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Role of knit fabric as wound dressing

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Abstract

Fibrous biomaterials are widely used in the design and fabrication of antibacterial wound dressings. Two strategies are used to make anti-infective dressings: antibacterial and probiotic therapies, which have potential biotoxicity and other side-effects. Herein, we report a new strategy for fabricating wound dressings to combat infection. Poly(4-Methyl-1-Pentene) (PMP) fabric can remove bacteria from infectious wounds through dressing changes based on its efficient bacterial adhesion. The maximum adhered count of *S. aureus* and *E. coli* on the PMP fabric was 1.63×10^6 CFU/cm^2 and 4.77×10^5 CFU/cm^2, respectively. In addition, the PMP fabric could inhibit the twitching motility of bacteria, which is beneficial for inhibiting infection. The ability of the PMP fabric to accelerate wound healing was demonstrated *in vivo* in a rat wound model. After treatment with the PMP fabric dressing, pathogenic bacteria in the wound were removed through dressing change; therefore, the wound exhibited better healing speed than when the commercial dressing was used. The low bacterial concentration effectively stimulated the expression of growth factors and suppressed wound inflammation, thereby accelerating wound healing. PMP fabric has three advantages: (1) It has been approved for use in clinical treatment by the Food and Drug Administration; (2) No antibacterial agent or probiotics were used; (3) The fabric could be manufactured through an industrial production process. These results indicate that the new strategy can be used in the design of new-generation wound dressings for antibacterial applications.

Keywords: Anti-infection; Bacterial adhesion; Healing mechanism; Knitted fabric; Wound dressing.

Introduction

Fibrous materials are strong, soft, flexible, and elastic, and the anatomical structures of the human body are in the form of fibers and fiber composite materials [1,2]. Several novel fibrous biomaterials have been designed and fabricated owing to the afore mentioned excellent properties of fibrous materials [3-5]. Three crucial points should be considered in the research on fibrous biomaterials: application, chemical composition, and physical structure. Fibrous biomaterials have been widely used for health protection, disease diagnosis and treatment, and tissue repair [6-9]. Extensive nano materials and drugs have been used to fabricate fibrous biomaterials, which are designed with complex and delicate structures to achieve desirable biofunctions, such as antibacterial properties, drug delivery, tissue regeneration, and intelligent healing abilities [10-12]. However, most drugs and nanomaterials used in this research have not been approved for clinical use by the Food and Drug Administration (FDA). In recent years, nanofibrous membranes have become a research hotspot as wound dressings because they have several advantageous properties, including high specific surface area, high reactivity, and they mimic extracellular matrix structures [13,14]. The shortcomings of nano fibrous membranes, such as their low preparation efficiency, low air permeability, and poor mechanical strength, make large-scale clinical applications difficult. For convenient clinical application, fibrous biomaterials should be fabricated through a mass-production method using FDA approved materials. Skin trauma is the most common cause of injuries in humans. Wound dressings can temporarily seal wounds and promote healing. Bacterial infection is a major cause of delayed wound healing and scarring [15]. Several novel antibacterial dressings have been

developed to treat wound infections. Two strategies are used to make anti-infective dressings: (1) Antibacterial strategy where various types of antibacterial agents are used to kill pathogenic bacteria in the wound, including antibiotics, natural antibiotics, antibacterial polymers, and nano antibacterial materials [16-21]; (2) Strategy for regulating colony balance where probiotics are inoculated at the wound to regulate wound flora balance to promote wound healing [22,23]. However, the first strategy increases bacterial resistance, potential biotoxicity, and other side effects. For the second strategy, the colonization efficiency is a significant obstacle, and there is potential biosafety risks associated with inoculating microorganisms at the wound.

In this study, we propose a new strategy for fabricating anti-infective dressings without the use of antimicrobials and probiotics. The Poly(4-Methyl-1-Pentene (PMP) knitted fabric demonstrated excellent ability to adhere to bacteria. Owing to the strong adhere to bacteria, pathogenic bacteria on the wound can be removed by changing the dressing. The above process can effectively reduce the concentration of pathogenic bacteria in the wound, thereby controlling the wound infection. The ability of the knitted fabric to promote wound healing was demonstrated using a rat wound model.

This mechanism of wound infection treatment is completely different from the traditional methods that rely on antimicrobials. The knitted fabric did not contain any additional ingredients, and it did not cause other side effects, such as bacterial resistance and potential bio toxicity. PMP was used to fabricate the dressing through melt spinning and knitting, which can enable large-scale manufacturing. Furthermore, PMP has been approved for the fabrication of biomaterials, and no other chemicals were added to the fabric. PMP is a commercially available material with excellent bio safety in the preparation of hollow-fiber gas-separating membranes in bioartificial lungs [24,25]. However, the contact of blood with hydrophobic PMP membrane materials induces nonspecific adhesion of proteins and activates the coagulation cascade. Therefore, the highly hydrophilic surface modification of PMP membranes is one of the main research topics in the synthesis of PMP gas separation membranes [26,27]. The highly nonspecific adhesion property of the PMP membrane is a disadvantage for a bio artificial lung; however, it is beneficial for the removal of bacteria as a wound dressing. These excellent properties of PMP-knitted fabrics indicate that they can become the next generation of wound dressings that can be widely used in clinical treatment.

Technical details

The following procedures have been followed

a) Fabrication of PMP knitted fabrics

b) Wound adhesion property in vitro model

c) Bacterial adhesion performance of the PMP fabric

d) Cytotoxicity

e) In vivo study

Design and fabrication of the PMP fabric

In the design and fabrication of wound dressings, three criteria were considered: (1) The material has been approved to fabricate biomaterials by the FDA; (2) No antibacterial agents and probiotics could be added to the wound dressing to treat wound infection; (3) The manufacturing method meets the requirements of industrial production. PMP has been approved for the fabrication of biomaterials by the FDA, and its hydrophobicity enables high bacterial adhesion.

Thewes et al. [31] proved that short-range hydrophobic interactions of the involved surfaces are mainly responsible for bacterial adhesion through single cell force spectroscopy, compared with long-ranged van der Waals and electrostatic forces. Maikranz et al. [32] studied the binding mechanisms of *S. aureus* to hydrophobic and hydrophilic surfaces. They observed that several macromolecules are involved in adhesion on hydrophobic surfaces; however, only a few macromolecules tether strongly to hydrophilic surfaces. Wang et al. [33] studied the bacterial adhesion mechanism on temperature- responsive surfaces, and observed that hydrophobic interactions contributed to bacterial adhesion. Previous studies have confirmed that hydrophilic surfaces are not conducive to bacterial adhesion [34]. Hydrophilic materials could form a hydration layer on the surface to resist nonspecific adsorption from biomolecules and microorganisms [35,36]. The wound dressings with appropriately hydrophobicity have the potential to remove pathogenic bacteria from the wound by dressing changes, thereby reducing the concentration of pathogenic bacteria on the wound and treating wound infection.

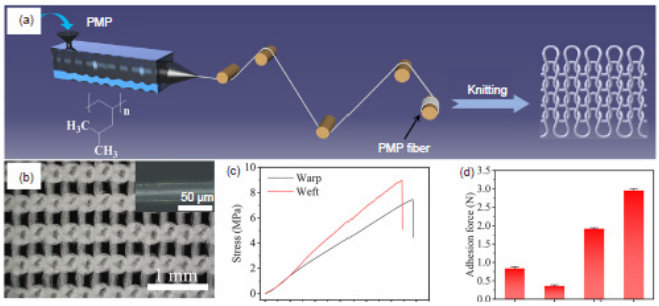

Figure 1: Study selection process.

Melt spinning and knitting fabrics are conventional manufacturing methods in the textile field; therefore, they were used to fabricate the fiber and fabric. The knitted fabric was manufactured using a PMP multifilament, which consisted of 48 PMP fibers with a diameter of approximately 30 μm (Figure 1b). According to previous studies, the fibers exhibited maximum bacterial adhesion when the fiber size was almost equal to that of bacteria [37], and the diameter of the bacteria is about 1 μm. Thus, the textile has the highest bacterial adhesion when the fiber diameter is approximately 1 μm. However, a fiber with a diameter of 1 μm cannot be obtained using the conventional melt-spinning method. The lowest fiber diameter that can be obtained through melt spinning process in our laboratory is 30 μm. Therefore, the knitted fabric with a fiber diameter of 30 μm was used for further study. The tensile strength of a single fiber was 1.62±0.23 cN/dtex, and the elongation at break was 35.58% ±1.40%. Owning to the excellent mechanical properties, the fabric also exhibited excellent mechanical properties. The tensile strength of fabric was 7.46 and 8.80 MPa in warp and weft; and the elongation at break was 58.77% and 54.42%, respectively (Figure 1c). The air permeability of the PMP fabric was 6157 L/(m^2S). Low wound adhesion helps to prevent secondary damage to the wound. The wound adhesion of the PMP fabric was 0.84 N, which was higher than the classic anti-adhesion dressing (vaseline gauze, 0.36 N); however, it was lower than the normal cotton dressing (2.95 N) and commercial anti-infective dressings (1.92 N) (Figure 1d). These results confirmed that the PMP fabric was successfully prepared, and it has potential application as a wound dressing.

Bacterial adhesion performance of the PMP fabric

In the propose design, PMP fabrics were used to remove bacteria from wounds and treat infections based on their high bacterial adhesion. In addition, the high bacterial adhesion can inhibit twitching motility of bacteria, which is also beneficial for the treatment of bacterial infections. Therefore, the bacterial adhesion property of the PMP fabric is directly related to its ability to treat wound infections. As shown in Figure 2a, the bacterial adhesion on the PMP fabric increased gradually over time; and the number of adherent bacteria did not increase significantly after 8 h. The maximum bacterial adhesion number was 1.63×10^6 CFU/cm^2 and 4.77×10^5 CFU/cm^2 against *S. aureus* and *E. coli*, respectively (Figure 2). The surface zeta potential of the PMP fabric was −38.81 mV, and the surface zeta potential of bacteria was also negative. Generally, negatively charged surfaces repel bacteria with a negative surface [38]. Therefore, the mechanism of strong bacterial adhesion is nonspecific adhesion caused by the hydrophobicity of the PMP fabric, and not the charge interaction between the bacteria and fiber. The high bacterial adhesion of the PMP fabric indicates that it has the potential to remove pathogenic bacteria from wounds.

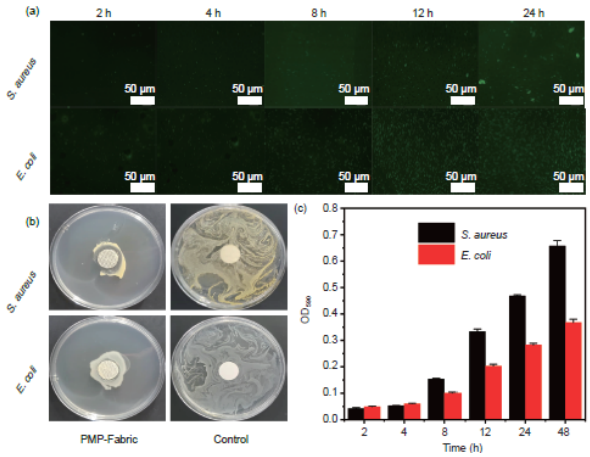

Figure 2: (Color online) Interaction between bacteria and PMP fabrics. **(A)** Fluorescence microscopy images of *S. aureus* and *E. coli* after incubating with the PMP fabrics; **(B)** Twitching motility of adhesion bacteria on PMP fabrics and filter paper; **(C)** The formation of S. aureus and E. coli biofilm.

As reported, the twitching motility of pathogenic bacteria also plays an important role in the wound cell infection [30]. As shown in Figure 2b, the diffusion radius of the bacteria was significantly smaller than that s control group. These results indicated that the twitching motility of *S. aureus* and *E. coli* was significantly inhibited based on their high bacterial adhesion. They also demonstrated that the PMP fabric could reduce wound infection by inhibiting the twitching motility of bacteria, in addition to removing the concentration of pathogenic bacteria. The performance of the PMP fabric was beneficial for inhibiting infection and accelerating healing.

However, high bacterial adhesion also promotes the bio film formation in the PMP fabric. The biofilm can exacerbate infections and have adverse effect on wound healing. As shown in Figures 2c and 3, the number of biofilms increased rapidly after 12 h. It is beneficial that the PMP fabric has a maximum bacterial adhesion number at 8-12 h. Therefore, the PMP fabric dressing should be replaced at 8-12 h to avoid potential harm of bio film to wound. The PMP fabrics can be sterilized before use to ensure that their use does not cause excess bacteria. Therefore, PMP fabrics do not cause redundant pathogenic bacteria on the wound, thus causing multiple bacterial infections.

In vivo study

The ability of PMP fabric dressing to accelerate wound healing *in vivo* was investigated in a rat wound model. Prior to the *in vivo* study, the cytocompatibility of the PMP fabric was evaluated *in vitro*. As shown in Figure 4, the cell viability was approximately 100% of HSF and HUVES cells in 24 h, which is similar to commercial wound dressing [31-38]. The proliferating cell count on the PMP fabric was significantly higher than that on the commercial dressings. The results indicated that the PMP fabric excellent cytocompatibility, and could be used in clinical medicine. In the *in vivo* study, wound bacterial infection was observed through Giemsa staining of the pathological slides. As shown in Figure 3a, no *S. aureus* was observed in the wound tissue of Group I as the uninfected control; the wound after treatment with PMP fabric dressing (Group II) and commercial antibacterial dressing (Group III) had little *S. aureus* on the pathological slides, and the tissue had the highest bacterial count after treatment with normal cotton dressings (Group IV). The number of adherent bacteria in the PMP knitted fabric and commercial wound dressing was compared using *S. aureus* and *E. coli* as the model bacteria. The number of adherent bacteria in the PMP knitted fabric was 1.42 times (*S. aureus*) and 1.25 times (*E. coli*) that of the commercial wound dressing. The results established that the PMP fabric could efficiently reduce wound pathogenic bacteria concentration based on its high bacterial adhesion, which proved that the propose design is reasonable. As shown in the representative wound images (Figure 3b), Group II shows the better healing ability than Groups III and IV. After 14 d of treatment, the wound areas were 5% (Group I), 9% (Group II), 19% (Group III), and 31% (Group IV) (Figure 3c). These results indicated that the PMP fabric dressing had excellent ability to promote wound healing.

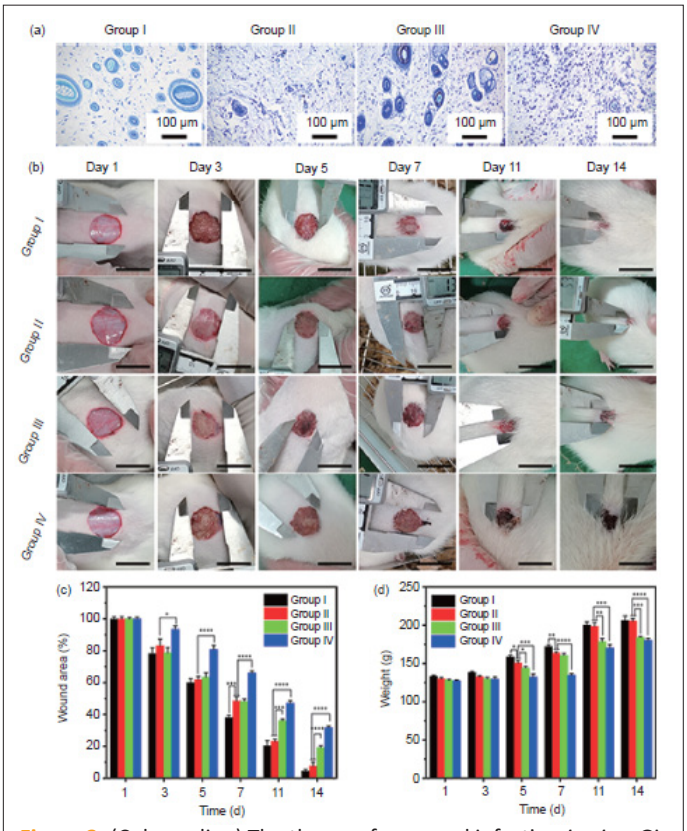

Figure 3: (Color online) The therapy for wound infection *in vivo*. Giemsa staining of the pathological slides of wound tissue, **(A)** Group I, Group II, Group III, Group IV; **(B)** Representative photographs of the wounds (the bar is 15 mm); **(C)** Wound area; **(D)** Weight of the rats. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

Body weight was used to assess the survival status of rats. After 14 d of treatment, the rat weights of Groups I and II were equal and higher than those of Groups III and IV (Figure 4d). The results indicated that the PMP fabric has an excellent therapeutic effect on infected wounds by removing pathogenic bacteria.

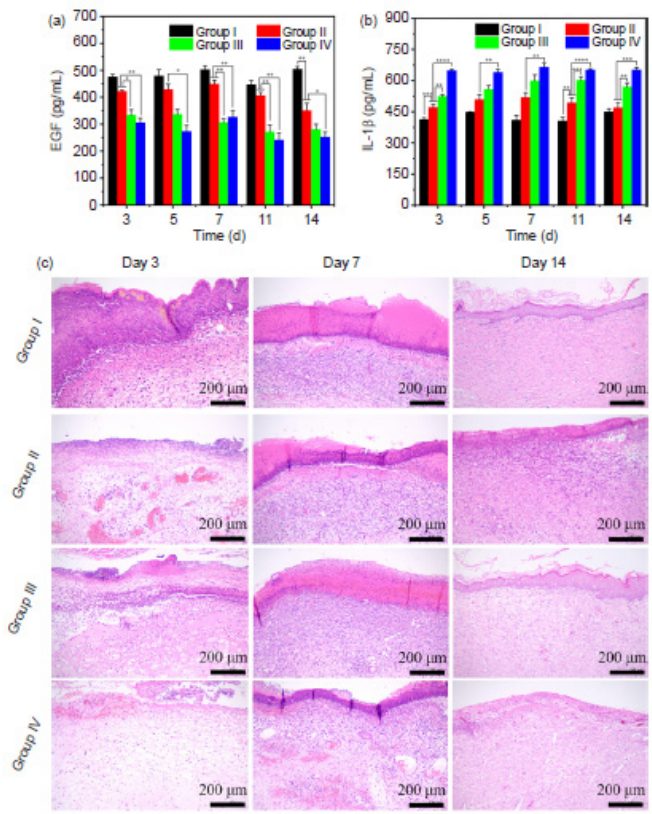

Figure 4: (Color online) The wound healing status after treatment with wound dressing. EGF **(A)** and IL1-*β* **(B)** Concentration of the rats after treatment with different dressings; **(C)** Representative H&E histological stained images of wound tissue after treatment with different wound dressings.

Growth factors can accelerate wound healing by promoting cell proliferation, migration, and regeneration, where excessive inflammatory factors inhibit wound healing. We also evaluated wound healing status by monitoring wound inflammation using inflammatory factors detection and H&E staining. EGF and IL-1*β* levels were assessed to determine the wound infection status and healing process (Figure 4a & b). There were significant differences in the expression of EGF and IL-1*β* after treatment with different dressings [40-53].

Group I, the negative control, exhibited the highest EGF concentration and lowest IL-1*β* concentration. The EGF concentration order of the infectious groups was Group II > Group III > Group IV, whereas that of the IL-1*β* concentration was Group II < Group III < Group IV. After treatment on the 3, 7, and 14 d, representative H&E staining histological images of the rat dermal wound were obtained, as shown in Figure 4c. After treatment for 3 d, Groups I and II had intact dermal tissue, Group III had partial necrosis, and Group IV had complete necrosis due to bacterial infection.

On the 14 d, fibrocyte were observed in Groups I and II, suggesting good wound healing; however, fibrocytes were observed in Groups III and IV. These results prove that the PMP fabric exhibits excellent performance in accelerating infectious wound healing.

Conclusion

In this study, a new strategy for fabricating wound dressings to combat wound infections was reported. The PMP fabric was demonstrated to remove bacteria from infectious wounds through dressing changes owing to its efficient bacterial adhesion. The adhered counts of *S. aureus* and *E. coli* on the PMP fabric were 1.63×10^6 CFU/cm^2 and 4.77×10^5 CFU/cm^2, respectively. In addition, the PMP fabric inhibited the twitching motility of bacteria, which is beneficial for inhibiting infection. The ability of the PMP fabric to accelerate wound healing was demonstrated *in vivo* in a rat wound model. After treatment with the PMP fabric dressing, pathogenic bacteria in the wound were removed during dressing change; therefore, the wound healed faster even than when using the commercial antibacterial dressing. Low bacterial concentrations effectively stimulated the expression of growth factors and suppressed wound inflammation, thereby accelerating wound healing. PMP fabric, as a wound dressing, has three advantages: (1) PMP has been approved for use in clinical treatment by the FDA; (2) No antibacterial agent or probiotics were not used; (3) The fabric could be manufactured through an industrial production process.

These results demons that this new strategy can be used in the design of a new generation of wound dressings for antibacterial applications.

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