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Potential application of menadione for antimicrobial coating of surgical sutures

Cheng Hong Yap [a], See Khai Lim [a], Yun Li Chan [a], Chin Fei Chee [b], Sun Tee Tay [a,*]

[a] Department of Medical Microbiology, Faculty of Medicine, Universiti Malaya, 50603, Kuala Lumpur, Malaysia
[b] Nanotechnology and Catalysis Research Centre, Universiti Malaya, 50603, Kuala Lumpur, Malaysia

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ABSTRACT

Staphylococcal-associated surgical site infections (SSI) are common nosocomial infections in healthcare facilities worldwide. The use of antiseptic-coated sutures has been recommended to minimise the risk of SSI in clinical settings. However, as there has been a growing concern over antibiotic resistance resulting from antiseptic usage, development of antimicrobial sutures using alternative compounds is necessary. In this study, menadione (2-methyl-1,4-napthoquinone), also known as Vitamin K_3, was evaluated as a potential antimicrobial compound for suture coating. The anti-staphylococcal activity of menadione was assessed using microbroth dilution method and biofilm inhibition assays. The low menadione minimum biofilm inhibitory concentration values against both methicillin-susceptible and -resistant *S. aureus* strains indicate its inhibitory activity against staphylococcal biofilm. Menadione-coated sutures were prepared by dip-coating surgical sutures in slurries containing poly(D,L-lactide-co-glycolide) polymers (either 65:35 or 75:25) and calcium stearate. Zone of inhibition assays showed dose-dependent antimicrobial effects of the sutures up to four days. A ~3 log10 colony forming unit/ml reduction of adherent bacteria ($p < 0.05$) on the sutures was demonstrated via bacterial adherence assays. The integrity and tensile strength of the sutures were unaffected by the coating procedure. In view of the increased antibiotic resistance and limited antimicrobials, menadione may be potentially useful for antimicrobial coating of surgical sutures.

1. Introduction

Wound contamination and insufficient disinfection prior to surgical closure are the main causes for surgical site infections (SSI), which are often associated with a high risk of hospital re-admittance, prolonged ICU stay, postoperative complications and substantial financial burdens.[1,2] Treatment and management of infections caused by *Staphylococcus aureus,* a major SSI pathogen, is complicated by the development of biofilm-associated infections and the emergence of methicillin-resistant strains. Currently, antiseptic-based (triclosan and chlorhexidine) antimicrobial sutures are commercially available to minimise the risk of SSI in clinical settings. Recent studies showed that antiseptic exposure can contribute to the emergence of multidrug-resistant bacteria.[3] Additionally, increased usage of certain antiseptics in clinical practice, has also raised health concerns.[4]

The increasing reports of antibiotic and antiseptic resistance globally has fueled research into finding alternative compounds for the development of antimicrobial sutures.[5,6] While most antimicrobial compounds target bacterial survival mechanisms, the selective pressure may induce the emergence of resistant bacterial subpopulations.[7] Meanwhile, compounds that do not directly affect bacterial viability may have less impact on the development of resistant strains.[8] A variety of antimicrobial compounds including antibiotics, natural products, and nanoparticles have been explored as antimicrobial materials for coating on surgical sutures.[6]

Menadione (2-methyl-1, 4-naphthoquinone, Vitamin K_3) is a synthetic lipid-soluble vitamin K_2 precursor which induces the production of reactive oxygen species (ROS). The ROS-mediated mechanism of menadione targets the bacterial biosynthetic machinery, causing direct damage to DNA, lipids and proteins. Menadione has also been recognised as a class of topical antibacterial therapeutic agents, demonstrating inhibitory effects against several Gram-positive pathogens including *S. aureus, Bacillus anthracis, Streptococcus pyogenes* and *Streptococcus agalactiae*.[9] Due to its lipid-soluble nature, menadione has shown the ability to modulate bacterial plasma membrane permeability, and enhance the activity of antibiotics such as aminoglycosides in

* Corresponding author. Department of Medical Microbiology, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.
E-mail address: tayst@um.edu.my (S.T. Tay).

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multidrug resistant strains of *S. aureus, Pseudomonas aeruginosa* and *Escherichia coli*.[10]

Currently, there are no reports regarding menadione inhibition and eradication of *S. aureus* biofilm. The unique properties of menadione has prompted us to explore its antibiofilm properties, and potential application in the development of antimicrobial sutures. The anti-staphylococcal activity of sutures coated with various concentrations of menadione were analysed using zone of inhibition (ZOI) and bacterial adherence assays.

2. Material and methods

2.1. Chemicals and reagents

Poly(D,L-lactide-co-glycolide) (PLGA 65:35, Cat. no. P2066, ester terminated, M_w: 40 000 – 75,000) and RESOMER® (PLGA 75:25, Cat. no. 769789, ester terminated M_w: 124 000 – 133 000) were sourced from Sigma Aldrich Co., USA and Sigma Aldrich Co., Germany, respectively. Calcium stearate (CaSt, Cat. no.102172042) was sourced from Sigma Aldrich Co., Netherlands.

2.2. Antimicrobial susceptibility testing of menadione

Methicillin-susceptible *S. aureus* (MSSA) strain ATCC® 29213™ and methicillin-resistant *S. aureus* (MRSA) strain ATCC® 33591™ were used in this study. ATCC® 29213™ is a standard quality-control strain used in microbiological assays.[11] Menadione (Sigma-Aldrich, Cat. no. M5625, China) was initially dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, France) to make a stock solution of 10 mg/ml and stored at −80 °C prior to use. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of menadione were determined against staphylococcal strains using microbroth dilution method in accordance with the standard protocol of Clinical and Laboratory Standard Institute (CLSI) M07-A9.[12] To determine menadione MIC, bacterial growth in U-shaped 96-well microtiter plates (Guangzhou Jet Bio-Filtration Co., Ltd., TCP012096, China) was observed using a reflective mirror after a 24 h incubation period at 37 °C. To determine menadione MBC, 10 µl of the culture fluid from wells without visible growth was cultured on Mueller-Hinton agar (MHA) (HiMedia Laboratories, India) and observed for growth after 24 h of incubation at 37 °C.

2.3. Determination of antibiofilm activity of menadione

In order to establish the minimum concentration of menadione required to inhibit 50% biofilm formation ($MBIC_{50}$), experiments were performed in sterile flat bottomed 96-well microtiter plates (Biologix Europe GmbH, 07-6096, Köln, Germany) as described previously.[13] A two-fold serial dilution ranging from 0.0625 to 512 µg/ml of menadione was prepared in Mueller Hinton Broth (MHB) (Difco Laboratories, USA) supplemented with 1% glucose. The working solution (100 µl) of menadione was transferred into designated microtiter wells prior to addition with an equal volume of 1×10^6 CFU/ml bacterial cell suspension in MHB supplemented with 1% glucose. The quantity of biofilm biomass in the presence of menadione was measured after 24 h using a crystal violet assay.[14] Briefly, after the content from each well was discarded, the wells were washed thrice with 200 µl of phosphate buffered saline (PBS), and fixed with 200 µl of 95% methanol for 20 min. All wells were dried and subsequently stained with 200 µl of 0.1% (v/v) crystal violet (CV) (Sigma Aldrich, Germany) per well for 20 min. The wells were then rinsed with distilled water thrice, air-dried and added with 150 µl of 30% acetic acid. The optical density of each well was determined at 570 nm using a microplate reader (Tecan's Sunrise™, Austria). The minimum concentration (expressed in µg/ml) which inhibited 50% of biofilm growth ($MBIC_{50}$) was determined using the following formula:

$$\text{Biofilm inhibition (\%)} = (1 - (A_{sample} - A_0) / (A_1 - A_0)) \times 100$$

whereby.

A_{sample} = absorbance of treatment solution wells at 570 nm
A_1 = absorbance of growth control at 570 nm
A_0 = absorbance of sterility control at 570 nm

The determination of minimum biofilm eradication concentration ($MBEC_{50}$) was performed similarly to that of the $MBIC_{50}$ assay with slight modifications. Briefly, each well was first inoculated with 200 µl of bacterial cell suspension (1×10^6 CFU/ml) in MHB supplemented with 1% glucose, followed by incubation for 4 h at 37 °C with constant agitation at 75 rpm in a shaker incubator (Stuart® S1500 orbital incubator, Bibby Scientific Ltd, Stone, England) to establish preformed biofilm. The wells were then washed gently with 200 µl PBS twice, added with fresh media and incubated for another 20 h. Varying concentrations (1–512 µg/ml) of menadione were then added to the preformed biofilm for further incubation at 37 °C for 24 h. The resulting biofilm biomass was quantified similarly as described in the $MBIC_{50}$ assay.

2.4. Preparation of menadione-coated sutures

Menadione was coated onto VICRYL® braided sutures (USP1, W9391, Ethicon LLC., USA) by a dip-coating method as described previously.[15] An initial 2 × stock solution containing 200 mg of either PLGA 65:35 or PLGA 75:25 and menadione (10, 20, 30, 40 and 80 mg) were prepared by dissolving the required amounts individually in 1 ml of ethyl acetate. The PLGA 65:35 and PLGA 75:25 stock solutions were allowed to homogenise overnight at −20 °C while the menadione solution was prepared freshly on the day of coating. The stock solutions of PLGA 65:35 or PLGA 75:25 and menadione were then mixed in equal volume and added with 16 mg/ml of calcium stearate which acts as a lubricant to facilitate the passage of the suture through tissue.[15,16] The resulting slurries were then subjected to sonication using an ultrasonic cleaner (Jie Tai, Shenzhen, China) at 20 kHz for 1 min at room temperature prior to coating.

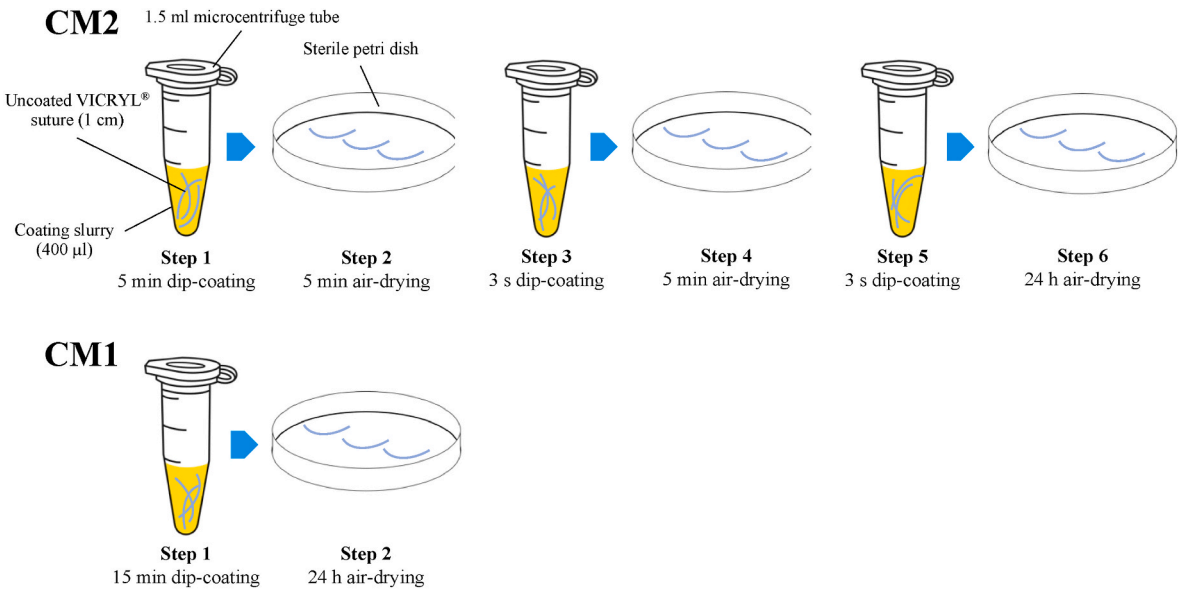
Two different coating methods were evaluated as illustrated in Scheme 1. The first coating method (referred as CM1) involved a layer by layer deposition of menadione onto the sutures, i.e., each suture was first subjected to 5 min of dipping in menadione slurry, followed by 5 min of air-drying, and subsequently two cycles of short dipping time (3 s) and 5 min air-drying, and subsequently left to dry overnight. The second coating method (CM2) involved dipping of the sutures in the slurry for 15 min, followed by air-drying overnight. Three sutures each measuring 1 cm in length were weighed (W_1) before and after the coating process to determine the amount of menadione coated over the length of suture (µg/cm) using the following equation:

$$\frac{W_2 - W_1}{1\text{ cm}} \times \frac{x\ \mu\text{g menadione in slurry}}{100\ \mu\text{g PLGA} + x\ \mu\text{g menadione in slurry} + 16\ \mu\text{g CaSt}}$$

For the convenience of reporting, each suture was denoted as M followed by the concentration of menadione used in the coating slurry i.e., M20 = suture dipped in 20 mg/ml of menadione slurry.

2.5. Determination of the antimicrobial activity of menadione-coated sutures via zone of inhibition (ZOI) assay

The menadione-coated sutures were tested against MSSA ATCC® 29213™ by using the zone of inhibition assay as described previously.[15] An overnight culture of *S. aureus* was adjusted to 0.5 McFarland turbidity standard and swabbed tri-directionally at a 60° angle onto a MHA plate using a sterile cotton swab. The menadione-coated sutures were embedded into the agar using forceps aseptically. The zone of inhibition around the sutures were measured in millimetres perpendicular to the suture placement after 24 h incubation at 37 °C. To determine the antimicrobial activity timewise, the same suture was re-embedded into another MHA plate freshly lawned with *S. aureus* after 24 h and ZOI



Scheme 1. Coating methods of menadione onto sutures.

measurements were taken the following day. The suture was re-embedded into new MHA plates freshly lawned with *S. aureus* for up to 8 days or until there were no further inhibition zones observed. The mean of three separate zone measurements represents the average diameter of the inhibition zone. Triclosan-coated sutures, VICRYL® Plus (V^+) (USP1, VCP359, Ethicon LLC., USA) and uncoated (V^0) sutures were used as positive and negative controls for the ZOI assay, respectively.

2.6. Determination of bacterial adherence on menadione-coated sutures

The assay was performed as described by Obermeier et al.[17] Menadione-coated sutures of 1 cm in length (n = 3) were first submerged in 1 ml of 1×10^8 CFU/ml *S. aureus* inoculum for 3 h in a shaker incubator (Stuart® S1500 orbital incubator, Bibby Scientific, England) at 37 °C with constant agitation at 75 rpm. The sutures were then washed 3 times in 1 ml of sterile saline to remove any loosely adherent bacteria. Subsequently, the sutures were sonicated for 3 min followed by vortexing (WiseSpin®centrifuge, Daihan Scientific, Korea) for 5 s. The resulting bacterial suspension was then serially diluted and plated onto MHA plates evenly using L-shaped spreaders. The colony-forming units (CFU/ml) were enumerated after 24 h of incubation at 37 °C. The number of viable adherent bacteria on the suture surfaces were compared to those obtained from V^+ (triclosan-coated) and V^0 (uncoated) sutures, respectively. A clinical strain of MRSA (R7) was also included in this assay.

2.7. Field emission scanning electron microscopy imaging of menadione-coated sutures

Field emission scanning electron microscopy (FESEM) was employed to visualise the surface morphology of coated and uncoated sutures. Each suture was first subjected to a critical point drying process in CO_2 (CPD 7501, Polaron, UK) for 1.5–2 h, followed by mounting onto an aluminium stub using carbon adhesive cement and coating with gold (Biorad E5100 Series 11, USA). The sutures were then observed under an FEI Quanta 450 FEG (USA) field emission scanning electron microscope at 200 × and 400 × magnification, respectively.

2.8. Evaluation of suture tensile strength

The tensile strength of the menadione-coated sutures (sutures dip-coated in slurries containing 20 mg/ml and 40 mg/ml menadione with PLGA and calcium stearate) were assessed using the Universal Testing Machine (UTM; AG-X Series, Shimadzu Corp., Kyoto, Japan) with a gauge length of 6 cm.[18] Each end of the suture (15 cm) was clamped to the respective arm-grip, and a knot was tied on the end of the arm to prevent slippage of the suture and to ensure consistent force distribution. Tensile strength assessment was evaluated at a cross-head speed of 5 mm/min with a full scale load of 10 N. Each suture was stretched to failure and the maximum force required to break the suture was recorded in Newtons (N).

2.9. Statistical analysis

All data were expressed as mean ± standard deviation. The Statistical Package for the Social Sciences (SPSS) software version 26.0 (SPSS Inc, Chicago, Illinois, USA) was used to statistically analyse the data. Independent *t*-test was used to compare the means between groups. One-way analysis of variance (ANOVA) and Tukey's Honesty Significant Difference test were used to compare means among groups of sutures with regards to the amount of menadione coated onto sutures and coating slurries containing different types of polymers and varying concentrations of menadione. . Although a $p < 0.05$ was generally used, a significance level of $p < 0.01$, $p < 0.001$, and $p < 0.0001$ were also used to indicate stronger significant differences.

3. Results

3.1. In vitro susceptibility of S. aureus strains to menadione

Table 1 shows the menadione MIC (ranging from 8 to 16 µg/ml), MBC (256 µg/ml), $MBIC_{50}$ (0.0625–0.25 µg/ml) and $MBEC_{50}$ (128–>512 µg/ml) values against MSSA ATCC® 29213™ and MRSA ATCC® 33591™ strains. As the menadione MBC to MIC ratio is > 4, this suggests that menadione is bacteriostatic.[19] Interestingly, the relatively low menadione $MBIC_{50}$ concentration against staphylococcal strains also suggests its potential application for staphylococcal biofilm inhibition (Table 1). Fig. 1 shows that ≥80% of MSSA and MRSA biofilms were inhibited upon exposure to 0.25 µg/ml menadione. The results of crystal violet staining assays for determination of menadione MBIC and MBEC against methicillin-susceptible (ATCC® 29213™) and methicillin-resistant *S. aureus* (ATCC® 33591™) strains are shown in Supplementary Fig. 1. DMSO alone (up to 2.56%) in the respective

Table 1
In vitro susceptibility of *S. aureus* reference strains to menadione.[a]

Bacterial strains	MIC (μg/ml)	MBC (μg/ml)	$MBIC_{50}$ (μg/ml)	$MBEC_{50}$ (μg/ml)
MSSA ATCC® 29213™	16	256	0.25 (79.53%)	>512 (28.14%)
MRSA ATCC® 33591™	8	256	0.0625 (55.06%)	128 (64.24%)

[a] Footnote: MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; $MBIC_{50}$, the lowest concentration that resulted in ≥50% inhibition of biofilm formation; $MBEC_{50}$, the lowest concentration that resulted in ≥50% eradication of preformed biofilm. Parentheses indicate the percentages of biofilm inhibition and eradication corresponding to the determined $MBIC_{50}$ or $MBEC_{50}$ values.

menadione preparation did not show inhibition effect to staphylococcal biofilm growth (Supplementary Fig. 2).

3.2. Dip-coating of menadione onto sutures

The results of the zone of inhibition (ZOI) assays showed that sutures coated with three layers of menadione using the method CM1 produced a larger ZOI (14 ± 1 mm) on day 1 and a lower ZOI (3.67 ± 0.58 mm) on day 2 when compared to sutures coated using CM2 method, whereby smaller zones were observed on day 1 (10 ± 1.73 mm) and day 2 (1.33 ± 0.58 mm), respectively (Supplementary Fig. 3).

3.3. FESEM and tensile strength analyses of menadione-coated sutures

As compared to the surface morphology of uncoated VICRYL® suture (V^0) (Fig. 2. A), sutures coated with PLGA with or without menadione demonstrated smooth surfaces (Fig. 2. B and 2. C, respectively). Comparatively, particles were seen distributed evenly on the surface of menadione-coated suture incorporated with CaSt (Fig. 2. D). The coating of menadione, CaSt and PLGA did not compromise the tensile strength of the resulting sutures. An increase in the break force (~10 N) was observed with menadione-coated sutures (Supplementary Table 1.).

3.4. Antimicrobial activity of menadione-coated sutures

3.4.1. Zone of inhibition (ZOI) assay

Fig. 3 (A) shows the chemical structure of menadione investigated in this study. Fig. 3 (B) exhibits the inhibitory effects of menadione-coated

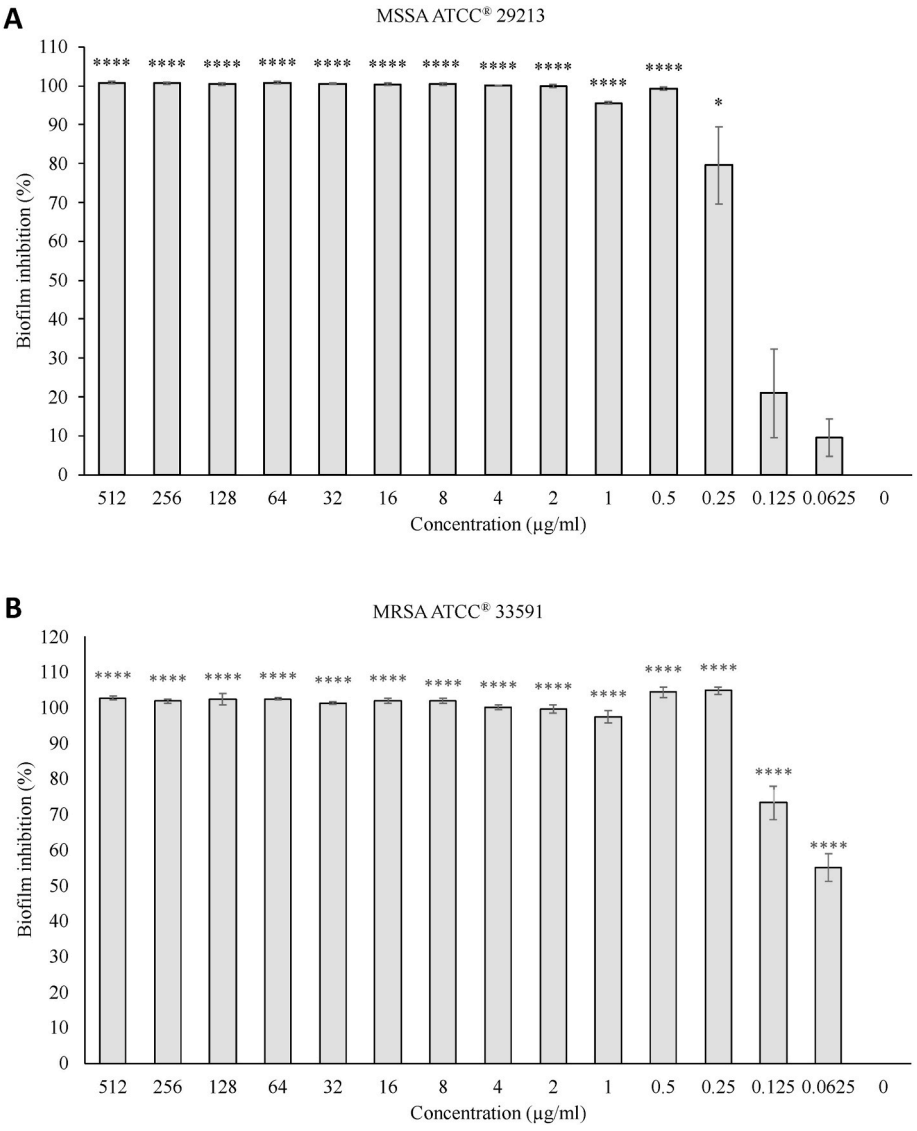


Fig. 1. The biofilm inhibitory effects of various concentrations of menadione against (**A**) MSSA ATCC® 29213™ and (**B**) MRSA ATCC® 33591™. The values are expressed as mean ± standard deviation, *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001 compared to the growth control.

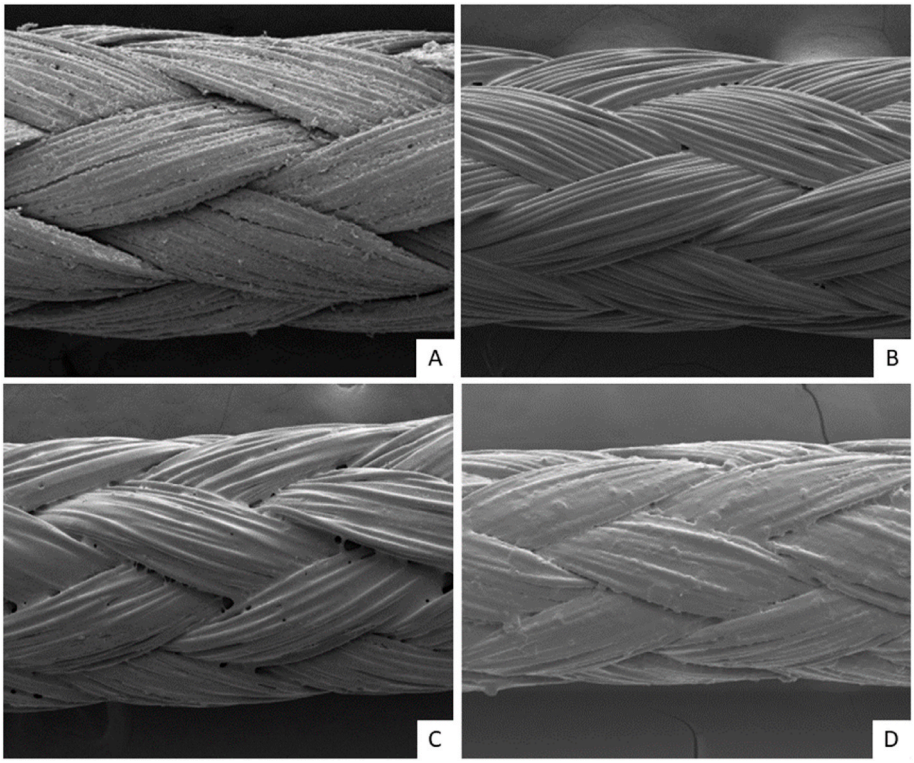


Fig. 2. SEM images of (**A**) VICRYL® polyglactin 910 suture, V^0, (**B**) VICRYL® polyglactin 910 suture coated with PLGA (65:35) only, (**C**) VICRYL® polyglactin 910 suture coated with PLGA (65:35) and menadione (5 mg/ml, M5), and (**D**) VICRYL® polyglactin 910 suture coated with PLGA (65:35), menadione (20 mg/ml, M20) and CaSt (16 mg/ml). Magnification: 200×

sutures (M40) coated with two different types of PLGA polymers (65:35 and 75:25) against *S. aureus* after 24 h of incubation. The zones of inhibition generated by both sutures were almost similar albeit slightly smaller as compared to that of V^+ suture.

Fig. 3 (C) shows the dose-dependent effect of the antimicrobial activity of menadione-coated sutures. Comparative analysis of sutures showed that M20/PLGA 75:25 (132.35 ± 16.98 μg/cm menadione) and M40/PLGA 65:35 (122.51 ± 9.87 μg/cm menadione) sutures produced the largest zone of inhibition after 24 h of incubation. This was then followed by M15, M10 and M5 sutures, regardless of the type of PLGA used. The inhibitory effects of the M20/M40 sutures coated with either PLGA 75:25 or 65:35 sustained up to 3–4 days, as compared to V^+ suture, which lasted for 7 days (Fig. 3C).

Fig. 4 shows that the amount of menadione incorporated per cm of suture were parallel with the concentration of menadione in the coating slurries. In general, PLGA 75:25 was found to hold significantly more menadione (ranging from ~50 to 300 μg/cm), as compared to PLGA 65:35 (ranging from 38 to 100 μg/cm) ($p < 0.05$).

3.4.2. Bacterial adherence testing with menadione-coated sutures

Bacterial adherence assay was performed using M20 (PLGA 65:35) and M40 (PLGA 65:35) sutures against reference strains of MSSA, MRSA and a clinical staphylococcal strain. VICRYL Plus (V^+) and menadione-coated (M20 and M40) sutures reduced the adherence of MSSA and MRSA reference strains and a clinical strain (R7) significantly, by at least 3 $\log_{10}$ reduction in CFU/ml ($p < 0.05$) (Fig. 5).

4. Discussion

Sutures are used to facilitate wound healing and hence, play an important role during surgical interventions. Patients are at higher risks of SSIs when sutures are contaminated with staphylococci from an external source, which eventually lead to persistent infection due to biofilm growth on the suture. The formation of biofilm allows bacteria to stay dormant within matrices that are characteristically impermeable to antibiotics and host defence, and thus, is particularly difficult to be eradicated.[20–25]

The low menadione MIC values (8–16 μg/ml) against MSSA and MRSA strains (Table 1) are in line with a previous study on menadione inhibition of staphylococcal planktonic cultures.[10] In addition, menadione exhibits low MBIC values (0.0625–0.25 μg/ml) against MSSA and MRSA strains (Table 1), suggesting its potential to inhibit biofilm growth. The *in vitro* susceptibility data has provided the foundation in developing antimicrobial coating using menadione since only a minimal amount of menadione will be required to prevent bacterial colonisation and subsequently biofilm formation on sutures. However, at least 2000-fold higher concentration of menadione would be required for the eradication of *S. aureus* mature biofilms, as indicated by the higher MBEC values (>512 μg/ml) as compared to the MBIC values (Table 1), thus, demonstrating the difficulty in staphylococcal biofilm eradication, as reported in previous studies.[6,14]

The antimicrobial coating incorporated PLGA, an FDA-approved copolymer, in the delivery of menadione on the suture. The high biocompatibility and biodegradable properties of PLGA has made it a good choice for fabrication of medical devices, including sutures. The findings in this study showed that multilayer coatings of PLGA (either PLGA 75:25 or 65:35) with menadione produced sutures with significantly higher antimicrobial activities, as compared to single coatings (Supplementary Fig. 3). Additionally, the integrity of the menadione-coated sutures was not affected as evidenced through the investigation by FESEM (Fig. 2) and tensile strength analyses (Supplementary Table 1).

The antimicrobial activity of menadione-coated sutures demonstrated dose-dependent effects and reached the maximum antimicrobial effect with the use of 20 mg/ml menadione coating slurry (Fig. 3C), as further increase of menadione concentration (40 mg/ml) did not result

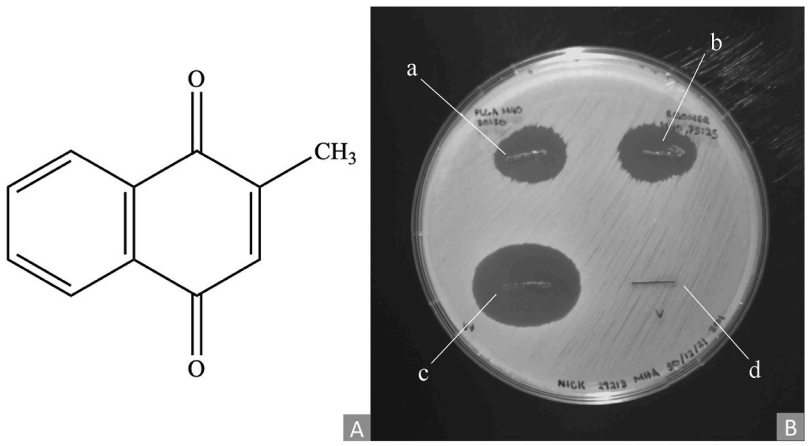
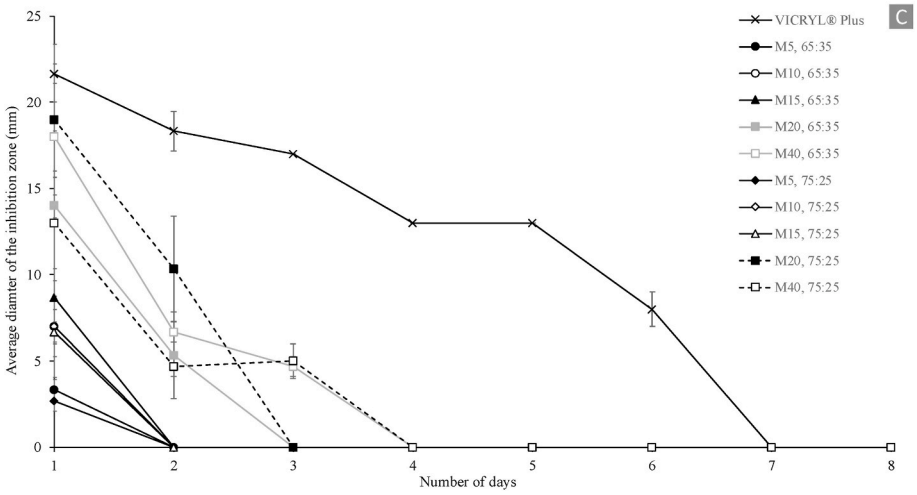


Fig. 3. (**A**) Chemical structure of menadione, (**B**) Zones of inhibition generated by menadione-coated sutures against *S. aureus* ATCC 29213 on a Mueller Hinton agar plate after incubation for 24 h, (a) M40 suture (PLGA 75:25); (b) M40 suture (PLGA 65:35); (c) V^+ suture; and (d) V^0 suture, (**C**) Sustainability of antimicrobial activities of menadione-coated sutures against MSSA ATCC® 29213™ reference strain. Sutures were coated in slurries containing 5, 10, 15, 20 and 40 mg/ml menadione with either PLGA 65:35 or 75:25. Triclosan-coated (V^+) sutures were used as positive controls. For the convenience of reporting, each suture was denoted as M followed by the concentration of menadione (mg/ml) used in the coating slurry.



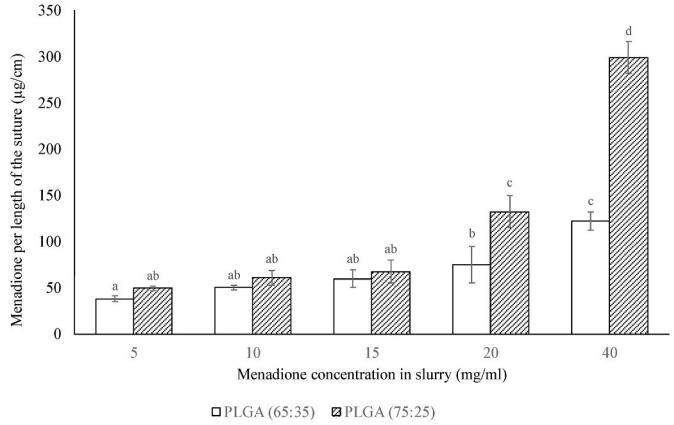


Fig. 4. Comparison of the estimated amounts of menadione per 1 cm length of sutures prepared by coating sutures in slurries containing either PLGA 65:35 or PLGA 75:25 with different concentrations of menadione. Columns with different lower-case letters ($^{a, b, c, d}$) are significantly different at $p < 0.05$ between varying coating concentrations.

in significantly higher ZOI results (Fig. 3B). Nevertheless, menadione-coated sutures (M20/M40) had a more immediate front-loaded compound release profile as the zone of inhibition against *S. aureus* strains sustained for 3–4 days as compared to V^+ suture which lasted for 7 days (Fig. 3C). It is good to note that the amount of menadione coated onto the sutures are determined via a gravimetric analysis which represents a rough estimation on the amount of menadione coated onto the sutures. Future studies may utilise UV-Vis spectrometry, Fourier transform infrared (FTIR) spectroscopy or high-performance liquid chromatography (HPLC) analyses to determine the exact amount of menadione coated onto the sutures.

Besides ZOI assay, a reduction in the number of bacteria adhering on sutures provides an important indicator on the efficacy of antimicrobial sutures.[28] In this study, the menadione-coated sutures demonstrated a significant reduction (~3 $\log_{10}$ CFU/ml) in the adherence of *S. aureus* reference and clinical strains (ATCC® 29213™, ATCC® 33591™ and R7) in comparison with uncoated sutures (Fig. 5), thus providing further evidence on the potential application of menadione for antimicrobial coating of sutures.

The use of ROS-inducing antibacterial strategies has been proposed as a promising approach for pathogen clearance.[29] The inhibitory effect of menadione towards bacterial pathogens such as *S. aureus*, *B. anthracis, S. pyogenes*, and *S. agalactiae,* has been attributed to ROS induction, which affects bacterial plasma membranes, biosynthetic machinery, two-component systems, respiration, and macromolecular synthesis.[9] Photosensitization of menadione by UV-A light has been reported to cause growth inhibition to *S. aureus* and Gram-negative bacteria.[30] Another study showed the antimicrobial activity of menadione towards

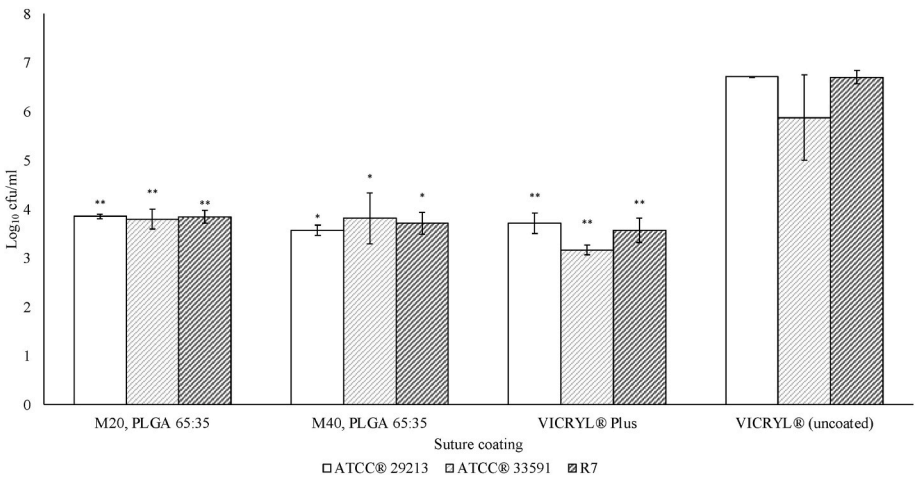


Fig. 5. Comparison of viable bacteria (log_{10} CFU/ml) adhered to menadione-coated sutures in comparison to VICRYL® Plus sutures (positive control) and uncoated VICRYL® sutures (negative control). Three staphylococcal strains, i.e., MSSA (*S. aureus* ATCC 29213). MRSA (ATCC 33591 and R7) were included in this study. (*) and (**) indicate significant difference at $p < 0.05$ and $p < 0.01$, respectively, between the uncoated (negative control) and coated sutures. M20, suture coated in slurry containing 20 mg/ml menadione; M40, suture coated in slurry containing 40 mg/ml menadione. V+ sutures demonstrated a ~3.5 log_{10} reduction in CFU/ml ($p < 0.01$) against MRSA ATCC® 33591™ and a ~3 log_{10} reduction against MRSA R7 ($p < 0.01$), while no reduction in log_{10} CFU/ml was observed for uncoated sutures.

Helicobacter pylori, a notorious cause of gastric cancer, and its anti-inflammatory effects by decreasing the injection of virulence factors into the host cells.[31] Previous studies have also shown that menadione enhances the effect of antibiotics through efflux pump inhibition, by suppressing *NorA* pump gene expression in *S. aureus*.[32,33] The modulation of plasma membrane permeability in bacteria has also rendered it a potential adjuvant for antimicrobial therapy of infections caused by a plethora of Gram-positive and negative organisms.[10,32,33]

Aside from the recognition of menadione as a novel class of topical antibacterial therapeutic agent,[9] menadione has been found useful to accelerate re-epithelialization of cornea following wounding from surgery or trauma.[34] It has also been investigated as an option for topical treatment of cetuximab-induced rash.[35] Menadione exhibits potent anticancer activity as evidenced via studies of the breast, bladder, hepatic, mammary, oral cavity, pharyngeal, blood cancers, parental and multidrug resistant leukaemia cell lines.[36,37] Recently, the antiviral potential of menadione against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by inhibition of the SARS-CoV-2 $3CL^{pro}$ activities has been described.[38,39]

Although all these findings suggest potential application of menadione for clinical use, DNA damage and mitochondrial dysfunction and fragmentation in human corneal endothelial cells and bovine lenses *in vitro*, have been reported at high concentrations (50 μM and 200 μM, respectively).[40,41] Menadione is likely to be cytotoxic at its minimum bactericidal (MBC: 256 μg/ml, equivalent to 1.5 mM) and biofilm eradication concentrations ($MBEC_{50}$: >512 μg/ml), and relatively less cytotoxic at its minimum biofilm inhibitory concentrations against MSSA ($MBIC_{50}$ = 0.25 μg/ml, equivalent to ~1.45 μM) and MRSA ($MBIC_{50}$ = 0.0625 μg/ml, equivalent to ~0.363 μM) (Table 1). Hence, despite findings supporting the potential application of menadione for staphylococcal biofilm inhibition, its antimicrobial benefit should be weighed against the adverse effects to humans or animals especially when higher concentrations are required for bactericidal and biofilm eradication effects. The small amount of menadione coated on suture surface (Fig. 5) may have little or no adverse effects on host cells coming into contact with the sutures. Nevertheless, further evaluation of the safety and effectiveness of menadione-coated sutures using appropriate animal models is essential.

Surgical site infection, one of the leading causes of hospital-acquired infection, contributes significantly to the increased medical costs and length of hospital stay. Analyses of the economic impact of SSI have called for the implementation of infection prevention procedures including use of antimicrobial sutures to minimise the risk of SSI.[1,42] As the cost of menadione is relatively low (https://www.echemi.com/productsInformation/pid_Seven41388-menadione.html) compared to antibiotics commonly used for wound management, the delivery of menadione via surgical suture may reduce the overall healthcare cost for surgical revision, and readmission due to SSIs. The coating procedures for menadione-coated suture can be further refined for optimum sorption of menadione onto suture in a way to ensure the sustainability of the menadione-coated sutures for an extended period till a surgical wound has completely healed.

5. Conclusion

With the rise in the incidence of biofilm-associated infections due to increase use of indwelling medical devices for critically-ill patients,[1] the discovery of menadione as a potent antibiofilm molecule provides a promising alternative to minimise the risk of SSI and other device-related infections. The antibacterial and antibiofilm properties of menadione-coated sutures, as supported by *in vitro* susceptibility testing, ZOI and bacterial adherence assays against MSSA and MRSA strains in this study, has paved the way for development of new antimicrobial sutures.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

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