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Bio-inspired crosslinking and matrix-drug interactions for advanced wound dressings with long-term antimicrobial activity

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ABSTRACT

There is a growing demand for durable advanced wound dressings for the management of persistent infections after deep burn injuries. Herein, we demonstrated the preparation of durable antimicrobial nanofiber mats, by taking advantage of strong interfacial interactions between polyhydroxy antibiotics (with varying number of −OH groups) and gelatin and their *in-situ* crosslinking with polydopamine (pDA) using ammonium carbonate diffusion method. Polydopamine crosslinking did not interfere with the antimicrobial efficacy of the loaded antibiotics. Interestingly, incorporation of antibiotics containing more number of alcoholic −OH groups ($N_{OH} \geq 5$) delayed the release kinetics with complete retention of antimicrobial activity for an extended period of time (20 days). The antimicrobials-loaded mats displayed superior mechanical and thermal properties than gelatin or pDA-crosslinked gelatin mats. Mats containing polyhydroxy antifungals showed enhanced aqueous stability and retained nanofibrous morphology under aqueous environment for more than 4 weeks. This approach can be expanded to produce mats with broad spectrum antimicrobial properties by incorporating the combination of antibacterial and antifungal drugs. Direct electrospinning of vancomycin-loaded electrospun nanofibers onto a bandage gauze and subsequent crosslinking produced non-adherent durable advanced wound dressings that could be easily applied to the injured sites and readily detached after treatment. In a partial thickness burn injury model in piglets, the drug-loaded mats displayed comparable wound closure to commercially available silver-based dressings. This prototype wound dressing designed for easy handling and with long-lasting antimicrobial properties represents an effective option for treating life-threatening microbial infections due to thermal injuries.

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1. Introduction

According to the Multidisciplinary Alliance Against Device-Related Infections (MAADRI), medical device associated infections are the major cause of more than half of the hospital acquired infections. Together with the rise in antibiotic-resistant superbugs such as methicillin-resistant *Staphylococcus aureus* (MRSA), there is a pressing demand for next-generation anti-infective materials for protecting wounds and medical devices against microbial contaminations. The infections caused by drug-resistant pathogens prolong the treatment duration and increase the nursing costs [1,2]. The dearth of new candidate compounds in the pipeline and a sharp decline in the number of companies investing in antibiotic research further exacerbate problems [3,4]. In the United States alone, an estimated 6.5 million people suffer from chronic skin ulcers while 1.25 million burns have been reported annually [5,6]. Infections caused by microbial pathogens is one of the greatest concerns in the management of chronic wounds and burn injuries as it delays or impedes the healing process and induces unwanted inflammatory response, thus complicating the treatment modalities [6–8].

S. aureus and MRSA are the major aetiological agents responsible for invasive infections in burn wounds around the world. However, in tropical climates, higher incidences of infections due to Gram-negative bacilli such as *P. aeruginosa* and multidrug-resistant *Acinetobacter baumanii* have been reported [9–11]. The advent of topical antimicrobial agent impregnated wound dressings such as silver, iodine, polyhexamethylenebiguanide, octinidine has provided a tangible alternative in the management of wound infections [12]. However, a rapid increase in the antimicrobial concentration due to unrestrained release at the tissue sites enhances the systemic absorption of antimicrobials causing adverse effect on vital organs. In addition, commonly used wound dressings require frequent changing of dressings, thus raising concerns in their utility for deep burn injuries [9–11]. Therefore, there is an apparent need of materials that could help in continued release of antimicrobials for an extended period of time, thus minimizing reapplication (to the painful wound sites) and the tissue damage inflicted on the newly epithelialized wound area. Unlike topical antiseptics, antibiotics represent an effective treatment option to prevent microbial colonization/contamination in open wounds and their combination with biopolymers has shown to promote granulated tissue formation and epithelialization [13].

A number of research reports are available showing the development of electrospun nanofiber mats loaded with antimicrobials for their potential biomedical applications, such as wound dressing, drug delivery, tissue engineering and medical devices [14–16]. In particular, composite electrospun mats containing prophylactic antibiotics have been shown to provide adequate extended release kinetics, thus enabling high local concentration that could minimize microbial bioburden while averting systemic toxicity [17–19]. It has been demonstrated that the interaction between anti-glaucoma drug and drug carrier enhanced the encapsulation efficiency and maintained the intraocular pressure for at least 120 days in non-human primate model [20]. In our previous work, we have demonstrated that polyhydroxy antifungals such as amphotericin B (AmB) and natamycin incorporated within gelatin (Gel) nanofibers decreased the haemolytic properties of the antifungals without losing their antifungal activity [21]. More recently, we reported the electrospinning of gelatin containing linear and branched polyethyleneimines crosslinked with dopamine under alkaline conditions [22]. These cationic polymers loaded crosslinked mats maintained their antimicrobial properties and retained the biocidal properties for about 1 week.

Taking advantage of strong interfacial interactions between polyhydroxy antibiotics and gelatin and their *in-situ* crosslinking with polydopamine (pDA), here we demonstrate the preparation of durable antimicrobial wound dressings. We have incorporated 6 different antibiotics (Table 1) containing varied number of hydroxyl groups (N_{OH}) within polydopamine crosslinked nanofibrous gelatin (Gel_pDA) mat and investigated their antimicrobial properties, long-term antimicrobial activity, aqueous wettability and stability and finally their mechanical and thermal properties. We further show the direct electrospinning of wound dressings on bandage gauze and confirmed *in vivo* efficacy of these materials in a partial thickness burn injury model in piglets. Together, these results demonstrate that the prototype electrospun gelatin mats containing polyhyroxy antibiotics or their combination followed by crosslinking with polydopamine is an efficient approach that could potentially be utilized for treating life-threatening burn-related injuries/infections. Scheme 1 shows the overall strategy adopted to design polydopamine crosslinked polyhydroxy antibiotic loaded nanofibrous gelatin based wound dressings and various non-covalent and covalent interactions involved.

2. Materials and methods

2.1. Materials

Gelatin (Type A, Porcine skin), 2,2,2-Trifluoroethanol (TFE), Dopamine hydrochloride (DA), Amphotericin B (AmB), Caspofungin diacetate (Cas), Polymyxin B sulfate (PmB), Tobramycin (Tob), Vancomycin hydrochloride hydrate (Van), Ammonium carbonate $(NH_4)_2CO_3$, Hoechst, Flouromount™ and FITC conjugated anti-α-tubulin were purchased from Sigma-Aldrich, Singapore. Daptomycin (Dam) was purchased from Tocris bioscience, Singapore.

Table 1
List of various antimicrobials used in the present study.

Antimicrobials	No. of alcoholic −OH groups	Antimicrobial properties
Daptomycin (Dam)	1	Lipopeptide antibiotics, approved for skin and skin structure infections caused by Gram-positive bacteria and for bacteremia and right-sided endocarditis caused by *S. aureus* and MRSA [23].
Polymyxin B (PmB)	2	Lipopeptide antibiotics, active against a number of Gram-negative bacilli, including isolates that are resistant to other antibiotics [24].
Tobramycin (Tob)	5	Aminoglycoside antibiotic with broad spectrum against Gram-positive and Gram-negative bacilli. Indicated for ophthalmic infections and nebulized formulation is used for treating chronic infections with cystic fibrosis caused by *P. aeruginosa* [25].
Vancomycin (Van)	6	Glycolipopeptide antibiotics approved for life-threatening infections caused by methicillin-susceptible and methicillin-resistant S. aureus and infections caused by Clostodium difficile [26].
Caspofungin (Cas)	7	Lipopeptide antifungal that inhibits cell wall architecture of fungus. Used for candidemia, invasive candidiasis and aspergillosis [27].
Amphotericin B (AmB)	10	Macrolide antifungal for serious systemic fungal infections [28].