Anti-biofilm activity of antibiotic-loaded Hylomate®

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A B S T R A C T

Introduction: Antibiotic envelopes are being developed for cardiac implantable electronic device (CIED) wrapping to reduce the risk of infections.

Methods: Fifteen CIED infection-associated bacterial isolates of Staphylococcus aureus, Staphylococcus epidermidis and Cutibacterium acnes were used to assess in vitro biofilm formation on Hylomate® compared to titanium, silicone and polyurethane coupons pre-treated with vancomycin (400 μg/ml), bacitracin (1000 U/ml) or a combination of rifampin (80 μg/ml) plus minocycline (50 μg/ml). Scanning electron microscopy (SEM) was performed to visualize bacteria on Hylomate®.

Results: There was significantly less (p < 0.05) S. aureus and S. epidermidis on Hylomate® pre-treated with vancomycin, bacitracin or rifampin plus minocycline after 24 h of incubation (<1.00 log10 CFU/cm²) compared with titanium, silicone or polyurethane pre-treated with vancomycin, bacitracin or rifampin plus minocycline. C. acnes biofilms were not detected (<1.00 log10 CFU/cm²) on pre-treated Hylomate® coupons.

Conclusions: This study showed that Hylomate® coupons pre-treated with antibiotics reduced staphylococcal and C. acnes biofilm formation in vitro.

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1. Introduction

There has been a 95% rise in numbers of cardiac implantable electronic device (CIED) implantations between 1993 and 2008 [1], which has, in turn, been associated with a higher burden of device replacement, generator change-outs, and upgrade/revision surgeries. The incidence of CIED infection has increased in parallel, with infection being a particular burden among those with underlying comorbidities [2]. CIED infections carry significant morbidity and mortality. The estimated annual rate of CIED infections is 1–6%, corresponding to ~8,000 to 13,000 CIED-related infections in the United States yearly [3]. The average cost associated with a single CIED infection event is ~$45,000–$83,000, representing a significant financial burden to the healthcare system [4,5]. Organisms associated with CIED infections attach to and grow in biofilms on generator and/or generator lead surfaces; the most frequently involved bacteria are Staphylococcus epidermidis, Staphylococcus aureus and Cutibacterium acnes [6]. The management of CIED-related infection usually includes complete device removal, including accessory hardware, as the use of systemic antibiotics alone will typically not suffice [7]. Given risks associated with treatment, prevention of CIED infections is desirable.

Several strategies to limit CIED infection have been proposed, including proper selection of patients for CIED placement, optimization of aseptic technique, administration of antibiotics at the time of device implantation, and use of antibiotic-coated implantable devices [2]. The last is intended to reduce or eliminate bacteria accessing device surfaces during implantation surgery, and includes antibacterial envelopes designed to release antimicrobial drugs directly into the CIED generator pocket [8,9,10]. Currently, the only antibacterial envelope available for use with CIEDs is TYRXTM (Medtronic, Minneapolis, MN), an absorbable envelope made of polypropylene and impregnated with rifampin and minocycline.

Hylomate® is a membrane made of cellulose synthesized by Acetobacter xylinum, which has been reported to be a well-tolerated material with potential biomedical applications, such as CIED wrappings [11], corneal bandages [12], wound dressings [13], treatment of oral diseases [14], and nerve repair [15].
According to Robotti and collaborators, Hylomate® is highly hydrophilic and able to decrease tissue fibrosis around CIEDs, facilitating implant removal or revision, if required [11,16]. The aim of this study was to evaluate biofilm formation on Hylomate® compared to other surfaces used in CIED generators and generator leads after pre-treatment of these surfaces with vancomycin, bacitracin or a combination of rifampin and minocycline.

2. Methods

Fifteen CIED infection-associated bacterial isolates, including five each of S. aureus (IDRL-9774, IDRL-11332, IDRL-11567, IDRL-11905 and IDRL-11992), S. epidermidis (IDRL-11532, IDRL-11770, IDRL-11889, IDRL-11913, and IDRL-12398), and C. acnes (IDRL-11914, IDRL-11980, IDRL-12431, IDRL-12532, and IDRL-12396), collected at Mayo Clinic, Rochester, MN from 2013 to 2020, and stored in the Infectious Diseases Research Laboratory biobank, were studied. Vancomycin minimum inhibitory concentration (MIC) values were ≤2 μg/ml for S. aureus and ≤4 μg/ml for S. epidermidis. Rifampin MICs were ≤0.5 μg/ml for S. aureus and S. epidermidis. Minocycline MICs were ≤0.5 μg/ml for all study isolates.

Ability to form biofilm after pre-treatment with antimicrobial agents was assessed on 12.7 mm diameter coupons made of Hylomate® (Hylomorph AG, Zurich, Switzerland) prepared with a biopsy punch, or of titanium, silicone, and polyurethane (Biosurface Technologies Corporation, Bozeman, MT), using an in vitro assay. Coupons received no pre-treatment (control) or pre-treatment for 15 min at room temperature with 1 ml vancomycin 400 μg/ml, bacitracin 1000 U/ml, or a combination of rifampin 80 μg/ml and minocycline 50 μg/ml [17,18] diluted according to CLSI guidelines [19]. Coupons were rinsed in sterile saline, inoculated with 103 Colony Forming Unit (CFU)/ml of bacteria in 2 ml tryptic soy broth (TSB) for staphylococci or brain heart infusion broth (BHI) supplemented with glucose 1% for C. acnes, and incubated at 37°C on an orbital shaker (110 rpm) with staphylococci incubated aerobically, and C. acnes incubated anaerobically. Three coupons were removed at each of 2, 4, 6, and 24 h for staphylococci, and 24, 36, 48 and 60 h for C. acnes. After removal, coupons were rinsed in 2 ml saline, placed in 1 ml saline, vortexed for 30 s, sonicated for 5 min, and then vortexed again to disaggregate biofilms and create bacterial suspensions. Sonicate fluids were serially sonicated for 5 min, and then vortexed again to disaggregate biofilm substrates.

SEM images (Fig. 5) show biofilms formed on Hylomate® with no antibiotic treatment, with bacterial cells apparently penetrating Hylomate® indicated by the red arrows.

4. Discussion

Results of this study demonstrate that Hylomate® pre-treated with antibiotics reduced the ability of S. aureus, S. epidermidis, and C. acnes to form biofilms. This may be facilitated by the hydrophilicity of Hylomate®, potentially enabling better absorption of antibiotics used as pre-treatments when compared with the other materials studied. We note that these are in vitro results [20].
Several studies have been carried out to test the activity and cost effectiveness of antibacterial envelopes in various patient groups undergoing de novo CIED implantation, revisions, or upgrades. The most comprehensive CIED clinical trial was the Worldwide Randomized Antibiotic Envelope Infection Prevention Trial (WRAP-IT), which evaluated the antibacterial envelope and do not imply in vivo activity, such as with CIED implant surgery.

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Fig. 3. Quantitative culture of *Staphylococcus aureus* (a, b, c, d, e), *Staphylococcus epidermidis* (f, g, h, i, j) and *Cutibacterium acnes* (k, l, m, n, o) on Hylomate®, titanium, silicone and polyurethane pre-treated with bacitracin (1000 U/ml). *Represents significant difference (p < 0.5) between Hylomate and other materials 24 h incubation.

Fig. 4. Results of quantitative culture of *Staphylococcus aureus* (a, b, c, d, e), *Staphylococcus epidermidis* (f, g, h, i, j) and *Cutibacterium acnes* (k, l, m, n, o) on Hylomate®, titanium, silicone and polyurethane coupons pre-treated with rifampin (80 μg/ml) plus minocycline (50 μg/ml). *Represents significant difference (p < 0.05) between Hylomate and other materials after 24 h or 60 h incubation for staphylococci and *C. acnes*, respectively.
TYRXTM impregnated with rifampin and minocycline in 6,983 patients at high risk for infection. There was a 40% reduction in the incidence of major CIED infections within 12 months of initial procedures, in comparison to standard-of-care infection prevention strategies [9]. In a follow-up study, beneficial effects of the TYRXTM envelope on reduction of the risk of CIED infection were sustained beyond the first year post-procedure without no apparent increased risk of complications [21].

A meta-analysis review of 11,897 high-risk patients from six studies showed risk reductions of CIED infections among patients with absorbable and non-absorbable antibacterial envelopes (TYRX™ and AISCIRx™) impregnated with rifampin plus minocycline compared with those managed conventionally [22]. There was a reported trend of lower mortality in those with antibacterial envelopes, although this finding did not reach statistical significance. How rifampin- and minocycline-loaded Hyloamate® might compare to TYRX™ impregnated with rifampin and minocycline is unknown.

Using an extracellular-matrix envelope derived from porcine small intestinal submucosa hydrated with gentamicin, Sohail

Fig. 5. Scanning electron micrographs of *Staphylococcus aureus* (a, d, g, m), *Staphylococcus epidermidis* (b, e, h, k, n), and *Cutibacterium acnes* (c, f, i, l, o) biofilms on cellulose after 24 (S. aureus and S. epidermidis), and 60 h (C. acnes) of incubation at different magnifications. Red arrows indicate bacterial cell penetration on Hylomate®. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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et al. demonstrated in vitro elimination of microorganisms when envelopes were incubated with *S. aureus*, *S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, or *Serratia marcescens*. In the same study, the authors showed bacterial reductions when the envelope was tested in a rabbit cardiac device pocket infection model after seven days of implantation compared with controls [23].

Here, the most active pre-treatment was the combination of rifampin and minocycline. Clinical studies incorporating rifampin and minocycline into central venous catheters, cerebrospinal fluid drains, and hemodialysis catheters have demonstrated reductions in device-related infections [24–26].

5. Conclusion
This study showed that Hylomate® coupons pre-treated with antibiotics reduced staphyloccocal and *C. acnes* biofilm formation in vitro. This suggests that antibiotic-impregnated Hylomate® should be further evaluated as a potential strategy to prevent CIED infections, including animal model and potentially human studies.

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CRediT authorship contribution statement
Mariana Albano: Methodology, Investigation, Validation, Writing - original draft. Keryll E. Greenwood-Quaintance: Conceptualization, Methodology, Writing - review & editing. Melissa J. Karau: Conceptualization, Methodology, Writing - review & editing. Jyawan N. Mandrekar: Formal analysis. Robin Patel: Funding acquisition, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest
Dr. Patel reports grants from Merck, ContraFect, TenNor Therapeutics Limited, Hylomorph and Shionogi. Dr. Patel is a consultant to Curetics, Specific Technologies, Next Gen Diagnostics, PathoQuest, Selux Diagnostics, 1928 Diagnostics, PhAST, and Qyella; monies are paid to Mayo Clinic. Dr. Patel is also a consultant to Netflix. In addition, Dr. Patel has a patent on a borderetella pertussis /parapertussis PCR issued, a patent on a device/method for sonication with royalties paid by Samsung to Mayo Clinic, and a patent on an anti-biofilm substance issued. Dr. Patel receives an editor's stipend from IDSA, paid by Samsung to Mayo Clinic, and a patent on an anti-biofilm substance issued. Dr. Patel has a patent on C. acnes biofilm formation in vitro. This suggests that antibiotic-impregnated Hylomate® should be further evaluated as a potential strategy to prevent CIED infections, including animal model and potentially human studies.

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