

Evaluation of the Effect of a New Fully Biodegradable External Dressing for Promoting Diabetic Ulcerative Wound Healing

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Abstract: In order to verify the effect of the new fully biodegradable external dressing on the repair of diabetes skin wounds and the promotion of wound healing, this study applied sodium alginate hydrogel dressing products which are supplemented with phage and conotoxin analgesic peptides to the skin lesions of diabetes mice, and comprehensively used the quantitative measurement of serological indicators, Image J Tissue section and other techniques to evaluate the effect of the new hydrogel dressing on the repair of diabetes wounds and the promotion of wound healing. The behavioral observation and analysis of mice did not show obvious regularity and difference. According to the results of serum cytokine analysis, functional hydrogel dressings can promote cell regeneration and wound healing in mice. Image J software analysis showed that the healing results of the experimental group were better than those of the negative control group, and the percentage of collagen fiber content showed an upward trend. Through comprehensive evaluation, it can be observed that the new fully biodegradable external dressing has obvious positive effects on wound healing, collagen fiber reconstruction and wound infection prevention of diabetes. The new biodegradable dressing not only promotes wound healing but also solves environmental pollution problems. It meets the sustainable development needs of society and has broad application prospects.

1. INTRODUCTION

As a temporary skin substitute, dressing provides the best healing environment for skin wounds [1], and it is widely used in burns, ulcers and other acute or chronic wounds. Although traditional dressings such as sterile gauze and cotton wool are inexpensive and readily available, they are prone to adhesion and infection. With the continuous concern about pollution, biodegradable materials have gradually gaining more attention for research. In the field of medicine and health especially, biodegradable medical materials have been vigorously developed, and are widely used in dressings, human tissue engineering materials, internal sutures, etc.. As a biodegradable material, hydrogel dressing stands out among many new modern dressings and has become a hot material in the field of dressings. Natural polymer hydrogel is a natural or synthetic cross-linked polymer with water content of more than 90%. As a three-dimensional network formed by high cross-linking of hydrophilic polymers, hydrogel can effectively absorb exudate, debride the wound, and provide an aerobic and

humid environment for wound healing [2]. Moreover, it can provide 3D skin-like mechanical properties to accelerate the healing [3]. The wet healing theory proposed in 1962 proved that wounds heal more than twice as fast in wet environments as in dry environments [4]. At the same time, the multi-hollow network structure of the hydrogel or the formation of nanoparticle-embedded drugs can help the drug released slowly, giving it excellent drug-carrying capacity [5], which can additionally transport a large number of related bio-active agents such as antibiotics, antioxidants, antimicrobial peptides, stem cells, growth factors and insulin [2]. Conotoxin is a small marine peptide that typically contains 10 to 50 amino acids and 1 to 5 disulfide bonds. MVIIA, the first ω -conotoxin, has been used to treat intractable chronic pain [6]. Conotoxin has potent and selective effects on many membrane receptors and ion channels and, therefore shows great potential as a neuropharmacological tool and therapeutic candidate [7]. The use of conotoxin to make peptides, to prepare medical dressings for wound treatment, through osmosis to achieve good analgesic effect, antibacterial, anti-inflammatory at the same time, has good biocompatibility, biodegradability,

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more suitable for diabetic foot wound treatment. Polylactic acid has unique biodegradability and biocompatibility [8], which can be decomposed into carbon dioxide and water in nature through the action of soil, water or microorganisms, and can be used as the outermost protective material to help solve the white pollution from the source, meeting the goal of building a green society in China [9].

Diabetes is a common and frequent disease that poses a serious threat to human health [10]. As a representative form of chronic, refractory trauma, diabetic ulcers are more common on the foot, and are the leading cause of non-traumatic amputation in diabetic patients [11]. Lesions of distal nerves and peripheral blood vessels of the lower extremities lead to varying degrees of foot ulceration, infection, and tissue destruction [12]. Management protocols for diabetic ulcers include glycaemic and infection control, wound decompression, topical debridement, vascular evaluation, and cover dressing [13]. According to the latest data provided by the IDF (International Diabetes Federation), 537 million people worldwide are living with diabetes and are expected to reach 700 million patients by 2045 [14]. Diabetic foot is a chronic complication of diabetes, which has the characteristics of high amputation rate, high recurrence rate and high mortality rate [12], seriously affecting patients' quality of life and causes heavy social and economic burden. Unlike acute trauma such as topical incisions, diabetic foot healing is not an orderly and timely process of coagulation, inflammation, migration, proliferation, and remodeling, but rather stagnates in the inflammatory phase and is highly susceptible to infection [15]. Currently, a variety of wound dressings in combination with insulin therapy and dietary control are used clinically for diabetic foot [16]. However, traditional passive dressings such as gauze can only cover and absorb wound surface exudates, lacking antimicrobial activity and requiring frequent replacement to prevent infection. They are greatly limited in their application in chronic complex wounds because adhesion wounds are prone to secondary injury during replacement [17, 18].

Starting from environmental problems, this study applied sodium alginate hydrogel dressing products which are supplemented with phage and conotoxin analgesic peptides to the skin lesions of diabetes mice, and evaluate their effect on diabetic skin tissue to promote wound healing and comfort through various indicators, in order to effectively absorb exudate, inhibit inflammation and control infection at the same time, through the role of phage and conotoxin analgesic peptides, etc.. It can also greatly reduce the pain of patients, promote granulation tissue production, accelerate wound healing and so on, providing a green new dressing technology for diabetic skin wound treatment.

2. MATERIALS AND METHODS

2.1. Laboratory animals

6 DB mice, 6-8 weeks old, males (Zhiyuan Biotechnology (Shandong) Co., Ltd.).

This study was approved by the Naval Medical University Ethics Committee. The institutional guidelines for the care and use of laboratory animals were followed throughout the study. DB mice were housed under a stable room temperature and relative humidity in a 12/12 h light/dark cycle. All animals had free access to tap water and food unless otherwise stated.

2.2. Reagent consumables

2.2.1. Experimental reagents

Phage, conotoxin analgesic peptides (Biotechnology Research Institute, Beijing Academy of Agriculture and Forestry Sciences), sodium alginate powder (Qingdao bright moon seaweed group co., Ltd.), normal saline, ribozyme-free water, double distilled water, chloral 4% hydrate, stationary solution, absolute ethanol, PBS buffer, chitosan (Laboratory of Marine Food Biotechnology, College of Food Science and Engineering, Ocean University of China)

2.2.2. Experimental consumables

10 ml centrifuge tubes, disposable needles, disposable 2 ml centrifuge tubes, disposable medical gloves, disposable micro-blood collection glass capillary straws, medical tape, alcohol cotton balls, cotton, linen gloves

2.3. Experimental instruments

Blood glucose meters, ultra-low temperature freezers, autoclaves, beakers, Erlenmeyer flasks, glass rods, mortars, epilators, topical scissors and forceps, centrifuges, pipette guns, light microscopes

2.4. Experimental protocol

The sodium alginate hydrogel dressing product containing *E. coli* obligate phage and conotoxin analgesic peptides was applied to the skin wounds of diabetic mice to evaluate the effect of functional hydrogel dressing on diabetic skin wound repair and healing.

2.4.1. Mice numbering and grouping

DB male mice, 6 to 8 weeks old, marked at the base of their tails with a black marker, numbered S5, S3, Y1, Y6, K4, K2, respectively. S5 and S3 were the experimental group, Y1 and Y6 were the negative control groups, and K4 and K2 were the blank control groups. Notably, S5 died on day 3 and S3 and Y6 on day 4. On the day 4, the adjustment number and grouping are: K2 is adjusted to experimental group and renumbered as S2, Y1 is still the negative control group, and K4 is still the blank control group. Y1 died on day 10.

2.4.2. Drug preparation

Phage at a concentration of 10 mg/ml were diluted at 1:20,

and conotoxin analgesic peptides at a concentration of 20 mg/ml were diluted at 1:20. Phage and conotoxin analgesic peptides dilution were prepared.

2.4.3. Functional hydrogel preparation

After preparing sodium alginate powder for high-temperature steam sterilization and cooling, 0.3 ml of phage and 0.188 ml of conotoxin analgesic peptides dilution per 0.025 g of sodium alginate were prepared to produce functional hydrogel, each serving for one wound.

2.4.4. Mice skin wound molding

Depilate the back of the rat with an epilator, using 4% chloral hydrate, anesthetize mice at a dose of 0.1 ml/10 g, use a sterilized skin tissue sampler, cut 2 round wounds with a diameter of 10 mm in size on the back of each rat, and excise the full thickness of skin. The wounds are numbered C1 and C2 in turn.

2.4.5. Mice administration and treatment

Mice were grouped on days 1, 2, 4, 6, 8, 10 and 12. Experimental group: each wound was applied with mixed functional hydrogel, and a fully degradable polylactic acid film was applied and fixed with medical tape. Negative control group: each wound was applied with an equal amount of blank hydrogel, and a fully degradable polylactic acid film was applied and fixed with medical tape. Blank control group: no treatment.

2.4.6. Experimental data collection: Wound recording

On days 2, 4, 8, and 14, take pictures of each wound in each rat with a smartphone. General animal behavior: daily food intake and water intake, mice weight data were collected on days 2, 4, 8, and 14, and observe the mice arching their backs and scratching. Serology: On days 2, 4, 8, and 14, the whole blood of mice was collected after orbit, blood glucose was measured, and serum was isolated after standing centrifugation. Tissue section: On days 2, 4, 8, and 14, the skin tissue of each set of wounds was cut sequentially and fixed in fixative solution.

2.5. Data analysis methods

Due to the death of mice, there was only one mouse for each group in this study. Therefore, the analysis of the data did not involve error analysis. Image J was used to calculate each wound area, and equation (1) was used to calculate the wound healing rate. The gross behavior of mice were observed and expressed through line graph by using Microsoft Office Excel. Wuhan Servicebio Technology Co., Ltd. was commissioned to test TGF- β , IL-1 β and VEGF by Western-Blot quantification. Granulation tissue growth was evaluated by analyzing the percentage of average collagen fiber content of HE stained sections under the microscopic.

$$\text{Wound healing rate(\%)} = \frac{\text{Initial size} - \text{Current size}}{\text{Initial size}} \times 100\% \quad (1)$$

3. RESULTS AND ANALYSIS

3.1. Wound healing rate results

Due to the missing data on the day 4 of the experimental group, the trend line prediction method was adopted, and the value was predicted according to the formula $y = 0.095x^2 + 0.3986x + 0.2962$. The following table is plotted, and no obvious regularities and differences are found (Table 1).

Since S5 died on day 3 and S3 died on day 4, K2 was adjusted to the experimental group on day 4 and renumbered to S2, resulting in the absence of photos in the experimental group on day 4, and the wound healing effect analysis was carried out according to the existing photos (Figure 1): the wound healing

Table 1. Mice wound healing rate

| Group | Mice wound healing rate | | | | |
|------------------------|-------------------------|-------|---------|--------|--------|
| | Time (days) | 1 | 2 | 4 | 8 |
| Experimental group | | 0.00% | -18.92% | 38.21% | 38.31% |
| Negative control group | | 0.00% | 11.26% | 37.73% | 15.22% |
| Blank control group | | 0.00% | -11.89% | 11.93% | 26.18% |

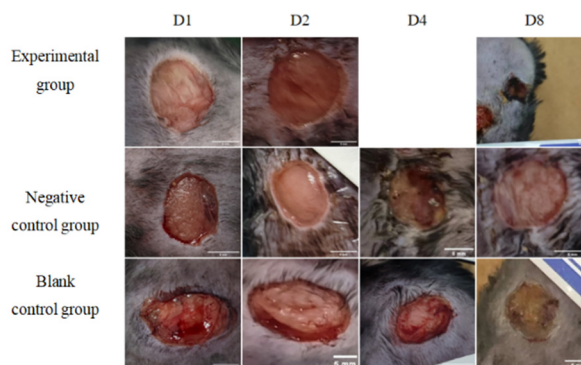


Figure 1. Comparative photos of wound size during wound healing in mice

results of all groups of mice were good, and the healing results of the experimental group and the blank control group were significantly better than those of the negative control group; In the experimental group, the wounds healed quickly, shrank more, the wounds were clean, and there was no infection or suppuration, the recovery was good. The recovery in the blank control group was good, and the wound neovascularization and tissue growth were obvious. The negative control group recovered moderately, the growth of wound tissue was relatively slow, and the size of the wound did not change significantly.

3.2. Behavioral observations of mice during wound healing

By observing and counting the behavioral data of mice, it was found that there was no significant difference in food intake and water intake between the experimental group, the negative control group and the blank control group (Fig. 2a,2b). Among them, the negative control group mice had a significant increase in water intake on day 10, which was presumably related to their death. All groups of mice lost weight, which was associated with diabetes (Fig. 2c). By observing the arched back of mice, it can be observed that there is no arched back in the experimental group and the negative control group, and continuous arching in the blank control group on day 4, and hydrogel dressings can promote wound healing and relieve pain in mice.

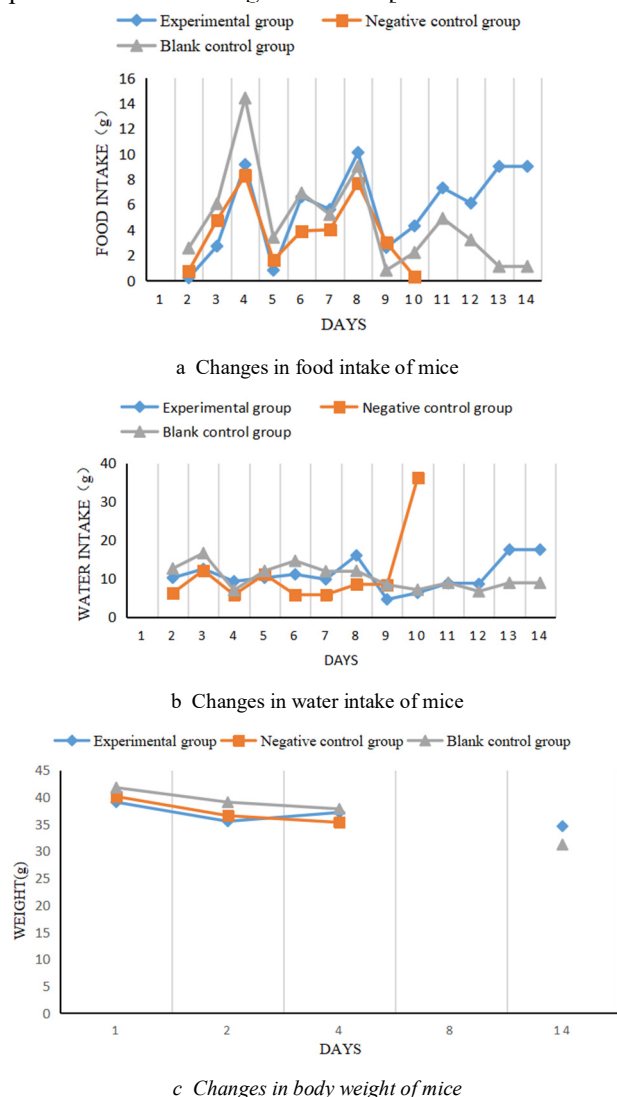


Figure 2. Behavioral observations during wound healing in mice (a. Changes in food intake of mice; b. Changes in water intake of mice; c. Changes in body weight of mice.)

3.3. Results of serum cytokine analysis during wound healing in mice

Since S5 died on day 3 and S3 died on day 4, the serological data of the experimental group on day 4 were missing, and analysis is now based on the existing data (Figure 3). TGF-

β (transforming cytokine- β) plays an important role in extracellular matrix synthesis and remodeling, and it can be observed that TGF- β is produced more in the experimental group in the late stage of wound healing, indicating that the hydrogel dressing of fully degradable polylactic acid film promotes cell regeneration and wound healing in mice.

3.4. Slice analysis results during wound healing in mice

Due to the different slice locations, the initial values of the average collagen fiber content percentage of wound sections in the experimental group, negative control group and blank control group were different.

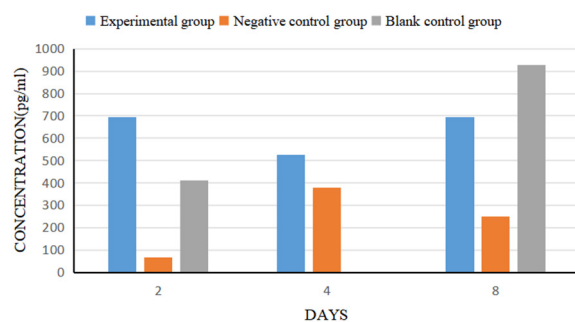


Figure 3. Changes of serum TGF- β during wound healing in mice

By analyzing the percentage of average collagen fiber content under the microscopic observation of wound sections, the change trend of collagen fiber reconstruction ratio in the three groups of wounds can be seen (Figure 4). The percentage of collagen fiber content in the experimental group showed an increasing trend, and the wound healing was good and the healing speed was faster. There was no significant change in the content of collagen fibers in the negative control group; The average percentage of collagen fiber content in the blank control group showed a decreasing trend, and the insufficient reconstruction of collagen fibers may be caused by the lack of protection of wounds and environmental infections in the blank control group. These results show that the new fully biodegradable hydrogel can promote collagen fiber reconstruction, and the effect of promoting wound healing is better than that of ordinary hydrogel.

The wound recovery process of each group was shown under the microscopic photos of HE stained tissue sections (Figure 5): on day 4, the experimental group was reconstructed with abundant collagen fibers, accompanied by a large number of new walled thin capillaries, and the wound healing was good; New hair follicles and capillaries were visible in the negative control group on day 1, and collagen fiber reconstruction was abundant on day 4, but it was slightly worse than that in the experimental group. The wound growth was slower in the blank control group, and the reconstitution of collagen fibers was worse than that in the experimental group and the negative control group, and inflammatory cell infiltration was visible. In summary, the new fully biodegradable hydrogel dressing has the effect of reducing inflammatory response, promoting collagen fiber reconstruction, and accelerating wound healing.

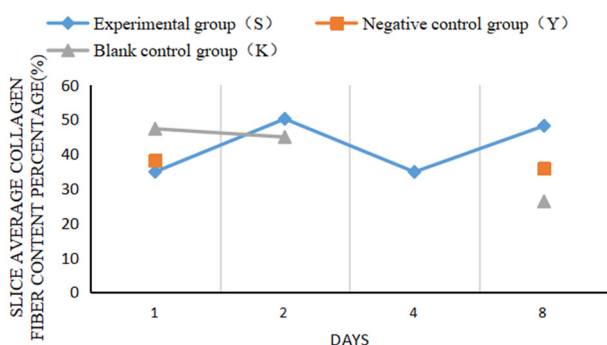


Figure 4. Slice analysis results during wound healing in mice

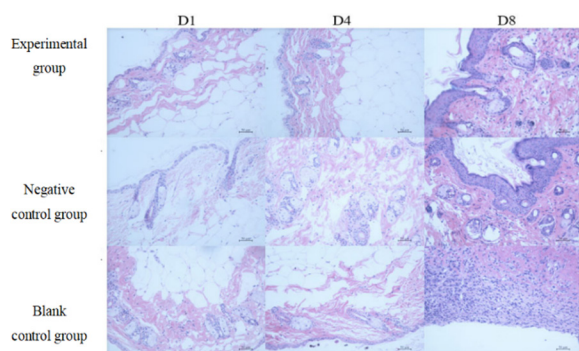


Figure 5. Hematoxylin-eosin stained section photographs during wound healing in mice (20×)

4. CONCLUSION

In the field of medical dressings, traditional dressings have shortcomings such as easily infection, inflammation, adhesion to wounds, poor air permeability, single function, and easily adhesion and secondary injury when used [19]. Polypropylene is difficult to degrade naturally and can cause extreme damage and pollution to the environment. Although traditional hydrogel can be superior to traditional gauze dressings in promoting wound healing, the materials that make them up are difficult to degrade, which can easily lead to waste of resources, and still cause environmental pollution as disposable medical waste [20]. Wound management is one of the important area in the medical system, among which chronic wounds such as diabetic foot are particularly difficult to heal [21]. Diabetic foot ulcer, as one of the serious complications of diabetes, has the characteristics of difficult to cure and easy to recur, and improper treatment can easily cause lower limb paralysis, disability and even amputation [22]. Wound dressings play a pivotal role in diabetic foot treatment, providing external protection and barriers against external contamination while promoting absorption of exudate around the ulcer site [23]. In the past few years, the research of diabetic wound dressings has progressed rapidly. Various new dressings have emerged, and hydrogel dressings are one of them. Silver nanoparticles dressings have been widely used in the treatment of diabetic foot ulcers in recent years, and have the advantages of controlling infection, reducing the pain of dressing changes, and reducing the number of dressing changes while promoting wound healing [24]. However, silver nanoparticles deposited in the body are

potentially toxic to various tissue cells such as skin, eyes, and kidneys [25], and gold metal and silver ions produced by dressing degradation also have certain toxicity to freshwater organisms [26], and some people may also be allergic to silver nanoparticles.

Natural polymer biomaterials come from a wide range of sources, hyaluronic acid, sodium alginate, chitosan, gelatin are commonly used natural bioactive polymer materials, they often used to make new medical dressings materials. The use of natural polymer biomaterials prepared new medical biological dressings can be used for the treatment and care of traumatic skin [27]. The inherent biodegradability and good bio-compatibility of natural polymer hydrogel make them widely used in biological, medical materials and other fields [28]. In promoting wound healing, new medical hydrogel dressings can provide an environment for wound wetness, and according to the wet healing theory, wounds in humid environments heal significantly faster than wounds in dry environments [29]. Bio-active hydrogel dressing can significantly shorten wound healing time by inhibiting infection, stimulating cell migration and proliferation, and promoting wound angiogenesis [30]. Hydrogel wound dressing also have strong drainage, anti-nonspecific protein properties [31].

In this study, obligate phage of *Escherichia coli* and conotoxin analgesic peptides were added to hydrogel dressings synthesized by alginate, applied to the skin of diabetic mice, and the effect of the new fully biodegradable hydrogel dressing on diabetic wound injury repair and wound healing was evaluated by wound healing rate and serum cytological factor analysis. Compared with ordinary hydrogel medical dressing, new hydrogel dressing has better antibacterial efficacy [32], and the effect of promoting wound healing is more obvious. The results of behavioral observations in mice showed that the new hydrogel dressing could relieve wound pain, calm and relieve analgesia, reduce scratching, and improve comfort and compliance. Serum cytokine analysis showed that the new dressing promoted the expression of cytokines such as transforming cytokine- β , regulated inflammatory response, cell proliferation, and wound reepithelialization [33]. HE and Masson staining showed that sodium alginate hydrogel dressings can promote fibroblast growth, increase the proportion of collagen fiber reconstruction, and accelerate diabetic wound healing [34]. In terms of environmental protection, the new hydrogel dressing in this study and the fully degradable polylactic acid outer packaging applied to the outer layer of dressings are biodegradable materials, which can be decomposed into small molecule harmless substances by physical photosynthesis, chemical biodegradation, oxidase and other forms [35], so as to minimize the environmental harm caused by a large number of applications. Polylactic acid film has good bio-compatibility and biodegradability, no toxicity and inflammatory reaction to the body, not only weak body rejection response, but also non-polluting, non-toxic environment-friendly material, widely used in the biomedical field in drug loading, human tissue engineering, application suture and other aspects [9,36].

The new biodegradable dressing can not only promote wound healing, but also solve the problem of environmental pollution, conform to the sustainable

development needs of society, and have broad application prospects for various acute wounds (such as burns, bleeding) and chronic wounds (such as diabetic foot ulcers, pressure ulcers) [37]. However, wound healing is a dynamic and complex process [38], making it difficult to develop a dressing that is suitable for all wounds. As one of the most competitive dressings [39], hydrogel dressings have excellent properties such as anti-infection and wound healing, and there are many challenges that need to be solved [40]. At present, many commercial alginate hydrogel dressings have been used for wound treatment [41], and further research on the physicochemical properties and biological properties of hydrogel dressings is still needed in the future, in order to achieve their wide application in more scenarios.

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