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Environmentally friendly polylysine gauze dressing for an innovative antimicrobial approach to infected wound management

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ABSTRACT

Infections frequently occur after skin injuries, posing a significant challenge in current clinical care. Frequently changing dressings to minimize wound infections and adhesions results in large amounts of medical waste. Therefore, developing environmentally friendly multifunctional dressings has considerable application and translational significance. This study aimed to prepare a wound dressing with favorable antimicrobial properties and biosafety by grafting a natural antimicrobial peptide, polylysine, onto a traditional cotton textile dressing. The cotton textile dressing offers excellent moisture absorption and softness, while polylysine provides excellent biocompatibility, a broad antimicrobial spectrum, and high stability. Furthermore, both materials are natural and biodegradable, making them ideal for environmentally friendly wound dressings.

As the body's largest organ, the skin serves as a protective barrier against microbial invasion [1–3]. Infections typically occur when this barrier is compromised due to skin injuries [4,5]. Despite strict enforcement of infection-control procedures and antimicrobial prophylaxis, their effectiveness has been limited because the frequent use of synthetic antibiotics fosters microbial resistance, leading to a paradoxical situation [6]. Hence, an urgent need is to develop effective antimicrobial strategies to combat these infections and expedite wound healing [7].

Traditional wound dressings, such as gauze and bandages, are commonly used to cover and protect wounds and absorb secreted exudates from the wounds; therefore, dressings should be changed frequently to prevent infection and adhesion [8,9]. Therefore, it is important to produce dressings with antimicrobial activity to promote healing and reduce inflammation by incorporating bioactive substances into their structure [10–14]. In a previous work, we explored antimicrobial dressings and achieved meaningful progress [15–19]. Among them, cotton gauze, made up of natural cellulose fibers, exhibits excellent moisture absorption, softness, and biodegradable properties. Additionally, the cost of preparing these dressings is relatively low. Therefore, novel cotton dressings are promising [20–22]. Polylysine is a naturally occurring peptide composed of lysine, one of the eight essential amino acids. It is water-soluble, biocompatible, and a broad-spectrum antimicrobial agent [23]. We incorporated polylysine into our modified cotton fabric dressings to enhance their antimicrobial properties and improve their overall performance in wound healing. The amino groups on the surface of polylysine have a positive charge, enabling it to attract and bind to the negatively charged surface of bacteria. This binding process disrupts the bacterial membrane, leading to the death of the bacteria. Furthermore, polylysine can interfere with the DNA structure and internal metabolic processes of bacteria, further inhibiting their growth and spread [24,25]. Polylysine is known for its stability, with an optimal pH range from 5 to 8, and it can withstand high temperatures without decomposing easily, making it an effective antimicrobial agent as it provides continuous and broad-spectrum bactericidal activity [26,27]. In the field of wound dressings, polylysine has been incorporated as an antimicrobial component in various forms, such as electrospun films, hydrogels, and nano-microspheres

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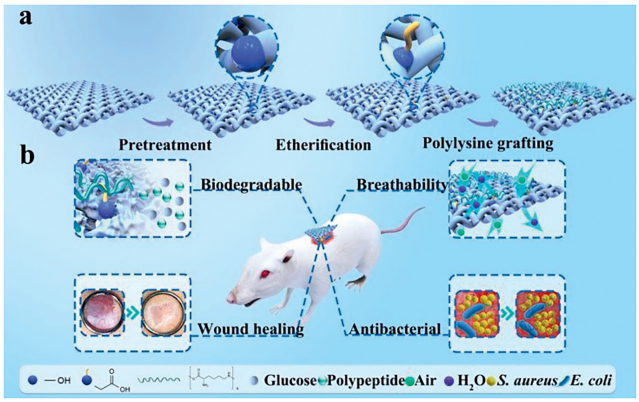

Fig. 1. Schematic illustration of the modification strategy of cotton-PL and its mechanism of operation. (a) Schematic diagram of the process of polylysine grafting on cotton fibers. (b) Cotton-PL promotes wound healing due to its breathable and antimicrobial properties. It is environmentally friendly with complete biodegradability.

[28–33]. These studies have demonstrated that polylysine is a potent antimicrobial ingredient in wound dressings, confirming its significant efficacy in treating infected wounds.

In the present study, to construct a dressing with safe and effective antimicrobial properties and degradability, the natural antimicrobial peptide polylysine was grafted onto commonly used cotton dressings using a non-toxic solvent in a green way (Fig. 1 and Fig. S1 in Supporting information). We conducted various tests to evaluate the modified dressing's physical and chemical properties, biocompatibility, and antimicrobial effectiveness. Additionally, we performed *in vivo* experiments to confirm its ability to prevent infections and promote wound healing. This experiment was approved by the Ethics Committee of West China Hospital of Stomatology, Sichuan University (No. WCHSIRB-D-2023-633).

The surface morphology of the original and modified gauze was characterized using scanning electron microscopy (SEM). As shown in Fig. 2a, the pristine gauze was composed of cotton fibers, which behaved smoothly and well defined and twisted in a spiral arrangement to form cotton threads. After carboxymethylation and grafting of the gauze with polylysine, the cotton threads on the surface of the gauze were still neatly and regularly arranged, and its fibers exhibited smooth surfaces without structural damage. The surface elements of the original and modified gauze were detected by energy dispersive spectrometer (EDS) (Fig. S2 and Table S1 in Supporting information). The nitrogen element evenly appeared on the surface of the cotton loading polylysine (cotton-PL) group. It was also characterized by Fourier transform infrared spectroscopy

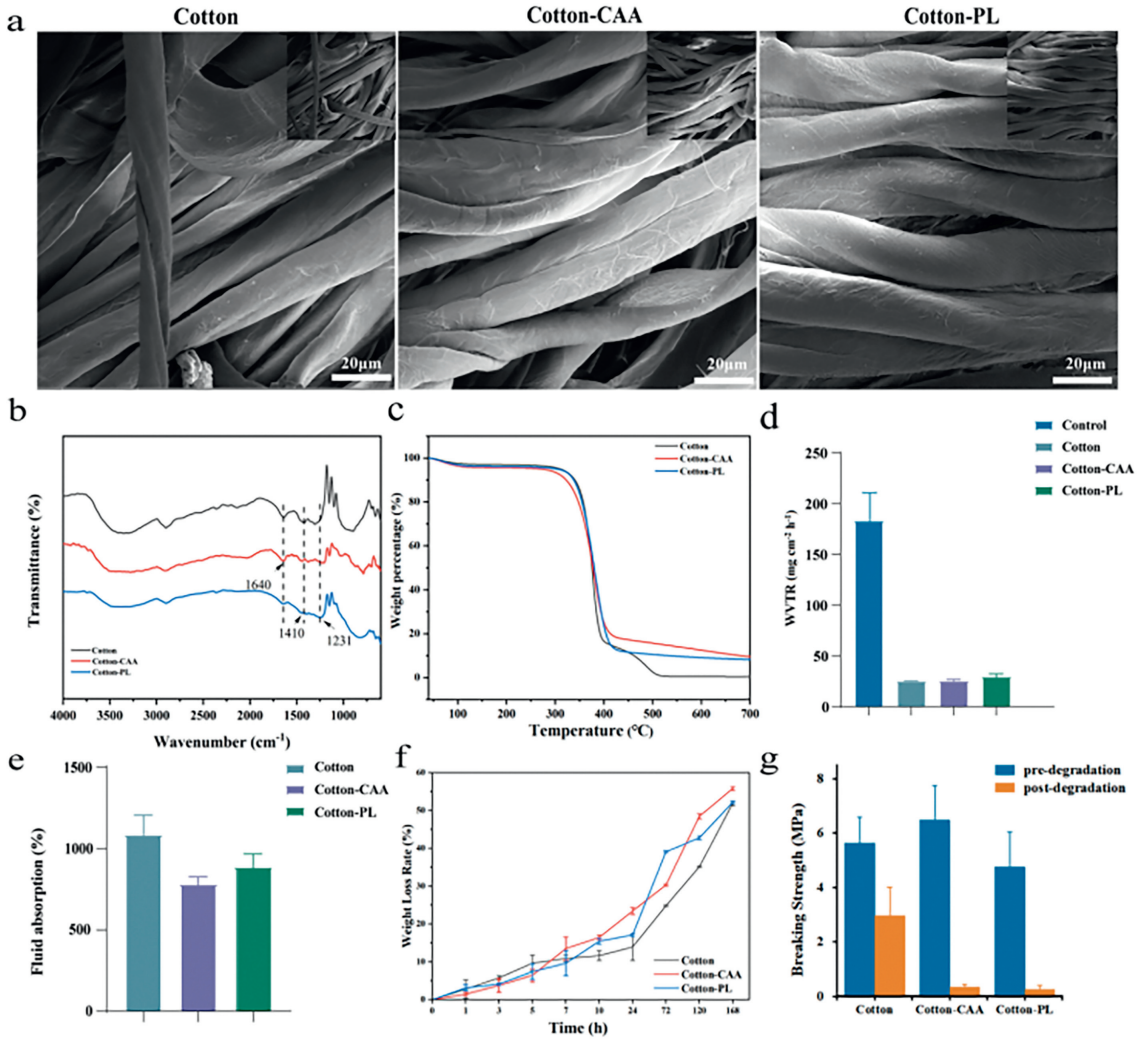

Fig. 2. (a) SEM images of the cotton, cotton-CAA and cotton-PL. Scale bar: 20 μm. (b) FTIR spectra of dressings. (c) TGA curve of dressings. (d) The WVTR of dressings. (e) The fluid absorption of dressings. (f) The weight loss rate of the dressings during 7 days of the degradation experiment. (g) Changes in the breaking strength of the dressings before and after 24 h of degradation. Mean ± standard deviation (SD) ($n \geq 3$).

(FITR) for modifying cotton fabrics. Fig. 2b presents the infrared images of the dressing before and after modification. The peaks near 1410 cm^{-1} are shifted, which might be attributed to the production of -CONH-, indicating that the polylysine has been successfully grafted onto the cellulose chain of cotton fabrics to produce polylysine-modified dressings. In addition, the analysis of the X-ray photoelectron spectroscopy (XPS) (Fig. S3 in Supporting information) confirmed the success of the surface modification of the cotton fabrics. Moreover, the grafting rate of polylysine was calculated at 0.19% by comparing the content of N-containing bonds in cotton-PL.

The thermal stability of the original and modified gauze was analyzed by thermo-gravimetric analysis (TGA). As shown in Fig. 2c, the maximum decomposition temperature of the three dressings was approximately 380 °C, with good thermal stability, indicating their feasibility of sterilization under high temperatures and utilization at room temperature.

The water contact angles (WCA) of the cotton dressings were measured using an optical contact angle tester. Fig. S4 (Supporting information) shows that the WCAs of the cotton fabric remained unchanged before and after treatment, measuring 0 degrees. Furthermore, the amount of polylysine grafted onto the fabric did not affect the contact angle, suggesting that the modification process did not alter the hydrophilicity of the samples. Regarding the water vapor transmission rate (WVTR), the cotton-PL dressings exhibited a slight increase compared to plain cotton dressings (Fig. 2d). In addition, the water absorption of the fabrics was examined, revealing that cotton-PL had higher water absorption than carboxymethyl acidified cotton (cotton-CAA) dressings but lower than untreated cotton (Fig. 2e). These findings can be attributed to the blocking effect of the long-chain polylysine, which prevents water molecules from coming into contact with the cotton fibers. As a result, water can easily escape from the fabric surface, leading to decreased water absorption and increased WVTR. However, based on the test results, it can be concluded that the modified cotton fabrics still possess favorable fluid management capabilities and can effectively provide moisture for skin wound management.

Incorporating synthetic polymers into wound dressings is a common practice, but these polymers often have slow degradation rates and require specific conditions for degradation [34], which can pose challenges for managing medical waste. In light of this, researchers have become increasingly interested in developing biodegradable wound dressings (Table S2 in Supporting information). The cotton fabric-modified dressing system designed in this study consists of natural fiber materials grafted with natural peptides, and the original material is environmentally friendly and biodegradable. Therefore, we verified the degradability of the material. As shown in Fig. 2f, after 7 days of cellulase treatment, the weight loss rate of all three groups of samples exceeded 50%, indicating significant degradation of the material. Furthermore, the surface structure of the cotton fabric was damaged, as observed in Fig. S5 (Supporting information), and its breaking strength decreased significantly within 24 h, as shown in Fig. 2g. In addition, the characteristics of the fabrics were examined before and after degradation. As illustrated in Fig. S6 (Supporting information), the degraded cotton fabrics exhibited noticeable wrinkles, an increased density of surface pores, and a rougher fiber surface compared to their original state. Additionally, the presence of spherical particles suggested the possible presence of unwashed cellulase. The XRD analysis results (Fig. S7 in Supporting information) demonstrated a decrease in the crystallinity index after degradation, and the disappearance of the characteristic peak at $2\theta = 23^{\circ}$ indicated a disordering of the cellulose structure due to degradation. These findings provide evidence of the material's degradability.

Infection is an important factor in delaying wound healing. This research aimed to construct a wound dressing with excellent anti-infection ability. To evaluate the impact of polylysine grafting on bactericidal abilities, we conducted experiments using *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). Fig. 3a demonstrates that the cotton fabric dressing exhibited antimicrobial effects following polylysine grafting. Specifically, the sample with 100% polylysine displayed superior antimicrobial efficacy, which might be attributed to the long molecular chain of polylysine. When the polylysine addition reached 200%, the space resistance was comparatively large, and the polylysine curled into a cluster on the surface instead of stretching. Consequently, the amino groups could not be fully exposed and come into contact with the bacteria.

We chose dressings containing 100% polylysine as the optimal group based on co-culture experiments and further investigated their antimicrobial properties. As can be seen in Figs. 3c and d, the antimicrobial rate of the dressing increased with an increase in contact time. Additionally, the concentration of the bacterial solution did not significantly impact the antimicrobial activity within the range of 10^6, demonstrating excellent bactericidal effects. At 10 min, the inhibitory effect on *S. aureus* was significantly better than that on *E. coli* since *E. coli* has an outer membrane mainly composed of lipopolysaccharides, which is more difficult to destroy than *S. aureus* [35]. Subsequently, we further investigated the antimicrobial properties of cotton dressings using the circle of inhibition method. As shown in Fig. 3b, no antibacterial zone was observed in any of the groups, indicating that the antimicrobial components do not dissolve and the bactericidal effect is achieved through contact.

To further verify the antibacterial mechanism, morphologic and fluorescent staining experiments were performed on the bacteria to observe any disruption in their cell membranes and cell walls. The morphology of *S. aureus* and *E. coli* exposed to cotton and cotton-CAA remained normal, while the morphology of bacteria exposed to cotton-PL dressings showed clear signs of deformation, wrinkling, and the formation of pores on their surfaces (Fig. S8 in Supporting information). The antimicrobial properties of the polylysine wound dressing were further evaluated by live-dead staining of bacteria with the two-color fluorescent dye Syto 9/PI. As shown in Fig. S9 (Supporting information), there was a significant increase in the number of bacteria stained by red fluorescence in the field of view after co-culturing with cotton-PL dressing compared to bacteria in contact with cotton and cotton-CAA, indicating that the cell walls of the bacteria in contact with cotton-PL were broken and the bacteria died.

The *in vitro* biocompatibility of the materials was evaluated by cytotoxicity and hemolysis assays. The L929 cell line was used to evaluate the cytocompatibility. Fig. 3e shows that the cell viability of cotton, cotton-CAA, and cotton-PL extracts was above 70% after 24 h. International standards consider materials with less than 70% cell viability to be potentially cytotoxic. Accordingly, the polylysine cotton dressings we designed have low cytotoxicity. The same results of live-dead fluorescent staining of the cells proved favorable cytocompatibility of the dressings (Fig. S10 in Supporting information). A hemolysis assay was performed using fresh rabbit blood. The results indicated that the cotton, cotton-CAA, and cotton-PL groups all had hemolysis rates below 0.5% (Fig. 3f), suggesting the non-hemolytic nature of cotton dressings.

To further confirm the feasibility of its application, we conducted *In vivo* experiments using a rat model of mixed infection with *S. aureus* and *E. coli* to evaluate the healing-promoting effect of the samples on infected wounds. The progress of wound healing was monitored (Figs. 4a and b), and it was observed that after 3 days, the cotton-PL group exhibited a wound contraction rate of over 40%, similar to that of the commercially available dressing 3M Tegaderm™, and significantly higher than the control groups. Between days 7 and 14, the wounds in the cotton-PL group showed

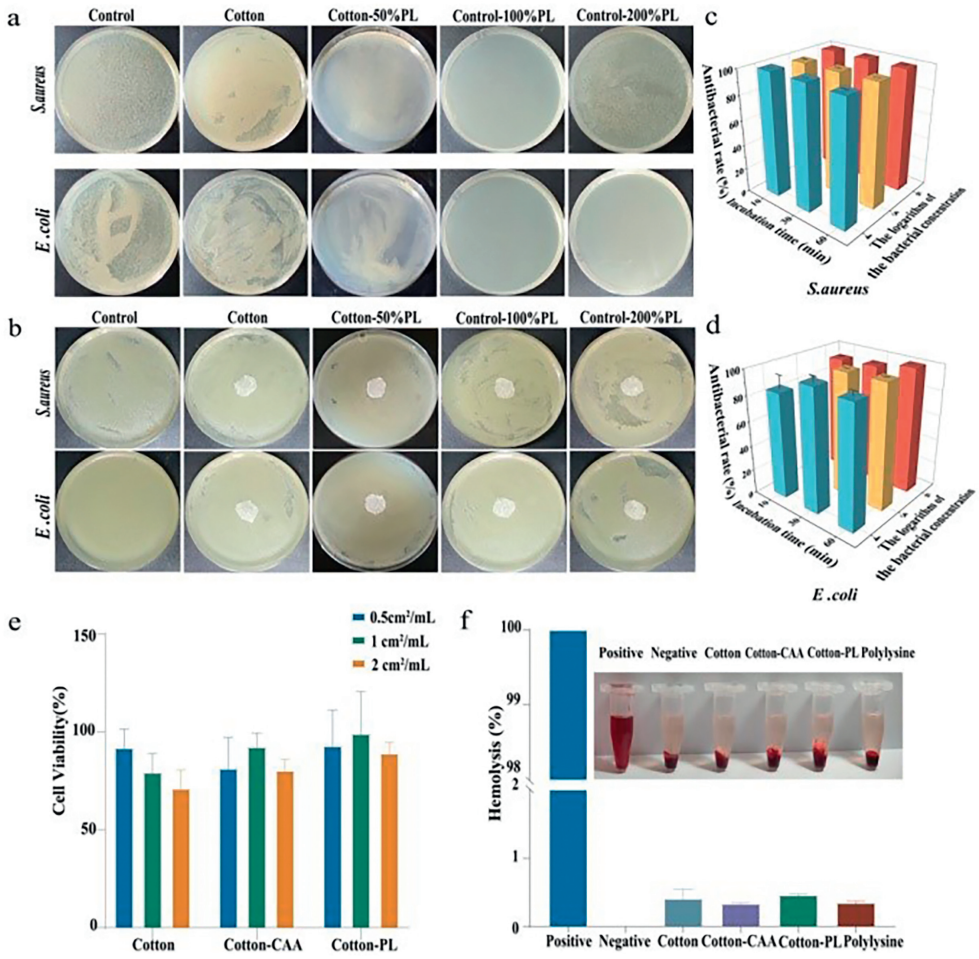

Fig. 3. (a) Antimicrobial results of co-culture of cotton dressings with different polylysine grafts against *S. aureus* and *E. coli*. (b) The results of the inhibition zone method. (c, d) The results of gradient antimicrobial experiments for *S. aureus* and *E. coli*, respectively. (e) Cellular activity of L929 cells after co-culture with dressings. (f) Hemocompatibility of dressings. Mean ± SD ($n \geq 3$).

visibly accelerated closure. By day 14 (Fig. 4c), the wound surfaces were almost fully healed, and the scabs had fallen off. The healing rate was significantly higher than the other cotton dressings and the commercial dressings. These findings suggest that the cotton-PL dressing could provide a long-lasting and continuous bactericidal effect, with a significant pro-healing effect on infected wounds.

The skin tissues were subjected to hematoxylin-eosin (HE) and Masson's trichrome (MT) staining to assess the histologic changes. As shown in Fig. S11a (Supporting information), on day 7, all the groups exhibited extensive wound damage, with inflammatory exudate covering the surface and the necrosis of the epidermis and local dermis. There was noticeable connective tissue hyperplasia in the dermis and subcutis and numerous neovascularizations surrounded by lymphocytes, granulocytes, and other inflammatory cells. On day 14, the wounds in the other control groups remained in an inflammatory state, with significant damage, inflammatory exudate, and inflammatory cell infiltration in the subcutaneous area. In contrast, the cotton-PL group exhibited almost complete healing of the wound epidermis. It had formed a well-organized epithelial structure, with only a small amount of lymphocyte infiltration in the subcutis, indicating that it was in the middle to late stage of inflammation. MT staining was performed to assess collagen deposition and maturation (Fig. S11b in Supporting information). On days 7 and 14, the ratio of the collagen-positive area in the cotton-PL group was 33.36% and 60.46%, respectively, which was significantly higher than the rest of the group, and the collagen was the most organized.

Tumor necrosis factor-alpha (TNF-α) is a critical mediator of inflammation. Hence, using immunohistochemical analysis, TNF-α was chosen to evaluate the efficacy of modified cotton dressings in preventing infection. As depicted in Figs. S12a and e (Supporting information), the expression of TNF-α in the wounds of the cotton-PL group was significantly lower compared to the other groups on days 7 and 14. This reduction can be attributed to the antibacterial properties of polylysine, which helps reduce the inflammatory response.

Angiogenesis plays a vital role in the wound healing process as it allows for the delivery of oxygen and nutrients and the removal of metabolic wastes and facilitates tissue rejuvenation and wound contraction. To evaluate trabecular angiogenesis, immunohistochemistry staining for CD31 and vascular endothelial growth factor (VEGF) was conducted. As shown in Figs. S12b and c (Supporting information), the expression of CD31 and VEGF was significantly higher than that of the control groups on day 7, whereas it decreased significantly on day 14, demonstrating that early utilization of cotton-PL dressing enhanced neovascularization and expedited the healing process and wound organization. On the 14th day, the wounds in the cotton-PL group entered the late stage of healing, at which point the connective tissue was gradually formed, the neovascularization was completed, and the blood and nutrient supply of the wounds was gradually restored to normal. Decreased

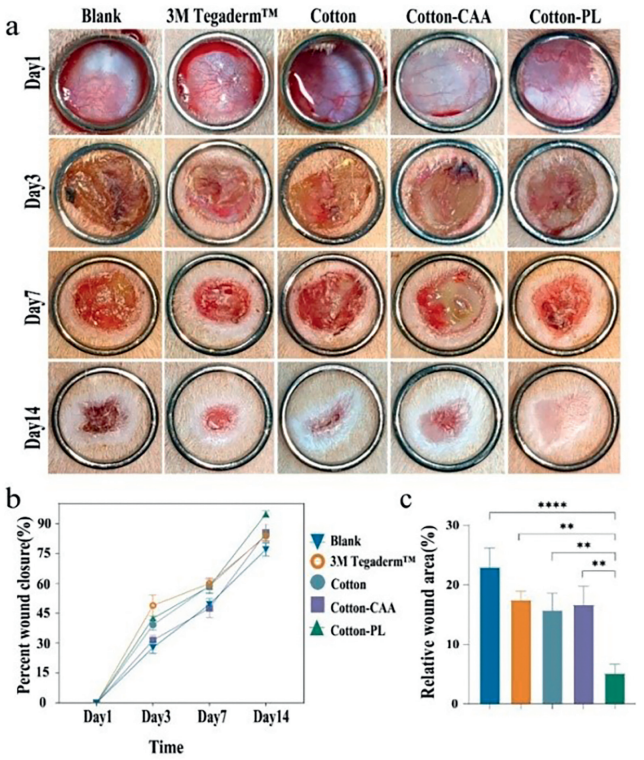

Fig. 4. *In vivo* assessment of infected wound healing with cotton fabric dressings. (a) Representative images of the infected wounds at different times. (b) Quantification of wound contraction during the healing process. (c) Quantification of relative wound area on day 14 after treatment. $^{**}P < 0.01$, $^{****}P < 0.0001$. Mean ± SD ($n \geq 3$).

CD31 and VEGF implied that the quantity and activity of vascular endothelial cells were diminished, and the angiogenesis process was almost complete.

Hypoxia-inducible factor alpha (HIF-α) is a crucial protein that becomes active in low oxygen conditions and controls various biological functions. During the initial phases of wound infection, hypoxia arises in the wound area due to the presence of bacteria, congestion, *etc*. This hypoxic environment can activate the expression of HIF-α. Immunohistochemistry (IHC) staining for HIF-α showed that the level of HIF-α in the cotton-PL group likewise exhibited a decreasing trend on day 14 compared to day 7, indicating that as the wound healed, the oxygen supply around the wound gradually improved, and the hypoxic environment improved, with a gradual decrease in the level of HIF-α, which reduced the effect on angiogenesis and inflammatory regulation (Figs. S12d and h in Supporting information).

Overall, this research developed a friendly environment for polylysine gauze dressing in infected wound management. A series of tests confirmed the feasibility of the dressing for application in skin wounds. Experiments showed that cotton dressings with different amounts of polylysine had significant bacteriostatic effects, confirming the potential for antimicrobial treatment. *In vivo* experiments using an animal model confirmed the dressing's ability to promote the healing of infected wounds. The mechanism behind this healing effect was investigated using histological methods, which revealed that the dressing primarily achieved wound repair by killing bacteria and reducing inflammation. Compared to modern wound dressings, cotton fabric, as a base material, offers exceptional breathability, moisture absorption, and comfort. It allows quick moisture absorption around the wound, keeping it dry and ensuring that the skin can breathe normally. The soft texture of cotton fabric prevents friction and irritation, providing enhanced comfort. Additionally, polylysine cotton dressings have significant antimicrobial properties and biocompatibility, aiding in the healing of infected wounds. Furthermore, both polylysine and cotton fibers are natural, biodegradable materials that are non-toxic and safe for the human body. They do not cause allergies or other adverse reactions, and their degradation products do not pollute the environment. However, it is important to note that this study had some limitations. Firstly, the experiments were only conducted in small animal models and have not yet been validated in clinical trials. Therefore, the effectiveness and safety of the dressing should be confirmed in real scenarios. Additionally, while the dressing showed favorable biodegradability, further research is necessary to investigate the safety and metabolic pathways of its degradation products. It is crucial to understand how these products interact with the body and whether they have any potential adverse effects. In summary, although there are still limitations to be addressed, the results of this study suggest that the biodegradable polylysine antimicrobial cotton dressing holds promise in promoting the healing of infected wounds. Further research and application are necessary to fully explore its potential and ensure its safety and effectiveness in clinical settings.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cclet.2023.109459.

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