

# Efficacy of NVC-422, a Stable Derivative of *N*-Chlorotaurine Against *Staphylococcus aureus* Biofilm

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## Abstract

**Background:** Microbial biofilms formed on the surfaces of indwelling medical devices can lead to antibiotic resistance resulting in acute and chronic infections. NovaBay has developed a non-antibiotic, anti-infective, small molecule (NVC-422; *N,N*-dichloro-2,2-dimethylpropane-1-sulfonamide), a stable derivative of *N*-chlorotaurine that exhibits broad spectrum antimicrobial activity with an excellent safety profile. Here we investigated its potential use for the prevention and reduction of *Staphylococcus aureus* biofilm colonization. The activity of NVC-422 formulated in 5 mM acetate saline pH 4 was examined in three *in vitro* biofilm models: Calgary Biofilm Device (CBD), CDC reactor, and Modified Robbins Device (MRD). **Methods:** The CBD was used to determine the minimum biofilm eradication concentration (MBEC) of NVC-422. The CBD 96-well plate was inoculated with *S. aureus* and placed on a shaker at 24 hrs at 35°C prior to treatment with NVC-422. Biofilm quantitation was performed by measuring the absorbance at 650 nm and correlated to viable plate counts. Absorbance values of less than 0.1 were considered to be evidence of biofilm eradication. High-shear biofilms were grown on polyurethane coupons in the CDC reactor. This reactor was inoculated with *S. aureus* for 24 hrs at 35°C, followed by continuous media flow for another 24 hrs. Coupons were removed, treated with NVC-422, and sampled for viable plate counts. The MRD reactor incubated at 37°C was fitted with catheter-like coupons. The reactor was continuously pumped with *S. aureus* for 24 hrs, prior to administration of NVC-422 according to a treatment protocol for seven days. Coupons and effluent were sampled for viable plate counts and effluent pH was measured. SEM and CSLM imaging were also performed. **Results:** *S. aureus* biofilms grew uniformly in the CBD and CDC reactors. The MBEC value for NVC-422-treated *S. aureus* biofilms was 5 mM. Treatment with 12 mM NVC-422 for 6 hrs was required to reduce *S. aureus* viable counts by 6 logs in the CDC reactor. In the MRD model, complete kill of *S. aureus* was observed within 8 hrs when 12 mM NVC-422 was administered. Re-growth of biofilm was not observed during the seven day study. **Conclusions:** NVC-422 significantly reduced *S. aureus* biofilms in three *in vitro* models. Treatment of biofilm with NVC-422 in the MRD model effectively eradicated *S. aureus* microorganisms and prevented re-growth. Due to its low potential for the development of bacterial resistance, NVC-422 is an attractive therapeutic agent for preventing biofilm colonization on indwelling medical devices.

## Introduction

The formation of microbial biofilms on the surfaces of indwelling medical devices is a salient feature that can lead to antibiotic tolerance resulting in medical device associated infections<sup>1,2</sup>, where they are accountable for more than half of all nosocomial infections<sup>3</sup>. Catheter-related infections, specifically, contribute to the escalating problem of these nosocomial infections<sup>4</sup>.

One of the organisms responsible in many of these device-related infections is *Staphylococcus aureus*<sup>5</sup>. With the increasing prevalence of antibiotic resistance among *S. aureus* isolates, current antibiotic therapies may no longer be sufficient to eradicate device-related infections. In this regard, NVC-422 is a promising therapeutic agent for the prevention and reduction of biofilm colonization on indwelling medical devices, due to its broad-spectrum microbicidal activity and low potential towards the development of resistance.

We have recently described the synthesis of a novel analog of *N*-chlorotaurine (NVC-422; *N,N*-dichloro-2,2-dimethylpropane-1-sulfonamide) with potent antimicrobial activity and long term solution stability<sup>6</sup>. In this study, we observed the activity of NVC-422 in 5 mM acetate saline pH 4 against *S. aureus* biofilm in three *in vitro* biofilm models: Calgary Biofilm Device (CBD) assay, CDC biofilm reactor and Modified Robbins Device (MRD) reactor. These results support the potential of NVC-422 as an antimicrobial lock solution for the treatment of catheter-related infections.

## Materials & Methods

**Calgary Biofilm Device (CBD):** The CBD was used to determine the minimum biofilm eradication concentration (MBEC) values of NVC-422. MBEC values were used to assess the concentration of an antimicrobial product required to kill bacterial biofilms. A standard culture of *S. aureus* ATCC 6538 was grown in Tryptic Soy Broth (TSB) and diluted to approximately 10<sup>7</sup> CFU/mL for inoculation of the CBD assay. Biofilm was grown for 24 hrs at 35°C prior to incubation with 0.08-40 mM NVC-422 in 5 mM acetate saline at pH 4 for 60 min at room temperature, followed by neutralization in Dey/Engley (D/E) Neutralizing broth and overnight incubation at 35°C in growth media. Biofilm quantization was performed by measuring the absorbance at 650 nm the following day. Absorbance values of less than 0.1 were considered to be evidence of biofilm eradication.

**CDC Biofilm Reactor:** The CDC reactor is a method that is approved to test antibiofilm efficacy by ASTM method E6-2196-02. ASTM International, 2002). Biofilm was grown under high shear on 1.3 cm<sup>2</sup> polyurethane coupons in the CDC reactor. The reactor was inoculated with CDC ATCC 6538 and allowed to grow for 24 hrs prior to continuous media flow of 1/10<sup>6</sup> strength TSB for an additional 24 hrs. Coupons were aseptically removed, treated with 12 mM, 20 mM and 40 mM NVC-422 in 5 mM acetate saline at pH 4 for 1 or 6 hrs. Biofilm was scraped off the coupons, neutralized in D/E broth and analyzed for viable cell counts by 10-fold serial dilutions and plated on TSA. Plates were incubated overnight at 35°C and CFUs were counted.

## Materials & Methods

**Modified Robbins Device (MRD):** The MRD reactor was used to study the efficacy of NVC-422 as a catheter lock solution. The reactor attached to a flow-through system was incubated at 37°C and was fitted with 7 mm diameter carbothane coupons. The reactor was continuously pumped with 10<sup>7</sup> CFU/mL *S. aureus* ATCC 700789 for 24 hrs, prior to administration of NVC-422 in 5 mM acetate saline at pH 4 as a catheter lock solution according to a treatment protocol for five days. Coupons and effluent were sampled for viable plate counts and effluent pH according to the sampling protocol (Table 1). SEM and CSLM imaging on coupons were also obtained. In a previous experiment, treatment protocol was continued for seven days to ensure no re-growth of biofilm (data not shown).

Day	Activity	Biofilm	Sampling	Planctonic
Day 0	Inoculation with 10 <sup>7</sup> CFU/mL <i>S. aureus</i> for 60 min			
Day 1	Infuse with 55 mL 0.3% of NVC-422 in acetate, lock system	Sample 4 random coupons prior to introduction of NVC-422	Sample effluent for pH and viable count prior to introduction of NVC-422	
4 hr post-lock	Locked	Sample 4 random coupons	Sample effluent for pH and viable count	
8 hr post-lock	Locked	Sample 4 random coupons	Sample effluent for pH and viable count	
Day 2	Locked	Sample 3 random coupons	Sample effluent for pH and viable count	
Day 3	Infuse with a modified Lactated Ringers solution for 4 hr at 200 mL/hr, followed by 60 mL (0.3% NVC-422 in acetate), then lock system	Sample 4 random coupons prior to introduction of dialysate	Sample effluent for pH and viable count prior to introduction of dialysate	
Day 4	Locked	Sample 2 random coupons for viable counts and effluent pH	Sample effluent for pH and viable count	
Day 5	Locked	Sample 4 random coupons	Sample effluent for pH and viable count	

Table 1. Outline of five day sampling protocol in the Modified Robbins Device (MRD) reactor

## Modified Robbins Device (MRD)

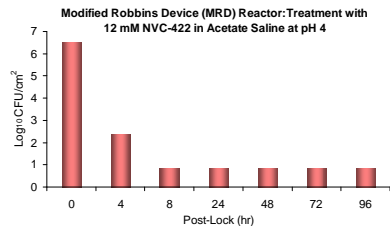


Figure 4. Mean Log<sub>10</sub> CFU/cm<sup>2</sup> of *S. aureus* in the Modified Robbins Device reactor. The administration for 4 hrs of 12 mM NVC-422 lock solution, 4 log biofilm reduction was observed. *S. aureus* biofilm was reduced by 6 logs after 8 hrs. No re-growth was observed in either effluent (planctonic) or biofilm samples throughout the duration of the experiment. Sampling of viable counts from effluent was consistent with biofilm sampling from the plugs. The pH of effluent sample shifted from pH 6 to pH 4 over time.

## Calgary Biofilm Device and CDC Biofilm Reactor

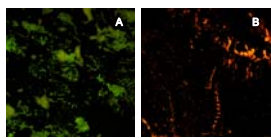


Figure 1. Confocal microscope images of Calgary Biofilm Device (CBD) pegs with *S. aureus* biofilm. CBD pegs were stained with Bac Light™ Live/Dead stain. Green corresponds to live cells, and red corresponds to dead cells. (A) Untreated pegs. (B) Pegs treated with 40 mM NVC-422 in acetate saline at pH 4. 10x magnification.

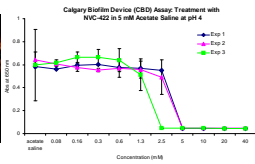


Figure 2. Minimum biofilm eradication concentration (MBEC) results for NVC-422 in acetate saline at pH 4 against *S. aureus* ATCC 6538 obtained from the CBD assay. MBEC value (expressed as the number of MBECs obtained from 3 plates) was 5 mM.

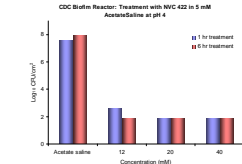


Figure 3. Mean Log<sub>10</sub> CFU/cm<sup>2</sup> of *S. aureus* ATCC 6538 following treatment with 12 mM, 20 mM, and 40 mM NVC-422 in acetate saline at pH 4. Once biofilm was established and coupons were removed from the CDC biofilm reactor, NVC-422 was applied for a treatment time of 1 or 6 hrs. Treatment with 12 mM NVC-422 for 6 hrs was required to reduce *S. aureus* viable counts by 6 logs in the CDC reactor.

## Modified Robbins Device (MRD)

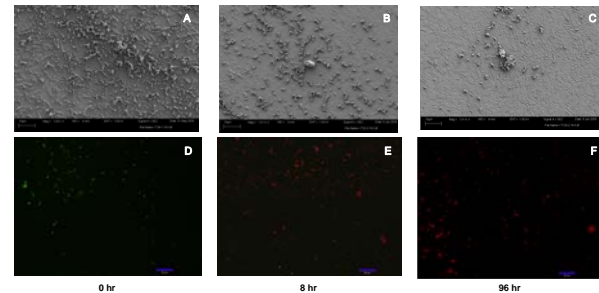


Figure 5. SEM and confocal microscope images of coupons from the Modified Robbins Device (MRD) study at 0, 8, and 96 hr post-lock. Coupons were stained with BacLight™ Live/Dead stain. Green corresponds to live cells, red corresponds to dead cells. SEM (Fig. 5. A-C) and CSLM (Fig. D-F) imaging show that some dead cells still remain intact on catheter material indicating NVC-422 does not completely remove cells from catheter surface even after 96 hr post-lock.

## Conclusions

- NVC-422 is an effective antimicrobial agent against *S. aureus* biofilms in three *in vitro* biofilm reactors: Calgary Biofilm Device (CBD), CDC biofilm reactor, and Modified Robbins Device (MRD) reactor
- NVC-422 has potent activity when tested against *S. aureus* biofilm in Calgary Biofilm Device assay, with an MBEC value of 5 mM
- Treatment with 12 mM NVC-422 for 6 hrs showed efficacy in reducing *S. aureus* biofilm by 6 logs in the CDC biofilm reactor
- S. aureus* biofilm was reduced by 6 logs after 8 hrs of exposure to 12 mM NVC-422 in the Modified Robbins Device (MRD) setup
- NVC-422 is a promising antimicrobial catheter lock solution for the prevention or reduction in colonization of the intraluminal catheter surfaces

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