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Comparison of Copper(II)-Ligand Complexes as Mediators for Preparing Electrochemically Modulated Nitric Oxide-Releasing Catheters

Kamila K. Konopi ska¹, Nicholas J. Schmidt¹, Andrew Hunt¹, Nicolai Lehnert¹, Jianfeng Wu², Chuanwu Xi², and Mark E. Meyerhoff^{1,*}

¹Department of Chemistry, The University of Michigan, Ann Arbor, MI 48109-1055

²Department of Environmental Health Sciences, The University of Michigan, Ann Arbor, MI 48109-1055

Abstract

Further studies aimed at examining the activity of different Cu(II)-ligand complexes to serve as electron transfer mediators to prepare novel antimicrobial/thromboresistant nitric oxide (NO) releasing intravenous catheters are reported. In these devices, the NO release can be modulated by applying different potentials or currents to reduce the Cu(II)-complexes to Cu(I) species which then reduce nitrite ions into NO_(g) within a lumen of the catheter. Four different ligands are compared with respect to NO generation efficiency and stability over time using both single- and dual-lumen silicone rubber catheters: N-propanoate-N,N-bis(2-pyridylethyl)amine (BEPA-Pr), N-propanoate-N,N-bis(2-pyridylmethyl)amine (BMPA-Pr), 1,4,7-trimethyl-1,4,7-triazacyclononane (Me₃TACN), tris(2-pyridylmethyl)amine (TPMA). Of these, the Cu(II)BEPA-Pr and Cu(II)Me₃TACN complexes provide biomedically useful NO fluxes from the surface of the catheters, $> 2 \cdot 10^{-10} \text{ mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$, under conditions mimicking the bloodstream environment. The Cu(II)Me₃TACN exhibits the best stability over time with a steady and continuous NO release observed for 8 d under a nitrogen atmosphere. Antimicrobial experiments conducted over 5 d with NO-releasing catheters turned “on” electrochemically for only 3 h or 6 h each day revealed > 2 logarithmic units in reduction of bacterial biofilm attached to the catheter surfaces. The use of optimal Cu(II)-ligand complexes within a lumen reservoir along with high levels of nitrite ions can potentially provide an effective method of preventing/decreasing the rate of infections caused by intravascular catheters.

Graphical Abstract

* mmeyerho@umich.edu.

Supporting Information

Synthetic procedures for copper(II) ligands, cyclic voltammograms of copper(II) complexes, long-term NO release measurements, and copper leaching test data.

Keywords

nitric oxide; copper(II) complexes; intravenous catheters; modulated NO-release; antimicrobial catheters; electrochemical nitrite reduction

1 Introduction

Intravascular catheters are devices commonly used in clinical practice to provide venous or arterial access for administration of fluids, infusion of medications and antibiotics, sampling of blood and facilitating hemodialysis¹. Their insertion into human blood vessels is, however, associated with increased risk of bloodstream infections contributing significantly to the patients' mortality, extending periods of hospitalization, and greatly increasing the costs of health care². The formation of microbial biofilms on the surface of the catheters poses a more difficult clinical problem, as the penetration of conventional antibiotics into such structured bacterial communities is significantly reduced^{3–6}. The high resistance of organized microbial biofilms to antibiotic therapy necessitates the need to examine anti-biofilm agents, both natural and synthetic, to overcome this significant health care problem.

Several technologies applied for use in catheter lock solutions to prevent biofilm formation have been reported. These include chelating agents, such as citrate and ethylene diaminetetraacetic acid (EDTA), often used as anticoagulants in lock solutions, but these agents also have significant antimicrobial activity^{7–9}. The main limitation of these agents is their significant toxicity when applied in high concentrations, as well as the need to combine with antibiotics in order to ensure a sufficient anti-microbial effect¹⁰. The other lock solution formulations that have proven to be effective at killing cells in biofilms contain ethanol^{11–13}, tauridine¹⁴ and surfactants¹⁵, which cause the bacterial cells detachment from the catheters' surface. Biofilm dispersal in intravenous devices has also been achieved by the bacteriophage pretreatment^{16–18} and the application of a N-acetyl-D-glucosamine-1-phosphate acetyltransferase (GlmU) inhibitor¹⁹.

The other major concerns related to the use of intravascular catheters are endothelial trauma, inflammation and formation of blood clots, which occur after several hours of catheterization and may lead to venous thrombosis²⁰. In most cases, antimicrobial agents are ineffective in preventing blood clotting unless anticoagulants such as heparin or citrate are added. Tissue plasminogen activator (tPA) has proven to be an effective agent to inhibit thrombosis²¹; however, its application is too expensive for a routine clinical use. Preferably, any new technology applied in IV catheters should provide both anti-microbial and anti-clotting activity.

The generation of nitric oxide (NO) constitutes a potentially attractive alternative to currently applied methods ensuring both anti-thrombotic and anti-microbial effects. The function of physiologically produced NO, released at low fluxes $(0.5 - 4) \cdot 10^{-10} \text{ mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ from the surfaces of endothelial cells that line in inner walls of all blood vessels is to inhibit platelet activation, which prevents clot formation^{22–24}. NO also is produced by macrophages as part of our immune response and exhibits potent micro-biocidal and micro-biostatic activity against a vast number of bacteria, viruses, mycobacteria, yeasts and protozoa^{25–26}.

Several strategies for NO generation have already been utilized for fabrication of NO-releasing catheters and other biomedical devices. This includes the use of NO donors, such as diazoniumdiolates and S-nitrosothiols (RSNO), incorporated into the catheters' polymer materials^{27–30}. This approach suffers, however, from certain limitations, including instability of such compounds, inability to completely control the NO release rate, a need to provide appropriate storage and delivery conditions, leaching of NO donors from catheter's walls into the bloodstream, and the potential toxicity of donor compounds or their decomposition products.

An attractive alternative method involves NO generation/release during the electrochemical reduction of nitrite ions in the presence of catalysts, such as iron porphyrins^{31–33} or copper(II) complexes. The potential application of a Cu(II) complex to generate NO in a catheter configuration, with a reservoir of nitrite ions in one dedicated lumen of a catheter, has been previously demonstrated by our group^{34–36}.

In this approach, the NO is generated as a result of electrochemical nitrite reduction to NO, which is mediated by the presence of mM levels of an appropriate copper(II)-ligand complex in the nitrite solution (along with working and counter wire electrodes). The Cu(II) complexes employed mimic the active site of the enzyme nitrite reductase (E.C. 1.7.99.3), which is capable of nitrite conversion to NO *via* a one electron³⁷. After the electrochemical reduction of Cu(II) to Cu(I) within the complex on the surface of the working electrode inserted into the NO generating lumen, and subsequent reduction of nitrite, NO will diffuse through the walls of the catheter in all directions. The main advantage of the electrochemical NO generation approach lies in its ability to precisely control the NO release rate. Indeed, it is not only possible to switch a catheter “on” and “off”, but the exact flux of NO from the outer surface of the catheter can be readily modulated within the physiologically relevant ranges by applying given values of potential or current to the electrochemical cell within a lumen of the catheter. This method enables “on-demand” NO release that preserves the nitrite ions reservoir for the extended periods of time.

Herein, we evaluate several selected Cu(II)-ligand complexes capable of catalyzing the electrochemical reduction of nitrite to produce NO. This encompasses: *i*) the electrochemical studies on the catalytic properties of four different Cu(II)-ligand complexes; *ii*) a comparison between new Cu(II)-ligand complexes and Cu(II)TPMA (the complex used in previous catheter studies of this concept) in terms of their efficiency of NO generation within catheter configurations (both single-lumen and dual-lumen silicone rubber catheters); and *iii*) experiments aimed at confirming the significant antimicrobial effect of

electrochemically generated NO on bacterial biofilm prevention/dispersal on catheter surfaces.

2 Experimental Section

2.1 Reagents and instrumentation

1,4,7-Trimethyl-1,4,7-triazacyclononane (Me_3TACN), tris(2-pyridylmethyl)amine (TPMA), copper(II) sulfate pentahydrate, disodium phosphate, HEPES buffer, potassium chloride, potassium phosphate monobasic, sodium chloride, sodium nitrite, and sodium phosphate were obtained from Sigma-Aldrich (St. Louis, MO). N-propanoate-N,N-bis(2-pyridylmethyl)amine (BMPA-Pr) and N-propanoate-N,N-bis(2-pyridylethyl)amine (BEPA-Pr) were synthesized in accordance with the procedures included in the Supplementary Information file.

Perfluoroalkoxy(PFA)-coated silver and platinum wires (both 0.127 mm o.d.) were purchased from A-M Systems (Sequim, WA). All solutions were prepared using Milli-Q water (Millipore Corp., Billerica, MA). Silicone rubber single-lumen tubing (o.d. 1.96 mm; i.d. 1.47 mm) and silicone rubber adhesive (RTV-3140) was purchased from Dow Corning (Midland, MI). Dual-lumen silicone catheters (7 Fr) were gifted from Cook Medical Inc. (Bloomington, IN).

All electrochemical measurements were conducted using a CH Instruments model 760e potentiostat/galvanostat (Austin, TX). A Sievers Nitric Oxide Analyzer (GE Instruments, Boulder, CO) was used to measure the flux rate of nitric oxide release from the surfaces of the catheters.

2.2 Fabrication of catheters

The catheters were fabricated in accordance with procedures reported previously³⁶. Single-lumen (o.d. = 1.96 mm; i.d. = 1.47 mm) and dual-lumen catheters (o.d. = 2.34 mm; i.d. of lumens = 1.27 mm and 1.14 mm) 7.5 cm in length were sealed at one end with silicone rubber adhesive. The Pt working electrode (4 mm² of surface area exposed) and a Ag/AgCl pseudo-reference electrode (8 mm² of surface area exposed) were inserted into single-lumen tubing or the larger lumen of the dual-lumen catheters that were filled with a solution containing 2 mM copper(II) complex, 0.4 M sodium nitrite, 0.3 M sodium chloride and 0.5 M HEPES buffer, pH 7.3. The other end of the catheter was then sealed around the wires with silicone rubber adhesive and left to harden at room temperature overnight.

2.3 Antimicrobial studies

The antimicrobial experiments were conducted using a CDC bioreactor (BioSurface Technologies, Bozeman, MT, USA). The bacteria strains used for these tests – *Pseudomonas aeruginosa* and *Staphylococcus aureus* – were maintained on Luria Bertani (LB) agar plates and then grown in LB broth at 37°C. Once the NO releasing catheters and the control catheters were fixed aseptically within the bioreactor holders, the bacterial culture was inoculated into the bioreactor at the final concentration of about 10⁶ CFU/mL. After 1 h of still (non-flow) incubation, a fresh LB medium was introduced at the constant flow of 100

mL/h using a peristaltic pump. Then, the bacterial biofilms were allowed to develop on the catheters' surface in continuous medium flowing for 5 d at 37°C. The NO release was turned "on" for either 3 h or 6 h each day during this period. After the 5 d time period, the catheters were cut into pieces and subjected to further analysis, including fluorescent live and dead cell imaging, as well as homogenizing pieces of the catheters and plate counting the number of live bacteria originating from the outer surfaces of the catheters.

3 Results and Discussion

3.1 Comparison of copper(II)-ligand complexes tested in catheters

As reported previously³⁴, the electrochemical NO generation approach from catheters has the ability to disperse bacterial biofilm and reduce *in vivo* clot/thrombus formation using Cu(II)TPMA as the electron transfer mediator. In this present work, we performed further studies in order to select/compare other ligands capable of potentially providing more efficient electro-catalytic activity for nitrite reduction. Three additional ligands beyond the Cu(II)TPMA species (see Fig. 1 for structures) were tested with respect to NO generation efficiency as well long-term stability of NO release.

The cyclic voltammograms (CV) for these four Cu(II) complexes examined in the presence of nitrite (0.4 M) confirmed the catalytic activity of all four species. The representative CV data obtained for the Cu(II)BMPA-Pr and Cu(II)TPMA complexes (at 2 mM) in bulk solution with different nitrite concentrations are shown in Figure 2. When no nitrite is present, the reversible peak corresponding to the one electron reduction/oxidation of the copper ion within the complex is observed. It has been previously demonstrated that the nature of the ligand donor atoms (e.g., nitrogens, oxygen, etc.), the number of the donor atoms, the chelate ring size, and the general ligand morphology affect greatly the stability of copper(II) complexes³⁸. Similarly, variation in the length of the bridge linking pyridine ring with amine nitrogen, type, and the number of donor atoms have a strong effect on the Cu(I)/Cu(II) redox potential, and likely also the rates of reaction of the Cu(I) species with nitrite to form NO. In the presence of nitrite, a cathodic catalytic current is observed in the CVs, which increases in proportion to the nitrite level in the solution. At lower nitrite concentration, a second-order reaction occurs on the electrode surface, whereas at higher nitrite concentration a pseudo-first-order reaction should be considered. Rates of diffusion (mass transfer) of both nitrite and the Cu(II) complex must also be taken into account, and also the Cu(II) complex is the limiting reagent in this system.

The reduction potentials are similar for both the TPMA and BMPA-Pr complexes with Cu(II), ranging from about -0.2 V to -0.35 V. A slightly wider range of potentials is observed for the Me₃TACN ligand, from -0.2 V to -0.4 V, whereas the electrochemical characteristic of BEPA-Pr complex with Cu(II) is significantly different. In this case, the redox potential is shifted about 150 mV towards a more positive potential, ranging from about -0.08 V to -0.3 V. The cyclic voltammograms (CV) obtained for the Cu(II)BEPA-Pr and Cu(II)Me₃TACN complexes are presented in Supplementary Information file in Figure S1.

The efficiency of NO generation and the stability of NO release over time for each of the four Cu(II) complexes was then examined in both single-lumen and dual-lumen silicone rubber catheter configurations. The NO flux from the outer surfaces of the catheters soaked in PBS buffer solution at 37°C was determined by a standard chemiluminescent NO detection method³⁹, by purging the buffer solution with a stream of nitrogen gas. By applying different values of current or potential to the wire electrodes immersed in Cu(II)-ligand complex/nitrite solution within a lumen of the catheters, the levels of NO release from the outer surfaces can be controlled. Figure 3 shows the modulation of NO levels obtained using varying constant currents (A) and constant potentials (B) using the Cu(II)BEPA-Pr complex (at 2 mM) in a single lumen silicone rubber catheter (with 0.4 M NO₂⁻ also present in the lumen). We use relatively high nitrite concentrations within the lumen of the catheter that serves as a nitrite reservoir in order to ensure long-term NO generation. The use of the electrochemical method for NO generation provides the possibility to control both the generated surface concentration and the longevity of NO release. Since short release periods, e.g., 2–3 h per day, are considered to be sufficient in preventing the microbial biofilm formation on the surface of the catheters, this goal can easily be accomplished *via* the electrochemical generation approach.

Figure 4 illustrates the NO fluxes measured for single-lumen silicone catheters filled with solutions of the four different copper(II) complexes (along with 0.4 M NaNO₂ in 0.5 M HEPES buffer) as a function of the applied potential (Fig. 4A) or current (Fig. 4B). Based on these measurements, we conclude that the more positive Cu(II)/Cu(I) redox potentials result in higher faradaic efficiencies. The highest NO generation efficiency is obtained using the BEPA-Pr ligand, with an NO flux exceeding $20 \times 10^{-10} \text{ mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ for both constant current and constant potential modes. A faradaic efficiency of > 90 % is observed for the current values ranging from 1 to 7 μA (see Table 1), with linear NO production from the surface of the catheter up to an applied current of 11 μA . When comparing the applicable potentials for each of the ligands, the catalytic activity of BEPA-Pr also occurs over the widest window of applied potentials, with a relatively more positive potential of –100 mV being sufficient to obtain a significant NO flux from the surface of the catheter.

Another complex that also ensures very significant NO fluxes is Cu(II)Me₃TACN, with the efficiency of NO production exceeding 80 % when relatively low currents are applied (up to 3 μA). Although the NO levels obtained using this ligand are almost 50% lower than for BEPA-Pr, the NO fluxes observed range from physiological to ca. $17 \times 10^{-10} \text{ mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$. In contrast, the Cu(II)TPMA complex (examined previously in preliminary studies³⁴), exhibits the lowest faradaic efficiency (around 54 %). As reported in that article, Cu(II)TPMA is a poor catalyst for selective NO generation, as it can further reduce the NO generated, from nitrite reduced, to nitrous oxide. The much higher Faradaic efficiencies of the Cu(II)Me₃TACN and Cu(II)BEPA-Pr directly show that the Cu(I) species of these complexes must not catalyze further reduction of the generated NO to N₂O to a significant degree.

Similar findings regarding the comparison of Cu(II) complexes were observed when using dual-lumen silicone rubber catheters in which one lumen was dedicated for electrochemical NO generation. This is the configuration necessary for creating functional IV catheters, since

the second lumen remains open to enable access of the blood stream. Due to the diffusion limitations caused by an increased wall thickness, and the further distance for NO diffusion to the outside surface of the open lumen, the measured average NO surfaces flux levels are somewhat lower for the dual lumen devices than in the case of single-lumen catheters. The NO surface flux obtained using 2 mM Cu(II)BEPA-Pr complex in a dual-lumen catheter is ~71 % of the original value observed with a single-lumen tubing when the same constant potential is applied. For the constant current mode, more than 93 % of the NO fluxes found with the single lumen configuration are observed. However, the range of applicable currents is significantly reduced in the case of dual-lumen catheters compared to single-lumen devices (see Figure 5). Indeed, the NO fluxes measured with Cu(II)Me₃TACN complex are lower, ~83 % and ~87 % for the constant potential and constant current, respectively. Despite the decrease of the average NO surface fluxes observed for dual-lumen catheters, the levels of generated NO are still sufficient to ensure fluxes that are physiologically relevant to decrease microbial growth (see below) and inhibit thrombosis.

In order to evaluate the long-term stability of electrochemical NO release in catheter configurations, we turned “on” the electrochemical NO generation continuously by adjusting the applied potential or current to obtain a physiologically relevant flux. The tests for all ligands were performed using the same concentration of Cu(II)-ligand complexes with working electrodes of equal surface areas in single-lumen silicone catheters. Although the BEPA-Pr ligand provides the highest NO flux values, the continuous release appears to be stable only for short periods of time (see Fig. 6A). A relatively high flux of 17×10^{-10} mol·min⁻¹·cm⁻² can be obtained by applying a constant current of 7 μ A, but stable NO release is observed from the catheter for only ~8.5 h with this applied current. After the adjustment of current to obtain a flux of 10×10^{-10} mol·min⁻¹·cm⁻² (4 μ A), the NO release flux is continuous for 24 h (Fig. 6B). A further decrease in the applied current to 0.9 μ A leads to a prolonged period of stable NO release for up to ~4.5 days (at flux $\sim 2 \times 10^{-10}$ mol·min⁻¹·cm⁻²), which is more acceptable for the final intended application of preparing NO release intravascular catheters.

The Cu(II)Me₃TACN complex exhibits catalytic activity for a longer time period, resulting in a useful NO flux ($\sim 5 \cdot 10^{-10}$ mol·min⁻¹·cm⁻²) with stable release for over 8 days (see Fig. S2). After this time, a rapid drop of NO flux is observed due to a dramatic increase of the internal solution's pH value. Despite using a buffered solution (pH 7.3), the reduction of nitrite causes an almost 2 unit change in the pH of this solution over time. When the pH is > 8, the catalytic activity of copper(II) complex decreases significantly leading to an immediate decrease in NO generation levels. However, the observed NO release is maintained for a couple of days and is still considered to be sufficient in preventing not only the microbial biofilm formation but also clot formation, without the necessity to change the catheter or refill with fresh nitrite/copper complex solution.

All of the data reported above were obtained under a nitrogen atmosphere, which allows for the direct comparison of the tested Cu(II) complexes in terms of the efficiency of NO generation and stability of release with time. In order to evaluate the performance of catheters under the conditions mimicking a physiological blood environment, the NO release was further examined in the presence of 3 % oxygen, which is close to the concentration of

free oxygen in venous blood. Under such conditions, the competitive reaction between the electrochemically formed Cu(I)-ligand species generated at the wire electrode surface within the catheter lumen and oxygen can occur, and this greatly hinders the reduction of nitrite to NO, leading to a significant decrease in the observed NO flux from the surface of the catheters. Moreover, since our initial studies were performed using silicone rubber catheters that exhibit high permeability towards both NO and oxygen, the ability of oxygen to react with the Cu(I) complex is enhanced.

Indeed, in the presence of oxygen, the amount of NO release from the catheter containing Cu(II)BEPA-Pr complex is ca. 21.4 % of the original value obtained in a N₂ atmosphere. For the Cu(II)Me₃TACN and Cu(II)TPMA, the NO fluxes were found to be 25.1% and 32.4%, respectively, of that in the nitrogen environment. Although the decrease of NO level is quite significant, the remaining surface NO flux ($> 2 \cdot 10^{-10} \text{ mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$) is still more than sufficient to significantly reduce clotting and infection when using both the BEPA-Pr and Me₃TACN ligand complexes with Cu(II) as the NO generating catalysts. Representative data showing the influence of oxygen on the NO generation of these two complexes within a catheter configuration are shown in Figure 7.

Another possible concern regarding the final application of the E-chem NO releasing catheters for clinical practice is the possibility of copper ion leaching into the bloodstream through the walls of the catheters. Therefore, ICP-MS analysis of the soaking solution after 8 d of continuous NO release from silicone rubber catheter containing Cu(II)Me₃TACN complex was conducted to determine the extent of Cu(II) leaching. After this time, the level of copper in the soaking solution (5 mL) that leaches through the catheter walls into a PBS solution was found to be $90.83 \pm 7.89 \text{ ppb}$ (n=3), which is 0.072% of the original total Cu(II) level within the lumen of the catheter. The concentration of copper in the soaking solution in the case of catheters containing Cu(II)BEPA-Pr complex after 1.5 days of NO release was found to be $5.39 \pm 0.04 \text{ ppb}$ (n=3), or only 0.0042% of original Cu(II) level (Tab. S1). The background levels of Cu(II) in the soaking solutions without the catheters was found to be $2.56 \pm 0.03 \text{ ppb}$. It is not clear at this point whether this very low level of copper ion leaching is coming from gaps in the seal at the distal end of the catheters, or actual migration of small amounts Cu(II)-ligand complex through the walls of the catheters.

3.2 The antimicrobial activity of E-chem NO releasing catheters

In order to verify the antimicrobial effect of the proposed electrochemical NO generation catheters, we determined the total viable bacteria adhered to the outer surfaces of the catheters after placing them into a CDC bioreactor inoculated with a given bacterial strain and flowing media over the surface of the catheters for 5 d. Dual lumen silicone catheters with two different Cu(II)-ligand complexes were examined; Cu(II)BEPA-Pr and Cu(II)Me₃TACN. During the 5 d test period, the NO release was turned “on” for only 3 h or 6 h each day at an applied potential (−0.3 V) that yields a surface NO flux of approximately $0.7 \cdot 10^{-10} \text{ mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$, in the presence of ambient oxygen levels. The conditions of antimicrobial experiments were slightly different than is case of the chemiluminescent measurements reported above. In the NOA configuration, the solution, in which the catheter is submerged, is vigorously purged with a nitrogen stream to efficiently transfer emitted NO

into the gas phase. The lack of oxygen clearly increases the level of NO measured for this flux testing. However, the generated NO fluxes proved to be sufficient to reduce biofilm formation even in the presence of ambient oxygen within the bioreactor testing of the catheter devices (see below).

As shown in Figure 8, although the NO release was only turned “on” periodically for a few hours daily, such small periodic doses of NO coming from the catheter surface results in a significant reduction of bacterial biofilm formation on the catheters’ surfaces. In the case of *Pseudomonas aeruginosa*, the viable bacteria counts are > 1 log unit less with 3 h/day NO release, and > 2 log units of reduction using 6 h of NO release per day (with Cu(II)Me₃TACN as the active complex), when compared to control catheters (no NO release). The results obtained with *Staphylococcus aureus* – with the same Cu(II)complex are even more significant, with a reduction in bacteria counts of almost 2 log units with 3 h per day of NO release and almost a 3 log units reduction when turning on the NO release for 6 h per day.

The experiments performed with the Cu(II)BEPA-Pr complex exhibit slightly higher reduction of bacterial biofilm after 3 h of NO release compared to the catheters with Cu(II)Me₃TACN complex. These findings are similar for both examined bacterial strains, with > 2 log unit reduction observed (see Fig. 8). In the case of the Cu(II)BEPA-Pr complex, however, no further reduction of biofilm is observed with NO release turned “on” for 6 h vs. 3 h. This is most likely due to the low stability of this copper complex. After several hours of continuous NO release, the catalytic activity of the complex decreases significantly hindering the NO generation, which enables further biofilm development on the catheter surfaces.

4 Conclusions

The performance of four copper(II)-ligand complexes within electrochemically controlled NO generating/releasing catheters has been examined. The Cu(II)BEPA-Pr and Cu(II)Me₃TACN species were found to ensure both high efficiency of NO generation and sufficient stability of NO release over time; which is potentially useful for preparing a new generation of antimicrobial and thromboresistant biomedical IV catheters. These complexes appear to have significant advantages over the Cu(II)TPMA complex employed in the initial report of this NO release/generation concept for catheter applications³⁴. Although the catalytic electrochemical reduction of nitrite to NO is significantly affected by the presence of even low concentrations of oxygen, substantial NO release surface fluxes ($> 2 \times 10^{-10}$ mol·min⁻¹·cm⁻²) using selected copper(II) complexes under the conditions mimicking a physiological environment can be achieved. We suspect that under *in vivo* conditions, due to the presence of carbon dioxide in blood, the pH within the NO generating lumen of the catheter will not change so rapidly. Currently, we are investigating the CO₂ effect on NO release profile with various types of buffers within the NO generating catheter lumen, and further studies are being conducted to diminish the effect of varying oxygen levels on the electrochemical NO release catheters.

Moreover, the tested complexes in catheters provided adequate NO release to significantly reduce microbial biofilm formation on the catheter surfaces (up to 3 logarithmic units) over 5 d of exposure to bacteria growth with relatively low NO fluxes electrochemically generated for only 3 h or 6 h daily. This suggests that the use of such E-chem NO generating catheters could greatly lower the risk of bloodstream infections in hospital settings. Currently, long-term *in vivo* experiments in animals are being pursued in order to further verify that such electrochemical NO generation from catheters can exhibit significant thromboresistant activity (prevent clotting).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Cu(II)-tris(2-pyridylmethyl)amine **Cu(II)-*N*-propanoate-*N,N*-bis(2-pyridylmethyl)amine** **Cu(II)-1,4,7-trimethyl-1,4,7-triazacyclonane** **Cu(II)-*N*-propanoate-*N,N*-bis(2-pyridylethyl)amine** **Figure 1.**

The structures of the copper(II) complexes examined in this work.

Figure 2.

The cyclic voltammograms of 2 mM Cu(II)BMPA-Pr and Cu(II)TPMA obtained in 0.5 M phosphate buffer solution (pH 7.3) with different concentrations of nitrite (20 scanning cycles for each) using Pt disc working electrode (vs. Ag/AgCl reference electrode).

Figure 3.

The modulation of NO release obtained from catheter containing 2 mM Cu(II)BEPA-Pr and 0.4 M NaNO_2 solution using Pt wire as working electrode and Ag/AgCl reference/counter electrode with constant current (A) and constant potential (B) applied.

Figure 4:

The NO fluxes generated in the single-lumen silicone catheters using different copper(II) complexes with various potentials (**A**) and currents (**B**) applied to Pt working electrode (vs. Ag/AgCl). Inner solution composition was 2 mM Cu(II)-ligand complex and 0.4 M NaNO_2 in HEPES buffer, pH = 7.3.

Figure 5.

The NO fluxes generated in the dual-lumen catheters using copper(II) complexes with BEPA-Pr and Me₃TACN ligands when various potentials (A) and currents (B) applied to Pt working electrode (vs. Ag/AgCl). Inner solution composition was 2 mM Cu(II)-ligand complex and 0.4 M NaNO₂ in HEPES buffer, pH = 7.3.

Figure 6.

The long-term NO release from Cu(II)BEPA-Pr complex with constant current of 7 μA (**A**) and 4 μA (**B**) applied to Pt working electrode vs. Ag/AgCl electrode in single lumen catheter configuration.

Figure 7:
The NO generation from single lumen catheters with Cu(II)BEPA-Pr (**A**) and Cu(II)Me₃TACN (**B**) in the absence (N_2) and presence of 3 % oxygen.

Figure 8.

The plate counts of the number of viable bacteria attached to the entire surface of the dual lumen silicone catheters after 5 d of NO release (for 3 h per day) obtained for *Pseudomonas aeruginosa* (**A**) and *Staphylococcus aureus* (**B**) using dual lumen silicone catheters with one lumen containing the given Cu(II) complex at 2 mM and NaNO₂ at 0.4 M. NO flux of ca. $0.7 \cdot 10^{-10} \text{ mol} \cdot \text{min}^{-1} \cdot \text{cm}^{-2}$ was set by the constant applied potential of -0.3 V .

Table 1:

Faradaic efficiencies and NO fluxes obtained for examined copper(II) complexes applied current

applied current	faradaic efficiency /%				NO flux /mol·min ⁻¹ ·cm ⁻² ·10 ¹⁰			
	TPMA	Me ₃ TACN	BMPA-Pr	BEPA-Pr	TPMA	Me ₃ TACN	BMPA-Pr	BEPA-Pr
1 μ A	54.5	83.96	93.34	95.46	1.52	2.07	2.41	2.39
3 μ A	24.75	80.79	85.3	97.53	1.98	5.29	5.16	7.37
5 μ A	-	72.19	52.8	92.04	-	8.17	6.27	12.49
7 μ A	-	67.18	31.8	93.55	-	10.12	6.75	17.46
9 μ A	-	60.07	23.45	89.22	-	11.6	6.89	22.08
11 μ A	-	57.04	14.44	87.97	-	12.5	6.9	25.94