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Study on wound healing effect of low-carbon topical dressings with new green packaging

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Abstract: In order to verify the effect of the new green and low-carbon hydrogel dressing on promoting wound healing, this project applied a sodium alginate hydrogel dressing product containing Escherichia coli and taro toxin analgesic polypeptide (The specific ingredients of the dressing) to skin wounds in common rats. Effects of the hydrogel dressing on promoting skin wound healing was evaluated by observing the occurrence and frequency of behavioral changes in rats, observing wwhistological sections under a high-power microscope, changes in serum cytokine indicators, and Image J analysis of collagen fiber reconstruction ratios in tissue sections. Through comprehensive evaluation, it can be found that hydrogel dressing has analgesic, anti-inflammatory and anti-infection effects on rat wound surface, and acts on promoting wound healing, promoting the formation of new blood vessels in the damaged skin tissue area, promoting the growth of granulation tissue, and promoting the reconstruction of collagen fibers in wound tissue.

1 Introduction

With the increasing white pollution and the increasing shortage of natural resources, the environmental problems caused by medical packaging materials have become increasingly prominent. Degradable, green and environmentally friendly packaging materials have been paid attention to, becoming a development path for sustainable development in the future and solving the increasingly serious contradiction between resources and the environment[1]. It is urgent to find alternative sustainable green packaging materials[2]. Medical packaging materials have special standards for materials: while providing an effective bacterial barrier, allowing biocides to pass through to meet the needs of sterilization, the material components are non-toxic, odorless and color-free. Medical packaging materials are divided into reusable packaging materials and single-use packaging materials. Reusable materials include: cotton, rigid containers; Disposable packaging materials include: medical non-woven fabrics, medical packaging paper, medical packaging paper bags, paper-plastic packaging materials. Disposable medical packaging supplies such as medical gloves, medical protective clothing, medical consumables packaging, bandages, etc. are in huge demand every year, and the degradation rate of discarding in the natural environment after use is extremely slow. Therefore the plastic remaining in the soil and water can seriously damage the ecological environment and affect the stability of the ecosystem. The raw materials of plastic medical packaging products in the past were mainly based on fossil energy, while bio-based degradable packaging materials turned to non-fossil energy, and the development stage of fossil energy and non-fossil energy multi-architecture achieved coordinated complementarity, trade-off, and gradually moved towards a green, low-carbon, safe normal life. At present, degradable biomedical materials include natural polymer degradable materials, such as gelatin, collagen, polysaccharides, silk fibroin, etc., produced by animals and plants in nature, with good bio-compatibility, similar to the tissue structure of the human body, catalyzed by enzymes in the organism, non-toxic and harmless to the organism; In addition, degradable bio-materials synthesized through chemical production, including polylactic acid, polylactide, polycaprolactone, etc., can be completely degraded and eventually excreted, and the whole process does not cause any harm to the human body; Besides, there are biodegradable bio-ceramic materials, medical metal materials, medical metal

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materials, etc.[3]. The emergence of biodegradable materials provides a new direction for green and environmentally friendly medical packaging materials. Biodegradable materials are continuously applied, automatic decomposition ability is very strong, low environmental pollution, the technology has now covered medicine, biology and other fields, with the effect of preventing extravasation of intravenous infusion, preventing phlebitis, prolonging the use time of indwelling needles[4].

Medical packaging materials refer to materials that provide protection, safety, convenience and other functions for medical device products such as drugs, medical devices and their auxiliary materials and consumables. As one of the medical device products, topical dressings have their own medical use and can provide necessary protection and treatment for wounds, burns, abrasions and other emergencies. The materials it carries, such as tape, gauze, etc., are provided to provide the effect of dressing, which not only has medical properties, but also has the function of material packaging, with the dual effects of protection and convenience. As a kind of medical packaging, topical dressings have special requirements for their materials. Choosing the right dressing can protect the wound, reduce the risk of infection, and accelerate wound healing. In this study, a new hydrogel dressing was made by using a biodegradable system, and sodium alginate was selected to form a functional hydrogel dressing matrix. The new hydrogel dressing can give full play to the advantages of hydrogel as a drug carrier, and can carry out targeted drug delivery and slowly release the drug to prolong the efficacy of the drug, which can complement the function of hydrogel to create a wet healing environment conducive to wound healing and form a low pH and low oxygen environment that is not conducive to bacterial reproduction, thereby enhancing the efficacy of the dressing. Sodium alginate is mainly found in natural brown algae, is a polysaccharide. The huge source of marine natural resources can be completely biodegraded and will not burden the natural ecological environment, in line with the concept of respecting nature, follow its ways, and protect it. Sodium alginate is a mixture of alginic acid diman and formaldehyde acid, and its main components are alginic acid and sodium salt. Sodium alginate is widely used as a medical dressing to aid wound healing due to its hydrophilic, biocompatible, and biodegradable properties[5]. The design and characterization of alginate biomaterials are important in wound dressings and tissue regeneration. The ideal biomaterial for wound dressings must be biodegradable, biocompatible, non-inflammatory, and non-toxic, and alginate meets this requirement[6]. At the same time, sodium alginate also has antioxidant and immune enhancement effects, which is very suitable for the needs of wound healing. Hydrogel dressings can carry bacteriophages or biosynthetic active polypeptide substances to replace antibiotics. The new non-narcotic analgesic polypeptide drugs for the nervous system can be used to prepare medical dressings for wound treatment, and achieve analgesic, antibacterial, anti-inflammatory and promote wound healing through osmosis. Fully degradable polylactic acid (PLA) film applied to functional hydrogel, polylactic acid not only has good biocompatibility, but the final degradation products are water and carbon dioxide, and the intermediate product is lactose without any harm to the body[3].

This study implements the concept of green environmental protection, uses the key technology of biodegradable system to make new hydrogel dressing packaging, replaces the traditional wound topical dressing bandage packaging, combines the advantages of controlled drug loading, targeted drug delivery and sustained release of hydrogel, constructs a suitable pH conducive to wound healing, suitable for breathable humid environment, enhances the efficacy of dressing, and constructs an ideal surgical dressing packaging for various forms of wound treatment, which promotes green development, circular development and low-carbon development.

2 Materials and methods

2.1 Laboratory animals

We used 4 SD rats, 6-8 weeks old, males (Zhiyuan Biotechnology (Shandong) Co., Ltd.), marked at the base of their tails with a black marker, numbered T1 to T4 respectively. T1 and T4 were the experimental group, T2 was the negative control groups, and T3 was the blank control groups.

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. SD rats 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

Disposable needles,10 ml centrifuge tubes, 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

NanoDrop Ultraviolet Spectrophotometer (Thermo Scientific), Full-featured Microplate Detector (Synergy H1M), Automated Pipetting Equipment (Assist Plus), Four-Dimensional Rotary Mixer (BE-1100), PCR Instrument, Benchtop Refrigerated Centrifuge, Agarose Gel Electrophoresis Instrument, Qubit3.0 Fluorescence Quantitative Detector, Blood Glucose Meter, Ultra-low Temperature Freezer, Autoclave Sterilizer, Beaker, Erlenmeyer flask, glass rod, mortar, epilatator, surgical scissors and forceps, centrifuge, pipette gun, light microscope.

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 rats to evaluate the effect of functional hydrogel dressing on diabetic skin wound repair and healing.

2.4.1 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.

2.4.2 Functional hydrogel preparation

After preparing sodium alginate powder for high-temperature steam sterilization, 0.42 ml of phage and 0.22 ml of conotoxin analgesic peptides dilution per 1 ml of hydrogel were prepared to produce functional hydrogel, each serving for one wound. (Proportions of the dressing)

2.4.3 Rats skin wound molding

Depilate the back of the rat with a epilator, using 4% chloral hydrate, anesthetize rats at a dose of 1 ml/100 g, use a sterilized skin tissue sampler, cut 4 round wounds with a diameter of 1.5 cm in size on the back of each rat, and excise the full thickness of skin. The wounds are numbered C1 to C4 in turn.

2.4.4 Rats administration and treatment

Rats were grouped on days 1, 2, 4, 6, 8, 10 and 12. Experimental group: Each wound is coated with a functional hydrogel mixed with 0.42 ml phage and 0.22 ml conotoxin analgesic peptides dilution and 1 ml hydrogel, and a fully degradable polylactic acid film is applied and fixed with medical tape. Negative control group: 1ml of blank hydrogel without mixing any drugs was applied to each wound, and a fully degradable polylactic acid film was applied and fixed with medical tape. Blank control group: no treatment. The rat experimental group stopped the drug on day 6, and both the experimental group and the negative control group used blank hydrogel.

2.4.5 Experimental data collection

Wound records: On days 2, 4, 8 and 14, the wound of each rat was photographed with a smart phone. General animal behavior: daily food intake and water intake, rat weight data were collected on days 2, 4, 8 and 14 to observe the mental state, arched back, trembling, and scratching. Serology: On days 2, 4, 8 and 14, the whole blood of rats was collected after orbit, and the serum was isolated after standing centrifugation. Tissue section: On days 2, 4, 8 and 14, the skin tissue of one wound per rat is cut sequentially and fixed in fixative solution.

2.5 Data analysis methods

Due to experimental material limitations, there was only one rat each group, so repeated experiments have not been performed，and the analysis of the data did not involve error analysis. The wound area of each wound was calculated by Image J, and the wound healing rate was calculated according to the formula (%) = (initial area of the wound - current area of the wound) / initial area. Data records observing changes in the behavior and frequency of rats are plotted as line charts Microsoft Office Excel. Wuhan Servicebio Technology Co., Ltd. was commissioned to test TGF-β, IL-1β and VEGF by Western-Blot quantification. Plotted the bars using Microsoft Office Excel on the data for the statistical analysis. Perform Masson staining on tissue slices, observe under a microscope, analyze collagen reconstruction proportion using Image J, and then use Microsoft Office Excel to create a line graph to represent the dynamic changes and differences in collagen reconstruction during wound healing in each group of rats. HE stained tissue sections were observed under a 20x microscope to observe granulation tissue growth. In the analysis of each group of data, we adopted the method of multiple sampling and averaging, which reduced the chance error of the data and made the data more realistic and reliable. (We adopted different data analysis methods, and only the average method was used this time.)

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 = 0.1026x2 - 0.8331x + 1.7304. Predict its value, and plot the table below (Tab.1).

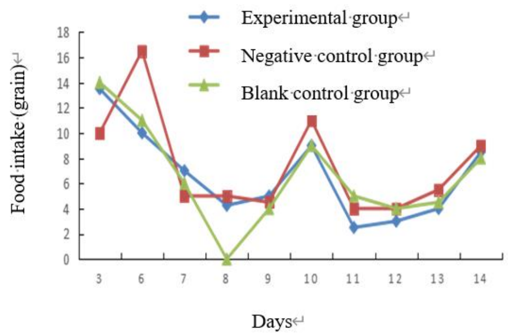
Tab.1 Rat wound healing rate

Group	rats wound healing rate			
Time (days)	2	4	8	14
Experimental group	0.00%	27.71%	34.68%	92.98%
Negative control group	0.00%	52.52%	84.55%	96.12%
Blank control group	0.00%	13.45%	47.46%	95.02%

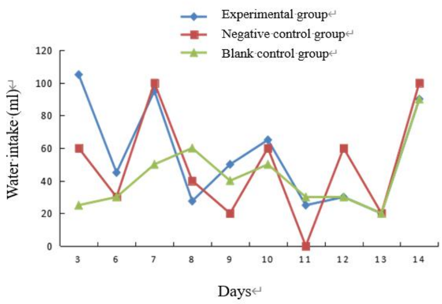
The wound healing results of all groups of rats were good, among which the healing results of T1 (experimental group) and T2 (negative control group) were better than those of T3 (blank control group) and T4 (experimental group) healing degree; T1 wound heals the fastest, shrinks the wound the most, the wound is clean, no infection and suppuration, and the recovery is the best. T2 wound recovery was good, and the wound was formed with neovascularization and tissue growth. T3 recovery was average, wound tissue growth was relatively slow, and wound shrinkage was good. The T4 wound is narrowed, the wound skin tissue grows well, and the neovascularization is the most.

3.2 Behavioral observations of rats during wound healing

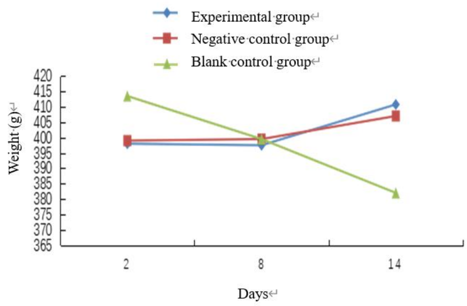
Observing and counting behavioral data from rats revealed no significant differences in food intake and water intake between the experimental, negative control, and blank groups (Fig.1).Among them, the food intake of rats in the negative control group increased significantly on day 6, which may be related to good wound healing and growth, increased caloric consumption, and increased feeding demand; in the blank group, the rats significantly reduced food intake on day 8, which may be related to the wound inflammatory response, increased pain and reduced appetite. The water intake of the rats in the blank group was significantly lower at the initial stage of wound healing than that of the experimental group and the negative control group, probably because the pain in the wound inhibited the water-harvesting due to the lack of the protective effect of the dressing. The experimental and negative control rats gained weight during the wound healing, and the increase was similar, which may be that the good wound recovery trend promotes the weight increase, while the blank control rats had weight loss, which may indicate the poor wound healing, a large amount of energy consumption in the rats, and the pain reduced the appetite, leading to the weight loss in the rats. The blank control group had higher mean stress levels of shivering and arch back than that in the experimental and negative control groups, which may be related to the lack of protection, more sensitivity to pain, and relatively slow wound healing in the blank control group. By observing the mental state of the rats, it was observed that the mood of the experimental group and the negative control group was more calm and stable than that of the blank group.



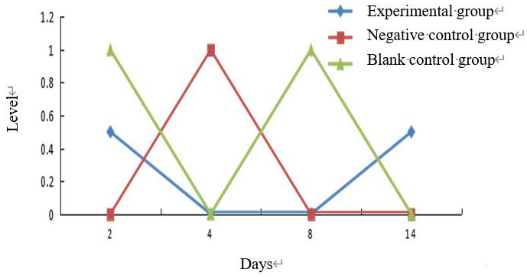
a Changes in food intake of rats



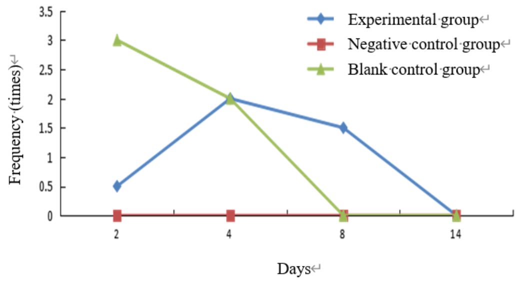
b Changes in water intake of rats



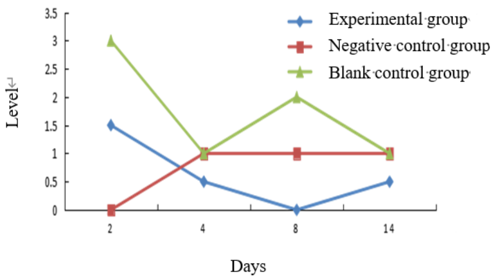
c Changes in body weight of rats



d Trembling situation



e Arching back condition



f Mental state

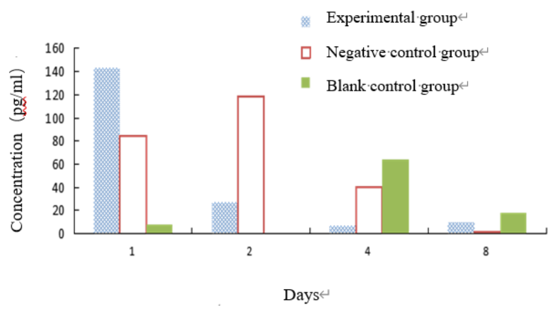
Fig. 1 Behavioral observations during wound healing in rats (a. Changes in food intake of rats; b. Changes in water intake of rats; c. Changes in body weight of rats; d. The trembling situation of rats; e. The condition of the arched back of rats; f. Rat mental state.)

3.3 Results of serum cytokine analysis during wound healing in rats

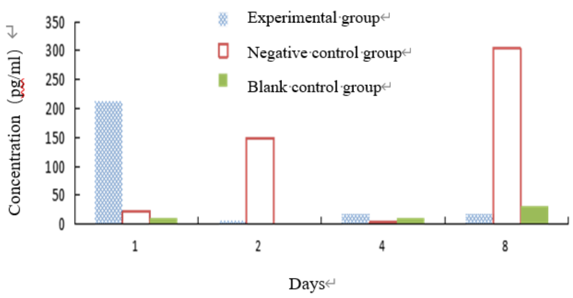
TGF- β (transforming growth factor- β) is widely distributed in the human body, and participates in the regulation of physiological and pathological processes such as the growth, differentiation and repair of many cells and tissues. TGF-β plays an important role in extracellular matrix synthesis and remodeling, and it can be observed through serological data analysis that the experimental group and the negative control group produced more TGF-β in the early stage of wound healing, and the TGF-β content in the negative control group was more (Fig.2-a).

IL-1β(interleukin-1β) is a key kind of proinflammatory cytokines, is also a typical multifunctional cytokines, it can not only participate in the coordination of a variety of autoimmune inflammatory response, associated with pain, inflammation, and autoimmunity, at the same time to participate in a variety of cell activities, including cell proliferation, differentiation and apoptosis, promote tissue defense and tissue repair, participate in neuroprotection, tissue remodeling and repair.IL-1β can play a role in wound healing, according to the serological data, it can be observed that the early IL-1β content in the experimental group is high, and the middle and late negative control group has a high IL-1β content (Fig.2-b).

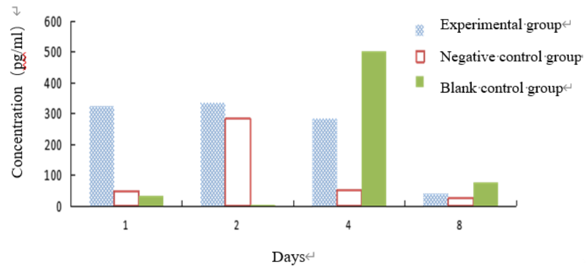
VEGF (Vascular endothelial growth factor) is a highly specific pro-vascular endothelial cell growth factor that promotes increased vascular permeability, extracellular matrix degeneration, vascular endothelial cell migration, proliferation, and blood vessel formation. VEGF also plays an important role in wound healing, and according to the serological data, it can be observed that the content of VEGF in the experimental group and the negative control group is higher in the early stage (Fig.2-c).



a Rat serum TGF-β Changes



b Rat serum IL-1β Changes



c Rat serum VEGF Changes

Fig. 2 Analysis of serum inflammatory factors during wound healing in rats (a. Rat serum TGF- β Changes; b. Rat serum IL-1 β Changes; c. Rat serum VEGF Changes.)

3.4 Slice analysis results during wound healing in rats

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 (Fig.3). 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. 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.

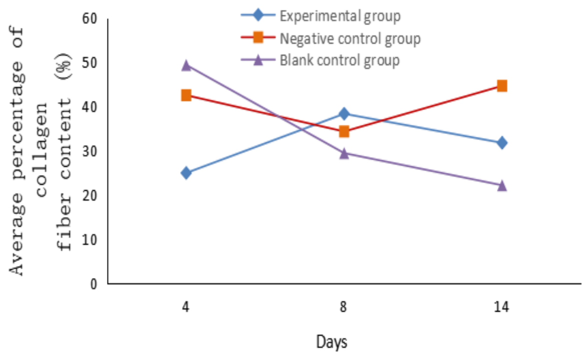


Fig. 3 Slice analysis results during wound healing in rats

The microscopic photos of Hematoxylin-eosin stained tissue sections (Fig.4) showed the wound recovery process of each group: T3 wound had infection and suppuration on day 4, and neutrogranular exudation could be seen under the tissue section, accompanied by different pus and tissue necrosis, exuding a large number of denatured cells, hyperplasia, necrosis and shedding, which may be caused by the lack of effective protection

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of the wound; On day 14, it was seen that T1 and T4 collagen fibers were abundant in reconstruction, wound growth was good, and T2 collagen fibers were looser, which was inconsistent with the expected collagen fiber reconstruction, which may be caused by the selection of slices and photographs. T3 collagen fiber reconstruction is good, but it is obviously not as large as T1 and T4, and wound growth is good.

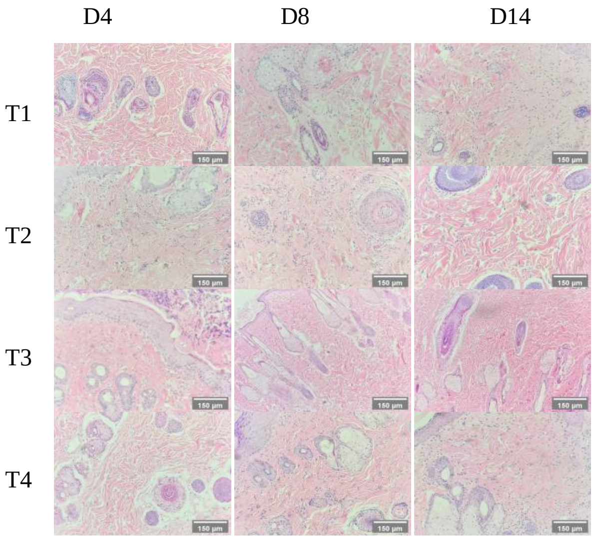

Fig.4 Hematoxylin-eosin stained section photographs during wound healing in rats (20×)

4 Conclusion

This study revealed that the new green biodegradable hydrogel dressing has the effect of preventing wound infection and suppuration, and the hydrogel dressing has a role in promoting early cell regeneration and wound healing in rats, promoting tissue remodeling and healing in rats during the whole stage of wound healing; compared with ordinary silver nanoparticles hydrogel topical dressings, it also has the effect of analgesic sedation and calming the patient's emotions, reducing the patient's pain[7], reducing the discomfort such as itching and stuffiness at the wound, reducing the scratching behavior of the injured and sick on the itching and pain of the wound, and avoiding the occurrence of silver ion allergy in some people[8]. The new green biodegradable hydrogel dressings of the wounded and sick have better acceptance, higher compliance and continuity, and can improve the treatment and nursing compliance of patients[9]; The results of serum indexes showed that in the early stage of wound healing, they expressed high cell transformation growth factor and vascular endothelial growth factor, which promoted the formation of new blood vessels and wound healing. Inflammatory factors showed a downward trend, and wound infection was well controlled; HE and Masson staining showed that the novel green biodegradable hydrogel dressing created a hypoxic environment, which had a certain effect on promoting granulation tissue growth and collagen fiber reconstruction, increasing fibroblast growth rate, increasing the proportion of wound collagen fiber remodeling, and accelerating capillary formation and wound healing[10].

As the first line of defense between the human body and the outside world, skin is vulnerable to damage by the external environment[11]. Skin injury is a common and frequent disease, and without timely and effective wound treatment protection, it often develops into infection and suppuration, and even deteriorates into sepsis secondary to systemic infection; Gauze, cotton pads and other dry dressings are inert medical packaging dressings, but because dry dressing gauze can only promote local dryness after absorbing exudate and cannot promote wound healing, wounds bandaged by traditional gauze often heal slowly, the barrier effect after penetration is poor, the wound can not be fully protected, easily causing exogenous infection. After drying, it is easy to make the wound and the dressing adhesion, and remove the dressing to bring secondary injury to the patient[12], causing the pain of dressing. Gauze dressings cannot control the continuous, slow release of the drugs carried on the wound, while biodegradable hydrogel has a porous storage structure that can hold a large number of drugs, and the release properties of hydrogel enable it to effectively control the drug concentration in target tissues[13]. Traditional gauze is discarded as disposable medical packaging waste after dressing, traditional disposable medical packaging waste treatment methods include incineration, landfill, etc.[14]. Incineration will cause plastic particles to be released into the air, water resources, and landfill often takes decades or even hundreds of years to completely degrade, bringing great burden to the environment. Although traditional hydrogel can promote wound healing and are superior to traditional gauze topical dressings, the packaging materials that make up the dressings are difficult to degrade, which can easily lead to waste of resources, and as disposable medical packaging material waste, it still pollutes the environment. Moverover, the metal silver ions produced by the degradation of silver dressings are also harmful to the environment[15].The problem of medical waste disposal has always plagued medical workers. Although the State Council has issued the *Regulations on the Management of Medical Waste* in June 2003, it is limited to the problem of simply dealing with medical waste. The development of polymer material science and technology has been applied to all aspects of life. With the emergence of various problems in medical waste treatment, degradable biomedical polymer materials have a certain application prospect in medical material packaging, medical supplies waste, disposable medical supplies[16].

Since the 21st century, the world’s medical field has developed rapidly, and bio-materials have also become a hot spot for researchers in the medical field in various countries. Biodegradable packaging materials play a vital role in patient surgery and recovery[17]. According to statistics, in 2020, the global biodegradable plastic production capacity reached 1.227 million tons per year, of which starch-based materials, polylactic acid and polyadipic acid/butylene terephthalate (PBAT) accounted for 32%, 32% and 23% of the production capacity of degradable plastics, respectively. The application of biodegradable biomaterials in biomedicine can make medical sutures, controlled release carriers, fracture fixation materials, tissue engineering scaffolds, suture reinforcement materials, etc.[18]. According to incomplete statistics, there are more than 90 varieties of degradable biopolymer materials and more than 400 kinds of products in medical applications, involving almost all fields of medicine, especially the diagnosis and treatment, replacement and repair of tissues and organs, and the special functional fields of inducing the regeneration of new tissues and organs account for a large proportion[19].

The new degradable bio-material polylactic acid raw material is renewable and is widely used in medical wound dressings. It has good air permeability and bacterial and microbial barrier effect on the fiber structure, and has good bio-compatibility, and will not irritate the skin. It has a good performance in the application of medical bio-topical dressings[20]. In recent years, the wet wound theory has been widely recognized, and practice has shown that this nursing mode can accelerate the healing of chronic wounds, prevent secondary infection, relieve pain, improve the quality of sleep and quality of life of patients, receiving a high degree of acceptance and recognition of patients[21-22]. Natural hydrogel material organisms are in direct contact with tissues and cells to promote wound healing, have good compatibility, can absorb large amounts of water, and allow wound water vapor to be exchanged with the outside world[23-24], which has unique properties similar to living tissues and shows unique advantages in the medical field[25].

Degradable packaging materials have been widely used in all aspects of food packaging in life, but antibacterial degradable films are mostly used in the storage, preservation, packaging of fruits and vegetables, meat and other foods, considering that they are non-toxic, degradable and the application to external excipients of medical packaging, they are expected to be widely used in non-food fields in the future. Personal hygiene appliances, medical supplies, environmental protection products and general consumer goods, etc., will be expected to replace traditional fossil packaging materials[26].

The new green biodegradable topical packaging dressing discussed in this study not only plays the advantages of hydrogel to protect wounds, prevent infection and promote wound healing, but also solves the problem of environmental pollution, exploring a new green and low-carbon medical path and bringing positive significance for the future green and low-carbon life and sustainable development.

However, the following shortcomings remain in the experiment:

(1) During the experiment, due to personnel and time factors, the area size and recovery of each wound in each group could not be measured in time, resulting in partial lack of data and incomplete experimental results.

(2) The number of experimental rats is small, which may cause some accidental errors due to the individual differences of rats, resulting in errors in experimental data, or problems that do not match the expected phenomenon.

(3) When observing, shooting slides and applying software such as Image J for data analysis under the microscope, accidental errors may occur in the experimental data due to subjective factors, resulting in data distortion.

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