studies of a Novel Antimicrobial Luer-Activated Needleless Connector for Prevention of Catheter-Related Bloodstream Infection

Dennis G. Maki
Section of Infectious Diseases, Department of Medicine, University of Wisconsin School of Medicine and Public Health and the University of Wisconsin Hospital and Clinics, Madison

Background. We report in vitro studies of a commercially available novel antimicrobial Luer-activated connector with the inner surface coated with nanoparticle-silver to prevent contaminants from forming biofilm and causing catheter-related bloodstream infection.

Methods. Sterile control nonmedicated connectors and antimicrobial connectors were filled with $\sim 1 \times 10^3$ cfu/mL of Staphylococcus epidermidis, methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus, Enterobacter cloacae, Pseudomonas aeruginosa, or Candida albicans; after 24 h of incubation, the numbers of remaining viable microorganisms were quantified and compared with the concentration in control connectors ($\sim 1 \times 10^3$ cfu/mL). In trials simulating clinical use, septal membranes of connectors were inoculated with E. cloacae, were allowed to dry, and were then actuated and infused with lactated ringer’s solution for 72 h, with sampling for microorganisms in downstream efferent fluid and for biofilm in the connector.

Results. Microorganisms suspended in the intraluminal fluid path of antimicrobial connectors were rapidly killed. For 5 species, there was a 5.23–6.80 mean log$_{10}$ reduction (\(>99.999\%\)), and with C. albicans, there was a 99.9% reduction. In clinical simulation trials, heavy contamination of downstream fluid was detected at all time points with control connectors, reaching $\sim 1 \times 10^3$ cfu/mL at 72 h, and heavy biofilm was uniformly present; with the antimicrobial connectors, there was complete prevention of downstream fluid contamination and total absence of biofilm formation.

Conclusions. These simulation experiments show that needleless connectors readily acquire an internal biofilm when microorganisms gain access to the internal fluid path and that biofilm formation allows an exponential buildup of internal contamination, with shedding back into the fluid path and downstream sufficient to cause bacteremia. Incorporation of nanoparticle silver into the lining surfaces of the novel connector kills microorganisms in the fluid pathway and prevents internal biofilm formation, even with high levels of introduced contamination and continuous fluid flow. This technology deserves to be evaluated in a prospective, randomized clinical trial to determine its capacity to prevent catheter-associated bloodstream infection.
as part of the national movement to reduce the risk to health care workers of biohazardous sharps injuries and exposure to bloodborne viruses, such as human immunodeficiency virus, hepatitis B virus, and hepatitis C virus, following the Occupational Safety and Health Administration’s Needlestick Safety and Prevention Act of 2000 [14–16]. A recent meta-analysis has shown that these devices have reduced the incidence of biohazardous needlestick injuries in health care workers [17].

Unfortunately, over the past 15 years a growing number of reports have suggested that valved needleless connectors are associated with significantly increased risks of CVC-associated BSI [18–27]. In most of these reports, the adoption of a new Luer-activated, valved needleless connector by a hospital or home care infusion therapy program—usually replacing a split-septum connector—has been associated with an abrupt and significant increase in the incidence of nosocomial BSI, particularly CVC-associated BSIs. Multiple manufacturers’ valved connectors have been implicated in these reports. In a number of institutions, increased rates of BSI persisted despite intensified efforts to achieve a high level of compliance with infection control practices to prevent CVC-associated BSI, including routinely vigorously disinfecting the membranous septum of the connector before actuation and entry, and BSI rates decreased only after discontinuing use of the new valved connector [18–27].

Frequent handling and access of catheter hubs, needless connectors, and injection ports has been shown to put patients at increased risk of CVC-associated primary BSI [18, 20–22, 28], presumably because they facilitate entry of microorganisms into the connector and fluid path. In support of this hypothesis, studies have shown a high incidence of biofilm formation on the interior surface of valved connectors that have been used clinically [29].

Although needleless connectors and injection ports are recognized sites of access for microbial contamination, no national standard exists defining the best and recommended form of antiseptic preparation to prevent microbial entry when the needleless connector or injection port is accessed. Although studies have examined the efficacy of various disinfectants to remove microorganisms from the membranous surface of needleless connectors or injection ports [28, 30–32], there have been no randomized clinical trials that have prospectively examined the efficacy of various approaches to disinfection of connectors for prevention of CVC-related BSI. Most importantly, numerous studies have shown that if there is significant contamination of the membranous septum prior to actuation, conventional disinfection may fail to prevent entry of microorganisms [28, 30–36].

It seems clear that there is an urgent need to develop new technologies that can implicitly reduce the risk of contamination of needleless connectors and associated BSI. We report in vitro studies of a novel antimicrobial luer-activated connector, with a nanoparticle-silver coating of the entire surface, to ascertain its capacity to resist internal fluid contamination and biofilm formation and potential to confer protection against catheter-associated BSI.

METHODS

The novel antimicrobial needleless connector. The novel antimicrobial connector evaluated in this study (V-Link; Baxter Healthcare) is an adaptation of the company’s nonmedicated single-use, disposable, luer-activated, valved connector constructed of polycarbonate (Clearlink; Baxter Healthcare), which is designed to connect an administration set or extension set to a vascular catheter and provide needleless access for administration of fluid or withdrawal of blood specimens. However, with the exception of the silicone membranous septum, the

Figure 1. Internal structure of the novel antimicrobial connector. The entire polycarbonate surface contains nanoparticles of silver.
entire surface of the antimicrobial connector (Figure 1), including the entirety of the internal fluid path and the external casing, has a novel nanoparticle silver coating (V-Link with VitalShield; Baxter Healthcare) to prevent introduced contamination and internal biofilm formation within the device, with the goal of reducing the risk of catheter-associated BSI. The device is not intended to be used as a treatment for established catheter-related BSI.

Study 1: Antimicrobial efficacy, 24-hour challenge. This study was designed to test the capacity of the antimicrobial device to resist contamination of the fluid path, by killing high concentrations of microorganisms present in the fluid path, when tested immediately after removal from the sterile package. Sterile control nonmedicated connectors and antimicrobial connectors were actuated (the administration set was attached to the device, penetrating the membranous septum, triggering the internal valve as the luer connection is completed) and flushed with sterile saline, then filled with \( \sim 1 \times 10^5 \) CFU/mL of test microorganisms in bacteriologic media containing 0.1% neoepitope, 0.25% glucose, and 1% bovine serum. After incubation for 24 h, the number of remaining viable microorganisms was quantified and compared with the final concentration in control connectors. Bacteriologic media containing large quantities of protein was employed to provide a worst-case challenge to the device, similar to what might be encountered in clinical use with total parenteral nutrition admixtures or blood, because ionic silver binds to thiol residues, quenching its antibacterial activity [37].

Tests were performed with suspensions of pure cultures of 6 recent clinical isolates of each of the following microorganisms: Staphylococcus epidermidis, methicillin-resistant Staphylococcus aureus, vancomycin-resistant Enterococcus faecium, Enterobacter cloacae, Pseudomonas aeruginosa, and Candida albicans. All devices to be tested were sterilized and flushed with sterile saline prior to inoculation. The internal fluid path of each device was challenged by aseptically infusing \( \sim 2 \) mL of the microbial suspension through the device with a syringe to ensure that the entire 0.25-mL fluid-path volume was exposed. After the inoc-

Figure 2. Design of Study 3, a clinical simulation with heavy contamination of the membranous septum of the connector before actuation and continuous use for 72 h, to assess the capacity of the connector to resist internal biofilm formation and downstream contamination of efferent intravenous fluid flowing into the patient. A sufficient number of control and antimicrobial connectors was studied in this trial to permit sampling of infusate as well as assess biofilm formation on the interior surface of the connector at each sampling period for 36 trials with control connectors and 36 with antimicrobial connectors.

Figure 3. Results of Study 1, the 24-h challenge of connectors with approximately \( 1 \times 10^5 \) CFU/mL of 6 species of nosocomial pathogens. The antimicrobial device demonstrated a rapid killing of suspended microorganisms within the intraluminal fluid path. With 5 of the 6 species tested, there was a 5.23–6.80 mean \( \log_{10} \) reduction (>99.999%), compared with controls, and with Candida albicans, there was a 99.9% reduction.
Figure 4. Results of Study 2, the microbial challenge after 96 h of repeated actuation and continuous infusion through the connector. It can be seen that the 96-h challenge had minimal effect on the capacity of the antimicrobial connectors to rapidly kill microorganisms within the intraluminal fluid path.

Study 2: Antimicrobial efficacy, 96-hour challenge. This study was designed to assess the persistence of surface antimicrobial activity on the interior of the device after exposure to stresses intended to simulate 96 h of continuous heavy clinical use. This was achieved via repeated chemical disinfection of the membranous surface with isopropyl alcohol, multiple actuations and prolonged continuous infusion of intravenous fluid through the connector. After exposing the test connectors to repeated actuations and continuous infusion with normal saline for 96 h, connectors were inoculated with the same 6 test microorganisms in bacteriologic media, incubated and sampled using the same protocol as in Study 1.

Study 3: Clinical simulation trial with assessment biofilm formation. This study was intended to simulate clinical use of the test connectors and to assess their capacity to resist contamination of the fluid path and internal biofilm formation, despite heavy contamination of the membranous portion of the connector at the time of actuation and initiation of continuous flow of intravenous fluid for 72 h. As depicted in Figure 2, the membranous surface of each test connector was disinfected with 70% isopropyl alcohol, which was allowed to dry, after which it was inoculated with ∼1 × 10^5 cfu/mL of E. cloacae (ATCC 2355) suspended in lactated Ringer’s with 5% dextrose solution with 1% fetal bovine serum and again allowed to dry. The connector was then actuated with the male Luer adapter of a Continu-Flo infusion set (Baxter Healthcare), which was connected to a 1-liter bag of 5% dextrose-in-lactated Ringer’s solution (Baxter Healthcare) containing 1% trypticase soy broth. Fluid was infused at 25°C at a rate of 0.5 mL/min for 72 h. Aliquots of infusate from the distal end of an extension tubing connected to the outflow portion of the connector, which were immediately frozen until culturing, were collected at baseline, during hours 1–5, and after 24, 48, and 72 h. Concentrations of viable E. cloacae in each aliquot were determined by plating and culturing serial dilutions in triplicate.

For ascertaining biofilm formation, the exterior surface of the connector was disinfected with sterile gauze saturated with...
Figure 5. Results of the clinical simulation trial to assess the capacity of the connector to resist internal biofilm formation and downstream contamination of intravenous fluid flowing into the patient. The control connectors rapidly became contaminated and significant contamination of downstream intravenous fluid was seen by 48 h of continuous infusion. In contrast, the antimicrobial connectors provided near-complete protection from downstream contamination of fluid and totally prevented internal biofilm formation.

RESULTS

Study 1: Antimicrobial efficacy, 24-hour challenge. With all of the control devices tested immediately after removal from the package, contamination of the contained fluid increased to $\sim 1 \times 10^6$ cfu/mL by 24 h. The antimicrobial device showed a powerful capacity to rapidly kill microorganisms within the intraluminal fluid path. With 5 of the 6 species tested, there was a $5.23 - 6.80$ mean log10 reduction ($\approx 99.999\%$) compared with controls; with C. albicans, there was a $99.9\%$ reduction (Figure 3).

Study 2: Antimicrobial efficacy, 96-hour challenge. In this experiment, despite heavy manipulation and continuous infusion of normal saline for 96 h before instilling the inoculum and incubating for 24 h, the antimicrobial connectors showed durable capacity to rapidly kill the initial inoculum and prevent growth of microorganisms within the intraluminal fluid path. There was a $3.0 - 6.8$ mean log10 reduction ($99.9\% - 99.9999\%$ reduction), compared with control nonmedicated connectors (Figure 4), which again demonstrated a rapid increase in contamination to $\sim 1 \times 10^7$ cfu/mL.

Study 3: Clinical simulation trial with assessment biofilm formation. In all 36 trials with control connectors, large numbers of microorganisms were detected in efferent infusate (Figure 5). Approximately $1 \times 10^2$ cfu/mL were detected at 1 h, $1 \times 10^3$ at 48 h, and $1 \times 10^3$ at 72 h. Commensurate biofilm formation was present at each of these sampling periods (Figure 6).

In contrast, only 1–3 cfu/mL were detected at 1 h in 5 of 36 trials with antimicrobial connectors, with no detectable fluid
Antimicrobial Needleless Connector for Prevention of BSI

• CID 2010:50 (15 June) • 1585

Figure 6. Scanning electron microscopy of connectors after 72 h of exposure in Study 3 (magnification, ×10,000). The control connectors all had a heavy biofilm observed on scanning electron microscopy, with ∼1 × 10^6 cfu recovered per connector, whereas none of the antimicrobial connectors demonstrated any biofilm formation on scanning electron microscopy, and all were sterile in culture.

contamination thereafter (Figure 5). There was no biofilm formation whatsoever detected at any of the sampling periods (Figure 6).

DISCUSSION

Numerous epidemics of nosocomial BSI have been traced to the entry of microorganisms through the lumen of the catheter into the patient’s bloodstream, most often in contaminated infusate [1]. However, in a growing number of recent outbreaks, needleless valved connectors for CVCs appear to have become extrinsically contaminated by microorganisms which traversed the septal membrane to colonize the internal surface of the connector, presumably resulting in the formation of a biofilm and shedding of microorganisms into the fluid path [18–27]. Unfortunately, there have not been adequate studies to assess the relative importance of this mechanism of microbial access as a cause of endemic CVC-related BSI. However, Donlan et al [29] found that up to 63% of randomly sampled needleless connectors in clinical use demonstrated viable biofilms.

The in vitro simulation studies we report demonstrate unequivocally the capacity of microorganisms to gain access to the interior of the connector at the time of actuation or, later, during continuous use to rapidly form a luxurious biofilm which, within 24–48 h, begins to shed into the fluid path downstream (Figure 5) and ultimately into the bloodstream of the patient receiving the infusion. Moreover, studies have shown that if there is significant contamination of the membranous septum prior to actuation, conventional disinfection often fails to prevent entry of microorganisms [28, 30–36].

With the recent reports that many hospitals in the United States have experienced increased rates of nosocomial BSI that are being used universally, particularly with CVCs or peripherally-inserted CVGs [1827], it seems clear that there is an urgent need for the development of novel technologies to reduce the risk of in-use contamination of connectors which can cause catheter-related BSI. It is noteworthy that the first technologic approaches to preventing contamination of CVC-administration set-catheter connections by use of a povidone-iodine “shield” [33] or a novel antiseptic-containing connector showed greatly reduced rates of CVC-associated BSI in trials in Europe, where there was a very high baseline incidence of CVC-associated infection [34–36].

The novel nanosilver-impregnated polycarbonate valved needleless connector we have studied was recently approved by the Food and Drug Administration and is now in use in many hospitals in the United States. The nanosilver particles are stably imbedded in the polycarbonate matrix and release minute quantities of bactericidal ionic silver off the surface into the fluid path. Simulation studies have shown that the total amount of ionic silver eluted into the fluid pathway with continuous infusion—and infused into the patient—is ∼0.80 mg per day, resulting in an incremental increase in the blood level of silver of ∼0.05 ng/mL. This is far below levels of silver exposure considered to pose a risk to human health; silver concentrations in drinking water up to 100 mg/L are considered to be safe by the United States Environmental Protection Agency, and mean blood silver levels in unexposed healthy humans range from 0.2–5 ng/mL [38, 39]. The amounts of silver entering the bloodstream of patients exposed to this nanosilver-impregnated connector are magnitudes less than occurs in patients treated with topical silver sulfadiazine or silver dressings, where blood levels of silver can reach hundreds of micrograms per milliliter [38]. Most absorbed silver is excreted in the feces and urine.
Our in vitro studies (Studies 1 and 2), which posed microbial challenges to the antimicrobial connector far beyond those likely to be encountered in clinical practice and used incubation temperatures of 37°C and high concentrations of protein in nutrient media, similar to what would be encountered with infusion of warmed blood or protein-containing total parenteral nutrition admixtures, demonstrate that incorporation of the nanoparticle-silver coating onto the interior surfaces of the polycarbonate connector rapidly kills microorganisms in the fluid path and prevents biofilm formation, even in the presence of high concentrations of protein that can sequester silver and neutralize its antibacterial activity [37]. With 5 of the 6 species tested, there was a 5.23–6.80 mean log_{10} reduction (99.999%), compared with controls (Figure 3), and with C. albicans, there was a 99.9% reduction. Stressing connectors for 96 h with repeated actuations and a continuous infusion had no material effect on the capacity of the connectors to rapidly kill microorganisms suspended in the internal fluid path (Figure 4). Even more importantly, in Study 3, which simulated clinical use by exposing connectors to heavy contamination and continuous flow for 72 h, there was near-total prevention of downstream fluid contamination (Figure 5) and complete suppression of biofilm formation (Figure 6).

In conclusion, this promising novel technology deserves to be evaluated in prospective, adequately powered, randomized clinical trials with catheter-associated BSI as the primary outcome measure [40], to ascertain its capacity to prevent bacteremia deriving from in-use contamination of needless connectors.

Acknowledgments

I am grateful to Winnie Kubey (Sr. Research Scientist-Microbiology, Baxter); Dustin Cawthon (Engineering Specialist-NPD, Baxter), and David Lamb, PhD, (Group Product Manager, Baxter) for insightful scientific input during the design and conduct of these studies, and to Paul Straka, Mark Hunter, Steve Strathmann, James Diorio, and Neal Zupec, Baxter Healthcare Technology Park, IL, for excellent laboratory support and professional consultation.


Potential conflicts of interest. D.G.M. designed studies reported, analyzed the data, and wrote the manuscript but has received no compensation from Baxter Healthcare for this research, for consultation, or for participation in company-sponsored continuing medical education programs. D.G.M.: no conflicts.

References

with needleless device use and the importance of infection-control prac-
bloodstream infection rates after the introduction of a new mechanical
valve intravenous access port. Infect Control Hosp Epidemiol 2006;
27:67–79.
bloodstream infection among patients with a needleless, mechanical
valve-based intravenous connector in an Australian hematol-ogy-on-
25. Salgado CD, Chinnes L, Paczesny TH, Cantey JR. Increased rate of
catheter-related bloodstream infection associated with use of a nee-
dleless mechanical valve device at a long-term acute care hospital. In-
infection temporally associated with the use of an intravascular nee-
27. Jarvis WR, Murphy C, Hall KK, et al. Health care-associated blood-
stream infections associated with negative- or positive-pressure or dis-
placement mechanical valve needleless connectors. Clin Infect Dis
barrier properties of a needleless and conventional needle-based in-
on needleless connectors attached to central venous catheters. J Clin
30. Salzman MB, Isenberg HD, Rubin LG. Use of disinfectants to reduce
microbial contamination of hubs of vascular catheters. J Clin Microbiol
1993; 31:475–479.
needleless and needle-access devices. Am J Infect Control 1997;
32. Menyhay SZ, Maki DG. Disinfection of needless catheter connectors
and access ports with alcohol may not prevent microbial entry: the prom-
ise of a novel antiseptic-barrier cap. Infect Control Hosp Epidemiol 2006;
27:23–27.
prevention of catheter sepsis in intravenous feeding. JPEN J Parenter
Enteral Nutr 1987; 11:159–162.
35. Inoue Y, Nezu R, Matsuda H, et al. Experimental study of hub con-
tamination: effect of a new connection device. The I system. JPEN J
prevention of catheter-related sepsis using a new hub model. Ann Surg
37. Lai SY, Read WJ, Pugh JR, Russell AD. Interaction of silver nitrate
with readily identifiable groups: relationship to the antibacterial activity
38. Wan AT, Conyers RA, Coombs CJ, Masterton JP. Determination of
silver in blood, urine, and tissues of volunteers and burn patients. Clin
39. Armitage SA, White MA, Wilson HK. The determination of silver in
whole blood and its application to biological monitoring of occupa-
40. Crnich CJ, Maki DG. The promise of novel technology for the pre-
vention of intravascular device-related bloodstream infection. I. Path-