Antibacterial activity of chitosan-based nanohybrid membranes against drug-resistant bacterial isolates from burn wound infections

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Abstract

Biocompatible and non-toxic properties of chitosan make it a candidate with excellent application prospects in developing wound dressing conjugate compounds. Six different chitosan-based nanohybrid membranes were evaluated against multi-drug resistant bacterial isolates. Twenty-seven drug-resistant *Staphylococcus aureus*, *Enterococcus faecalis*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* species were isolated from burn wound infections. Different combinations of chitosan, ciprofloxacin (CIP), biofunctionalized montmorillonite (MMT), and montmorillonite with sulfate chains (SMMT) were provided, and their antibacterial activity was assessed using the colony count method. Six Methicillin-resistant *S. aureus*, seven vancomycin-resistant *E. faecalis*, four *A. baumannii*, and 10 *P. aeruginosa* multi-drug resistant were identified. Chitosan and montmorillonite did not show significant antibacterial effect but, chitosan/MMT/CIP was the most effective nanocomposite. Chitosan-based nanocomposites with ciprofloxacin could effectively reduce the susceptibility of drug-resistant bacterial isolates. Bacterial targeting using nanosystems provides an opportunity for effective antibiotic treatment by improving antibacterial efficacy.

Introduction

Burn patients account for an essential source of life-threatening conditions around the world (Mock C). Loss of the natural cutaneous barrier, immune system dysregulation, and prolonged hospitalization predispose the patients to bacterial colonization and acquire infection. Spread of the infection throughout the body and subsequent complications as bacteremia, sepsis, and organ dysfunction result in an increased risk of patient death (Lachiewicz et al., 2017). The challenging issue in burn wound infection control is the increasing resistance to antibiotics (Lari et al., 2005, van Langeveld et al., 2017). The multi-drug resistant organisms (MDROs) are frequently reported from different countries including, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus*, which are resistant to several classes of antimicrobial agents and increase the risk of antibiotic treatment failure (Estahbanati et al., 2002, Lachiewicz et al., 2017, Lari et al., 2005, Heidari et al., 2016).

To overcome the drug resistance, antimicrobial biomaterials were introduced recently (Hall et al., 2020). In vitro and in vivo antimicrobial activity of Chitosan, a polycationic biopolymer, were demonstrated previously (Rashki et al., 2020). The biocompatible, biodegradable, non-antigenic, and non-toxic properties placed chitosan as an essential target at the Siebel Center for antibacterial research (Pangestuti and Kim, 2010). The antimicrobial activity of this natural polysaccharide was investigated in different formulations with different combinations, and wound dressing application of chitosan nanohybrids was proposed (Kravanja et al., 2019, Qin and Li, 2020).

Therefore, the antibacterial activity of montmorillonite (MMT) and ciprofloxacin conjugated chitosan membranes against multi-drug resistant (MDR) isolates of *S. aureus*, *Enterococcus faecalis*, *P. aeruginosa*, and *A. baumannii* from burn wound infections was evaluated in this study.

Material And Methods

Bacterial isolates and drug susceptibility testing

A total of 27 drug-resistant isolates of *S. aureus*, *E. faecalis*, *A. baumannii*, and *P. aeruginosa* were collected from burn wound infections. Drug susceptibility testing of the isolates was done using the disk diffusion method on Mueller-Hinton agar (MHA) plates (Hudzicki, 2009). The following antibiotic disks were tested; Ampicillin (10µg), Tetracycline (30µg), Ciprofloxacin (5µg), Linezolid (30µg), Cefoxitin (30µg), Gentamicin (10µg), Azithromycin (15 µg), Cefazidime (30µg), Cefotaxime (30µg), Imipenem (10µg), and Meropenem (10µg). The vancomycin and colistin antibiotics were tested using broth microdilution method using cation adjusted Mueller-Hinton broth (MHB) with concentration ranges of 0.25-32 µg/mL. After overnight incubation at 35°C, results were interpreted according to the CLSI guidelines (Aerobically; et al.). *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212, and *S. aeruginosa* ATCC 27853 standard strains were used to control the tests.

Nanohybrid membranes

Nanohybrid membranes of chitosan with ciprofloxacin (CIP), biofunctionalized montmorillonite (MMT), and sulfate chains (SMMT) were purchased from Amirkabir University of Technology where they synthesized. Totally six different nanocomposites were provided including, chitosan, chitosan/MMT, chitosan/SMMT, chitosan/CIP, chitosan/MMT/CIP, and chitosan/SMMT/CIP (Moghadas et al., 2016).

Susceptibility testing of nanohybrid membranes

Susceptibility testing of all nanohybrids was assessed using the colony counting method. Briefly, the 0.5 McFarland suspension of all bacterial strains were prepared from new colonies. 1 mL of a 1:20 dilution of 0.5 McFarland suspension was added to 9 mL of MHB having 5 mg of each membrane and put on the shaker incubator at 35°C/24 hours/100 rpm. Then, 100 µL of serially diluted culture suspensions were spread over the surface of MHA, and bacterial colonies were counted after incubation at 35°C for 24 h. At least one log reduction of bacteria load was considered an antibacterial activity of the nanohybrid membranes (Pinto et al., 2012).

Statistical analysis

The mean differences of the growth of *S. aureus*, *E. faecalis*, *A. baumannii*, *P. aeruginosa* isolates against the initial inoculation in the presence of chitosan, chitosan/MMT, chitosan/SMMT, chitosan/CIP, chitosan/MMT/CIP, and chitosan/SMMT/CIP nanohybrid membranes were analyzed by T-Test using SPSS statistical software version 20.

Results
Antibiotic resistance pattern

The distribution of the isolates was as follows: six Methicillin-resistant S. aureus (MRSA), seven vancomycin-resistant E. faecalis (VRE), four A. baumannii, and 10 P. aeruginosa multi-drug resistant (MDR) isolates were tested. Ciprofloxacin resistance of S. aureus, E. faecalis, A. baumannii, and P. aeruginosa was found in 2, 2, 2, and 4 isolates, respectively. The resistance pattern of the isolates is shown in Table 1.

Antibacterial activity of nanohybrid membranes

Pure chitosan showed a bacteriostatic effect on the growth of all the testing Gram-positive and Gram-negative isolates, but this is not significant (<1 log reduction). Four nanohybrid membranes, including chitosan/SMMT, chitosan/CIP, chitosan/MMT/CIP, and chitosan/SMMT/CIP had a significant effect against the testing isolates. E. faecalis, S. aureus, and P. aeruginosa were the most affected bacteria with significant growth reduction in the presence of nanohybrid membranes except for pure chitosan. However, despite >1 log growth reduction of A. baumannii isolates in the presence of different nanohybrid films, mean differences in these isolates’ growth were not statistically significant when chitosan/MMT chitosan/CIP and chitosan/MMT/CIP used. MMT had no significant effect in reducing the growth of bacterial isolates, and chitosan/SMMT/CIP was the most efficient compound. One CIP-resistant S. aureus, a CIP-intermediate susceptible E. faecalis, and A. baumannii isolates were completely inhibited when chitosan/SMMT/CIP was used. Growth of CIP-intermediate susceptible P. aeruginosa isolates inhibited using chitosan/CIP and chitosan/MMT/CIP compounds. Standard strains were almost affected as same as clinical isolates except when ciprofloxacin was used. Figure 1 shows the log reduction of six different nanohybrid membranes against Gram-positive and Gram-negative bacterial isolates.

Discussion

Antimicrobial resistance is a global health and developing threat that requires urgent multisectoral action to overcome resistance development (Mendelson and Matsoso, 2015). Nano-based drug delivery to targeting bacteria has apparent advantages over overexposing bacteria to higher drug concentration and enhancing existing antibiotics’ ability to treat infections (Yeh et al., 2020). Chitosan with polycationic properties attacks the negatively charged bacterial cell wall to demonstrate its antimicrobial activity, leading to reduced susceptibility and an effective combination with available antibiotics (Rogers et al., 2012). Herein, the antibacterial efficacy of different chitosan nanohybrid membranes was evaluated.

Pure chitosan showed bacteriostatic effects by inhibiting the growth of both Gram-negative and Gram-positive bacterial isolates tested, which agrees with previous experiments (Sobhani et al., 2017). Also, MMT did not significantly increase the antibacterial activity in the chitosan biocomponent structure. Sandri G et al. investigated the antibacterial activity of montmorillonite–chitosan–silver sulfadiazine nanocomposites against standard strains of S. aureus, Staphylococcus pyogenes, Escherichia coli, and P. aeruginosa (Sandri et al., 2014). Our experiment indicated that neither montmorillonite nor chitosan itself possesses significant antibacterial activity against targeting strains. However, SMMT showed better results in improving the antibacterial effect of the chitosan-based nanohybrids. Thus, better results were obtained when chitosan/SMMT was used with ciprofloxacin antibiotics.

Among the considering bacterial species, some ciprofloxacin-resistant/intermediate susceptible isolates became susceptible in the presence of chitosan/MMT/CIP. This observation is a clear benefit of nano-based composites to increase the efficacy of antibiotic therapy. Sobhani Z et al. showed that ciprofloxacin-loaded chitosan nanoparticles had 50% lower MICs than the ciprofloxacin hydrochloride alone in standard strains of E. coli and S. aureus (Sobhani et al., 2017). Furthermore, four- and two-times decreased MICs of P. aeruginosa and Klebsiella pneumoniae species were reported by Farhangi M et al. when chitosan-ciprofloxacin conjugates against the free drug (Farhangi et al., 2018).

Burn wounds can be quickly colonized by different bacterial species and are at risk for developing secondary sepsis to pneumonia, then an urgent management is needed (Bowler et al., 2012). Moreover, co-infection and co-existing within a biofilm community are often in burn wounds result in complicated conditions and limited therapeutic options (Church et al., 2006). Promising results of chitosan-based nanocomposites with a broad-spectrum antibiotic, ciprofloxacin, were obtained against different common bacterial pathogens of burn wounds in the present study. In addition, the anti-biofilm activity of chitosan conjugates was successfully investigated previously (Regiel et al., 2012).

In conclusion, chitosan nanohybrids showed satisfying improvement in drug susceptibility of Gram-negative/positive multi-drug resistant bacterial isolates. Despite the low antibacterial activity of chitosan and MMT, nanoparticle combinations of chitosan/SMMT/CIP significantly inhibited the growth of four bacterial species tested. The biocompatible and non-toxic nature of chitosan and obtained antibacterial activity of nanohybrid membranes converge to propose the chitosan/SMMT/CIP as a wound-healing potential especially.

Declarations

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Conflict of interest: The authors declare no conflict of interest.

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Table

Table 1. Resistance pattern of Staphylococcus aureus, Enterococcus faecalis, Acinetobacter baumannii, and Pseudomonas aeruginosa isolates.
| Species         | No. of isolates | Ampicillin | Azithromycin | Ceftazidime | Cefotaxime | Cefoxitin | Ciprofloxacin | Colistin | Gentamicin | Imipenem | Li |
|-----------------|-----------------|------------|--------------|-------------|------------|-----------|---------------|----------|------------|----------|----|
| *S. aureus*     | 1               | -          | R            | -           | R          | R         | -             | R        | -          | -        |    |
|                 | 1               | -          | I            | -           | R          | S         | -             | I        | -          | -        |    |
|                 | 1               | -          | R            | -           | R          | I         | -             | R        | -          | -        |    |
|                 | 1               | -          | S            | -           | R          | I         | -             | R        | -          | -        |    |
|                 | 1               | -          | R            | -           | R          | R         | -             | R        | -          | -        |    |
|                 | 1               | -          | S            | -           | R          | S         | -             | S        | -          | -        |    |
| *E. faecalis*   | 2               | R          | -            | -           | -          | R         | -             | -        | -          | -        | R |
|                 | 2               | R          | -            | -           | -          | I         | -             | S        | -          | -        |    |
|                 | 1               | R          | -            | -           | -          | I         | -             | -        | -          | -        |    |
|                 | 1               | R          | -            | -           | S         | -          | -             | R        | -          | -        |    |
|                 | 1               | R          | -            | -           | -          | S         | -             | -        | -          | R        |    |
| *A. baumannii*  | 1               | -          | -            | R          | R         | -          | S             | S        | R          | S        |    |
|                 | 1               | -          | -            | R          | R         | -          | R             | S        | R          | R        |    |
|                 | 1               | -          | -            | R          | R         | -          | I             | S        | I          | R        |    |
| *P. aeruginosa* | 3               | -          | -            | R          | R         | -          | I             | S        | R          | S        |    |
|                 | 1               | -          | -            | R          | R         | -          | R             | S        | R          | R        |    |
|                 | 2               | -          | -            | R          | R         | -          | R             | S        | I          | R        |    |
|                 | 1               | -          | -            | R          | R         | -          | I             | R        | R          | R        |    |
|                 | 1               | -          | -            | R          | R         | -          | S             | S        | R          | R        |    |
|                 | 1               | -          | -            | R          | R         | -          | I             | I        | R          | R        |    |

R: resistant, S: susceptible, I: intermediate susceptible

**Figures**
Figure 1

Antibacterial activity of chitosan nanohybrid membranes against standard and clinical isolates of Staphylococcus aureus (A), Enterococcus faecalis (B), Acinetobacter baumannii (C), and Pseudomonas aeruginosa (D). (ATCC, American tissue culture collection)