Study of Plastic Kaempferol Hydrogel for the Treatment of Hand Injuries Sustained During Rowing Training and Other Situations

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Abstract. The regular occurrence of hand injuries is a significant health issue in training, such as rowing. This research incorporated kaempferol components and developed a gel for protecting against trauma to prevent hand injuries, based on the author's expertise in training and protection. The gel is a hydrogel prepared by freezing and thawing polyvinyl alcohol (PVA). The gel provides a moist environment and cover protection for the wound, thereby accelerating wound healing. In addition, kaempferol possesses various biological activities, such as anti-inflammatory, anti-cancer, antibacterial, and antioxidant effects. Therefore, in this study, we used PVA and kaempferol to prepare kaempferol composite hydrogel and the effect of kaempferol hydrogel on cells was characterized. Kaempferol's antibacterial properties were confirmed, and the effectiveness of kaempferol composite hydrogel in healing full-thickness skin wounds in mice was studied. In addition, it proved that the new hydrogel has good tension and maintains a stable state at room temperature.

Keywords: Kaempferol, hydrogel, wound healing, cell activity, antibacterial effect.

1. Introduction

Wound infections commonly occur in daily life, as well as in accidents, disasters, wars, and surgeries. Bacteria are the main causative agent of skin infection. Once a skin wound is infected by bacteria, excessive pathogenic bacteria may form a layer of biofilm on the wound surface, affecting tissue cell migration, re-epithelialization, and tissue remodeling. The toxins and proteins secreted by the bacteria can damage the tissues around the wound. If not promptly or appropriately addressed, the skin's fundamental protective function will be compromised, leading to a larger wound size and more challenging treatment.

Hydrogels are extensively utilized in the fields of medicine and bioengineering due to their unique physical and chemical properties, as well as their potential biomedical applications. In particular, hydrogels have garnered significant attention in medical trauma research. Characterized by a three-dimensional network structure formed by cross-linked water-soluble polymers, hydrogels are highly porous and hydrophilic. These properties facilitate gas exchange and create a moist environment, essential for maintaining fluid balance in the wound area and promoting effective wound healing. Furthermore, the porous structure effectively imitates the structure and function of ECM, enhances cell migration, growth, and maturation, and plays a crucial part in wound healing, immune system regulation, tissue engineering, and other applications. In particular, hydrogels are suitable for all stages of wound healing, are non-reactive to biological tissues, promote collagen deposition and fibroblast proliferation, and promote re-epithelialization to achieve the purpose of wound healing. Because of the extensive range of hydrogels applied externally to address skin wound infections, the hydrogel matrix is diverse, and it can be functionalized by adding monomers, macromolecules, and even loaded drugs with certain characteristics.

Based on the advantages of the above compounds, a multifunctional hydrogel was created to enhance wound healing by preventing external bacterial invasion, promoting cell regeneration, and providing a suitable environment for local wound healing.

2. Topic Selection Basis

2.1 Skin Trauma

The skin represents the initial line of defense within the human immune system. It is composed of the epidermis, dermis, and subcutaneous tissue. Additionally, it incorporates a range of accessory organs, including blood vessels, nerves, and muscles (Figure 1). The skin serves a vital function in safeguarding the body from external threats and regulating body temperature. At the same time, the skin is vulnerable to damage and trauma in daily life. Trauma is the destruction of human tissues or organs caused by injury factors. Natural disasters, traffic accidents, instrument injuries, and clinical operations will all cause trauma. Despite its impressive ability to regenerate, there are instances when skin wounds do not heal promptly, leading to severe impacts on the health and life of patients.

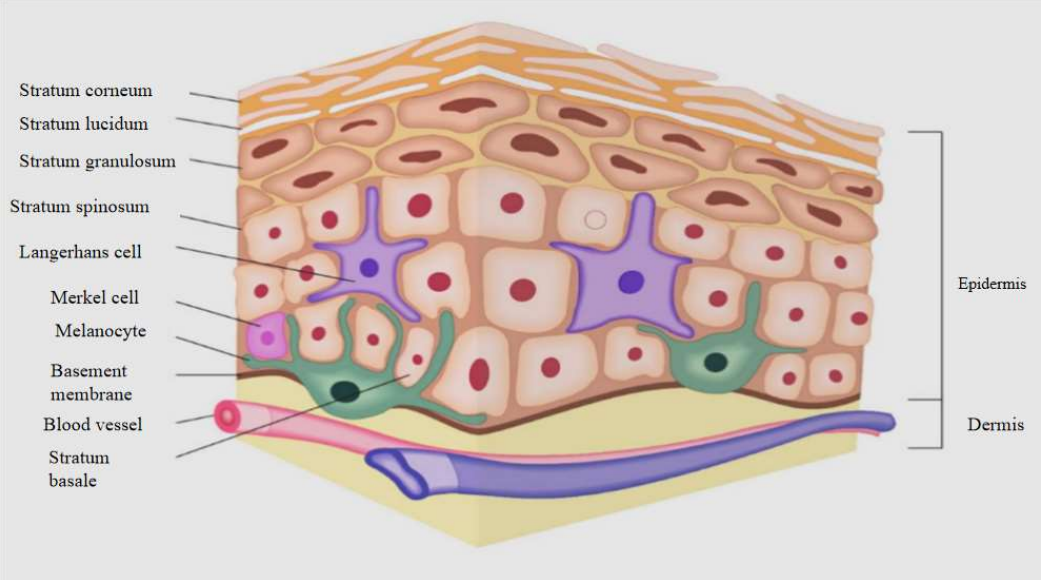

Figure 1. Skin structure

Infectious skin trauma is a disease in which pathogenic bacteria enter the epidermis, dermis, or subcutaneous tissue and multiply in the wound site, causing damage to the skin barrier and causing complications such as septic shock, sepsis, and septicemia. The skin controls the microbial community's stability on the skin surface and prevents subcutaneous tissue from being invaded and colonized by pathogens. Nevertheless, following skin injury, the skin wound becomes exposed, creating a damp environment for bacteria to colonize, facilitating their growth, and causing an infection in the wound. If left unattended, it can evolve into a dangerous systemic infection that could prove fatal in the end. Wound infection consists of three stages: contamination, colonization, and infection. For contamination, it means that bacteria do not reproduce at the site of the wound. Colonization refers to the presence of a limited amounts of bacteria in the wound, which does not affect wound healing. Infection refers to bacteria that can cause skin tissue swelling, fever, and purulent exudate, affecting wound healing. [1].

2.2 Hydrogel

Hydrogel is a network structure formed by three-dimensional water-soluble polymers that contain high moisture to maintain a moist wound environment. It shares physical properties and chemical composition with the extracellular matrix (ECM). Moreover, hydrogel has good biocompatibility and no cytotoxicity and plays an important role in wound treatment, immune regulation, and tissue engineering. Specifically, the hydrogel is appropriate for every phase of wound healing, does not react with biological tissues, creates a moist environment ideal for skin wound recovery, encourages collagen deposition and fibroblast growth, supports re-epithelization, and ultimately facilitates the

process of wound healing. At the infected site of the skin wound, the hydrogel will form a temporary barrier to cover the wound, keep moisture, create a closed hypoxia environment, and prevent microbial invasion. Moreover, it allows oxygen to enter the regenerative tissue, promotes the release of growth factors by phagocytes in the regenerative tissue, and accelerates the healing of skin wounds. In addition, wound exudate contains many inflammatory cytokines and chemokines that are beneficial to the growth of bacteria. Hydrogel can effectively absorb wound exudate without removing too much water from the wound area. Due to its excellent liquid absorption capacity can reduce the liquid evaporation rate, preventing the formation of a dry scab on the wound and minimizing the risk of secondary injury during the removal process [2].

2.3 Kaempferol

2.3.1 Introduction to Kaempferol

Kaempferol is a flavonoid compound derived from kaempferol and the rhizomes of some plants. It is also known as Kaempferide, kaempferol-3, and tetrahydroxyflavone. It is found naturally in tea and many common vegetables and fruits, including beans, broccoli, cabbage, currants, grapes, kale, tomatoes, citrus fruits, Brussels sprouts, apples, and grapefruit. The molecular structure formula of kaempferol is shown in Figure 2, and its relative molecular weight is 286.23. Its monomer is a crystalline yellow solid powder. Kaempferol is easily soluble in organic solvents such as dimethyl sulfoxide (DMSO) and ether and is slightly soluble in water. Kaempferol possesses diverse biological effects, including antioxidant, anti-inflammatory, anti-cancer, antibacterial, analgesic, cardioprotective, and diabetic preventative and therapeutic properties.

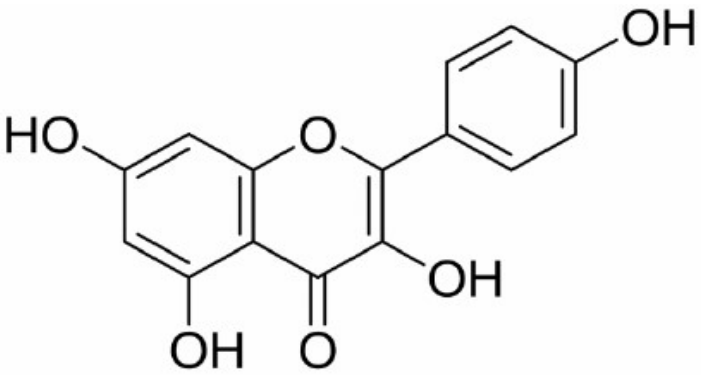

Figure 2. Kaempferol chemical structure [3]

2.3.2 Anti-inflammatory Effect of Kaempferol

In vascular tissue, inflammation is an important biological response that reflects tissue damage caused by different pathogens, irritants, or cell damage. The body heals damaged tissue and protects the body through anti-inflammatory responses. Studies have shown that kaempferol has a protective effect on chronic inflammatory diseases, including intervertebral disc (IVD) degeneration, postmenopausal osteoporosis, and colitis, as well as acute inflammatory diseases, including acute lung injury (ALI) [4]. Multiple mechanisms may mediate the anti-inflammatory activity of kaempferol. Studies have shown that kaempferol can inhibit the activity of nuclear transcription factor κ B (NF- κ B), reduce the levels of pro-inflammatory cytokines, and increase anti-inflammatory cytokines, including IL-10. Research indicates that kaempferol can inhibit the activity of tumor necrosis factor (TNF-α) and the expression of interleukins (IL-8, IL-1β). Studies have found that kaempferol can significantly inhibit the phosphorylation level of mitogen-activated protein kinase (MAPK) and exert an anti-inflammatory effect. In addition, kaempferol also inhibits the expression of lipoxygenases (LOX), cyclooxygenase (COX), and inducible nitric oxide synthase (iNOS) to participate in the pro-inflammatory response [5].

3. Project Design

Research purpose: Design a new type of healing-promoting anti-inflammatory hydrogel. Figure 3 is the experimental design roadmap:

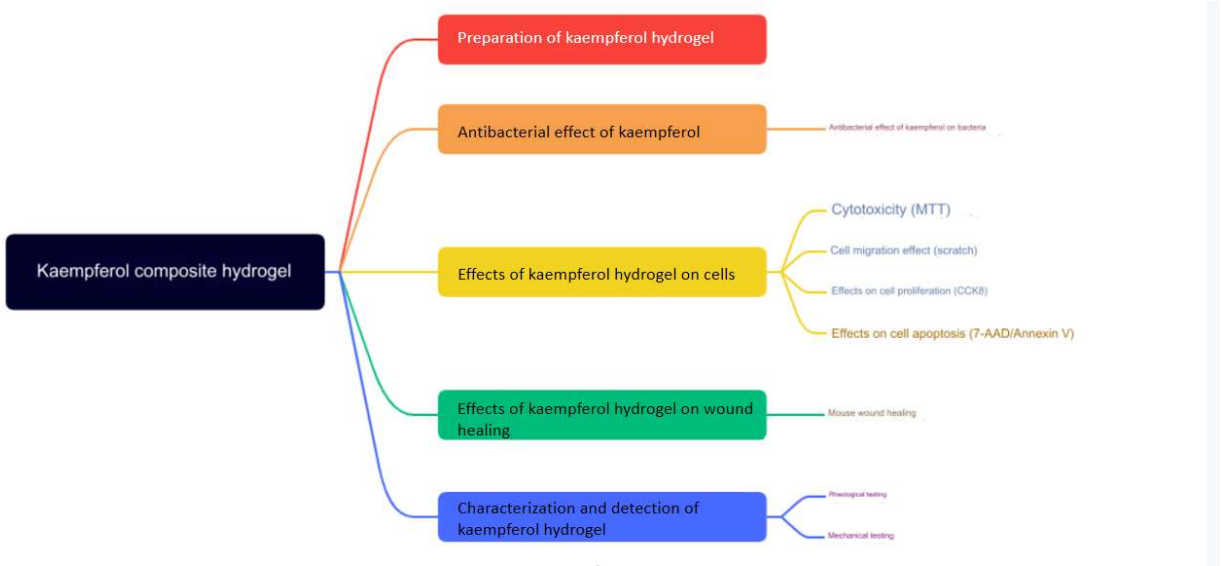

Figure 3. The experimental design roadmap

4. Experimental Materials and Procedures

4.1 Preparation of Kaempferol Hydrogel

Take 5% of PVA powder by mass. It's best to use ultrapure water to avoid impurities affecting the function of PVA. First, disperse the powder in cold water to swell it, and then slowly stir it at 90 degrees until it dissolves. It usually takes 1-2 hours and should be a clear, transparent liquid after dissolution. Note that the solvent will evaporate due to high temperature during the stirring process, so ultrapure water needs to be added to adjust the volume. The dissolved PVA is placed at -20 degrees and frozen and thawed repeatedly.

4.2 Effects of Kaempferol Hydrogel on Cells

(1) Cytotoxicity: L929 mouse fibroblasts were subcultured to 80% of the bottle bottom area and then seeded in a 96-well plate at 2*103 cells/well. They were divided into a control group and an experimental group. After 24, 48, and 72 hours of culture, 20 μL of 5 mg/mL MTT solution was added in the dark, and 150uL DMOS was added after incubation for 4 hours in the incubator. Shake in a constant temperature shaker at 37°C for 15 minutes and detect the absorbance at a wavelength of 490 nm. The relative cell proliferation rate (RGR%) is calculated using the following formula.

$$RGR(\%) = \frac{OD_{\text{Experimental group}} - OD_{\text{Blank group}}}{OD_{\text{Negative control}} - OD_{\text{Blank group}}} * 100\% \quad (1)$$

Table 1. Cytotoxicity Scoring

Toxicity score	0	1	2	3	4	5
RGR (%)	≥100	75~99	50~74	25~49	1~24	0

(2) Cell migration: We utilized a cell scratch assay to investigate the effect of kaempferol on the migration of L929 cells. To begin, we marked the positions evenly on the bottom of a 6-well plate

using a marker pen, creating three lines per well. The digested and counted L929 cells were then plated and allowed to reach approximately 90% confluence. Following this, the medium was replaced with a serum-free medium to induce cell starvation. After 24 hours, a 200 μL pipette tip was employed to create scratches in the cell layer. Subsequently, the plate was washed three times with PBS to remove the scratched cells, and serum-free medium was added for continued culture. Concurrently, kaempferol was introduced at a final 20 μg/mL concentration. A serum-free medium was added to the experimental group, and photographs were taken to document cell migration at 0, 12, 24, and 48 hours. We used Image J and Photoshop to analyze the scratch area change and pinpoint the cell migration location. We photographed from an identical location for records to guarantee the experiment's reliability.

(3) We used the CCK-8 assay to detect the effect of kaempferol on L929 cell proliferation. The concentration of L929 cells resuspended after trypsin digestion was counted using a hemocytometer and inoculated into a 96-well plate (5×103 cells/well, 90 μL). Following the adhesion of the cells to the wall, 10 μL of kaempferol was added to achieve final concentrations of 0, 10, and 20 μg/mL, respectively. The cells were then cultured in a constant temperature incubator at 37°C and 5% CO_2 for 24 hours. Subsequently, ten μL of CCK-8 was added to each well and incubated in a 5% CO_2 incubator at 37 °C for one hour in the dark. The absorbance was measured at 450 nm. The zero adjustment hole was set, and the absorbance value indirectly reflects the number of cells.

4.3 Antibacterial Effect of Kaempferol

We prepared kaempferol into a mother liquor of 100 mg/mL and filtered it for sterilization. The concentration of E.coli was diluted to 1*105 ~ 6 CFU/mL.

(1) Medium Allocation

Preparation of LB solid culture medium: Weigh approximately 4.00 g of LB broth and 4.00 g of agar into an Erlenmeyer flask. Add 200 mL of deionized water and stir thoroughly to achieve a 2% (w/v) LB broth and 2% (w/v) agar medium. Filter the mixture using a membrane-sealed Erlenmeyer flask and sterilize it at 121°C for 20 minutes. Subsequently, the sterilized culture medium was poured into plates, adding about 10 mL to each plate. Allow the plates to cool at room temperature to form solid culture medium plates, and store them in a refrigerator at 4°C for future use.

(2) MIC and Inhibition Rate

In a 96-well plate, 10 mg/mL kaempferol mother liquor was diluted with a culture medium to obtain solutions of different concentrations. We added 200 μL LB culture medium to the first well, 100 μL culture medium to the second well as a negative control, and 100 μL bacterial solution to the remaining wells with final concentrations of 100, 50, and 20 ug/mL respectively. As an experimental group, a mixture of LB medium and the extract was used as a blank sample, which was cultured at 37°C for 18 to 24 hours, and the growth of the bacteria in the liquid was observed. When the bacterial solution in the well is clear and transparent, the concentration of the drug solution is the MIC, and the absorbance at a wavelength of 600 nm is measured. Calculate the bacterial inhibition rate (%) according to the following formula.

$$\text{Inhibition rate } (\%) = \left(1 - \frac{OD_{\text{group}}^{\text{Experimental}} - OD_{\text{Blank group}}}{OD_{\text{Negative control}}}\right) \times 100\% \quad (2)$$

4.4 Effect of Kaempferol Hydrogel on Wound Healing

The experimental subjects were 6-week-old C57BL/6 female mice (n=10) weighing 23.79±0.12g, purchased from the Experimental Animal Center of Shanghai Jiao Tong University. Mice were raised under constant temperature conditions (22 ± 2°C) with a 12-hour day-night cycle, fed standard diets, and had free access to water. Ten mice were randomly divided into two groups: the first group was the control group (Con), and the second group was the hydrogel + kaempferol group (PVA +

Kae). After anesthetizing the mice, the hair on their backs was removed, the backs of the mice were disinfected with medical alcohol and iodophor, and a skin defect wound about 1 cm in diameter was incised on the backs of the mice. After completion of the procedure, the control group was not treated, and the latter group was fitted with kaempferol hydrogel.

4.5 Data Processing

Microsoft Excel 2016 and SPSS 26 were used for statistical and significance analysis of data, and GraphPad prism9 was used for drawing.

5. Experimental Results

5.1 Cytotoxicity of Kaempferol

Fibroblasts play a vital role in tissue repair. To evaluate the extract's safety, kaempferol's cytotoxicity was evaluated using mouse fibroblast cells L929. As shown in Figure 4, the histograms of proliferation and growth of L929 cells cultured for three days in media containing kaempferol at concentrations ranging from 0 to 100 μg/ml indicate that kaempferol has no cytotoxicity to the cells in this concentration range.

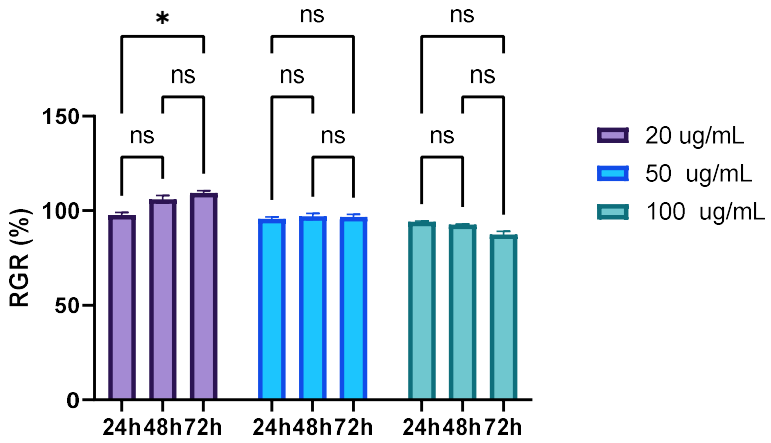

Figure 4. Histogram of the effect of kaempferol on L929 cell cytotoxicity (*p<0.05)

5.2 Effects of Kaempferol Hydrogel on Cell Proliferation

The viability of the hydrogel on L929 cells was examined using a hydrogel extract (CCK-8). Following incubation of the hydrogel extract with L929 cells for 1, 2, and 3 days, no discernible impact on cell proliferation was observed. Cell viability remained near 100%, with no notable differences between groups (Figure 5). These findings indicate that kaempferol hydrogel exhibits minimal toxicity to cells and demonstrates favorable biocompatibility.

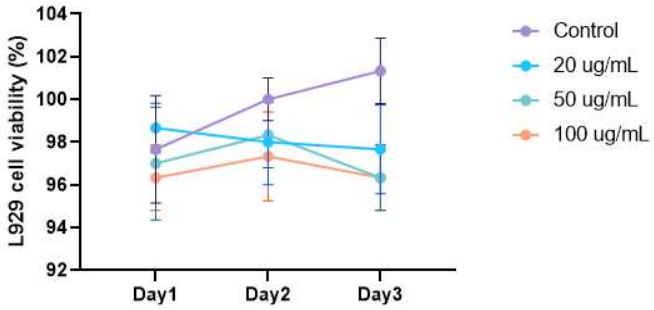

Figure 5. Viability of L929 fibroblasts after incubation with kaempferol hydrogel extract

5.3 Effects of Kaempferol Hydrogel on Cell Apoptosis

To further elucidate the effect of kaempferol hydrogel on cells, we utilized hydrogel extract to incubate L929 cells for 48 hours, subsequently employing 7-AAD and Annexin V to detect cell apoptosis. The experimental results demonstrate that the proportion of 7-AAD+ Annexin V+ cells exhibited a slight increase at a concentration of 100 μg/mL (Figure 6) following the action of the kaempferol hydrogel extract. However, this increase was not statistically significant, indicating that the hydrogel has no significant effect on cell apoptosis. It substantiates the hypothesis that the hydrogel causes minimal damage to cells.

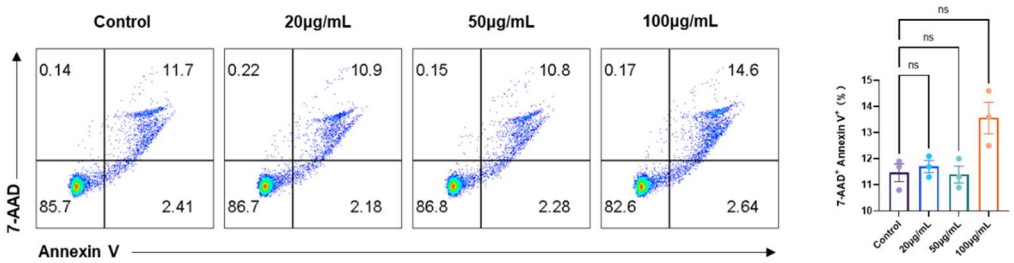

Figure 6. L929 Apoptosis detection of fibroblasts after 48 h incubation with kaempferol hydrogel extract

5.4 Effects of Kaempferol Hydrogel on Cell Migration

In addition, cell migration experiments demonstrated that the migration rate of kaempferol hydrogel to intermediate scratches was between that of complete medium and DMEM medium alone (Fig.7). The results show that kaempferol hydrogel can enhance the growth of L929 cells more effectively than DMEM alone. However, it is not as effective as a complete medium. The porous microstructure of kaempferol hydrogel can promote the proliferation and migration of L929 cells.

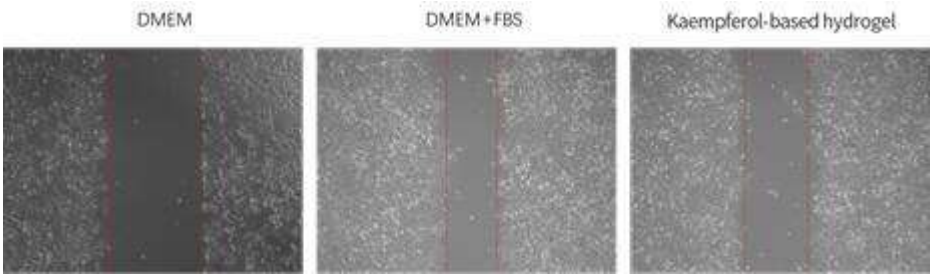

Figure 7. L929 fibroblast migration

5.5 Effect of Kaempferol Hydrogel on Antibacterial Effect

The OD values of kaempferol at different concentrations on E.coli are shown in Table 2 and Figure 8. When the concentration of EPL was ≥ 100ug/mL, the OD value was about 0.04, showing a significant inhibitory effect on drug-resistant bacteria.

Table 2. The initial OD value of E.coli at 630 nm after 24 hours of culture

Group	Control	20	50	100
1	0.296	0.255	0.203	0.040
2	0.301	0.227	0.229	0.044
3	0.299	0.268	0.215	0.043

(Kaempferol concentration: ug/mL)

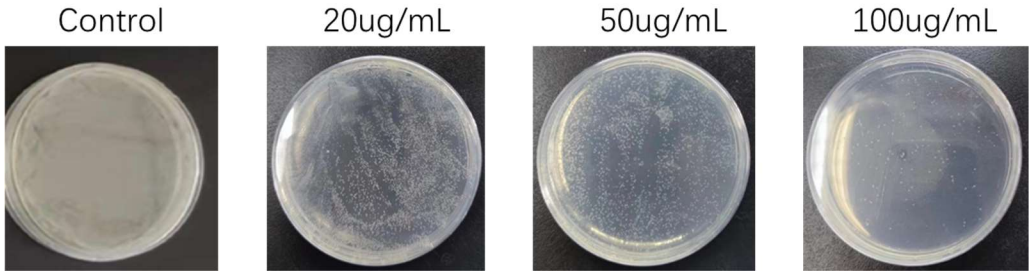

Figure 8. Antibacterial effect of kaempferol

5.6 Effects of Kaempferol Hydrogel on Wound Healing

We quantitatively assessed the wound area of mice on days 4, 8, and 12 during the healing process to further evaluate the benefits of kaempferol hydrogel in promoting in vivo wound healing. On the fourth day of healing, the wound areas of the mice in all groups were closed and smaller (Figure 9). On the eighth day, the mice coated with kaempferol hydrogel had a higher healing rate than the control group, which could be attributed to the better ability of kaempferol hydrogel to promote healing during the healing process. It shows that the skin wound environment that is too dry is not conducive to wound healing. On the 12th day of wound healing, the wounds of mice in the PVA+Kae group were almost completely healed.

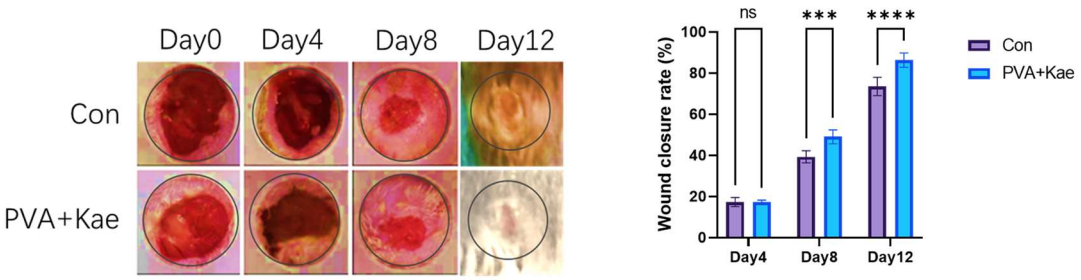

Figure 9. Wound healing process (***p<0.001, ****p<0.0001)

5.7 Characterization of Kaempferol Hydrogel

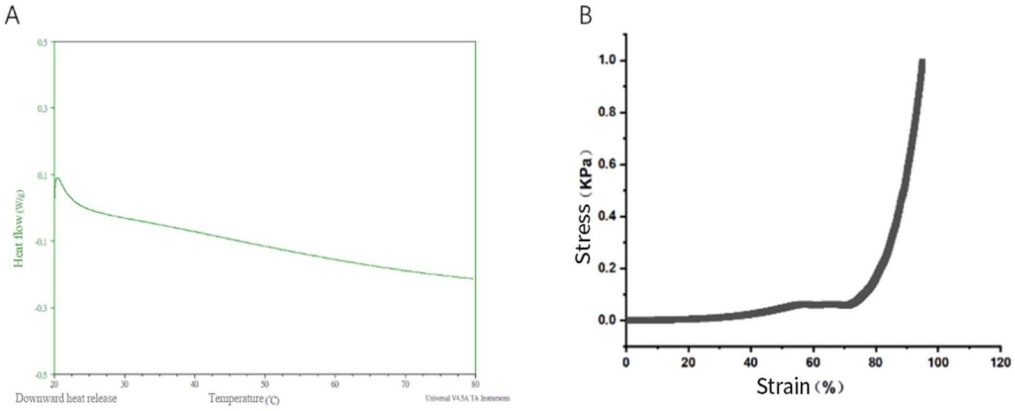

Figure 10. Characterization of kaempferol hydrogel

Rheological and mechanical testing was conducted to ascertain the characterization properties of kaempferol hydrogel and gain a comprehensive insight into its physical properties. The test results demonstrate a consistent decline in the heat flow value of the hydrogel as the temperature rises (Figure 10A). Moreover, mechanical characterization shows that the stress value increases rapidly when the strain value reaches about 72% (Figure 10B). It shows that this new hydrogel can maintain a gel-like solid state at different temperatures and is suitable for external wound repair. Moreover, mechanical testing results show that the hydrogel has good plasticity.

5.8 Summary of Experimental Results

Kaempferol hydrogel is beneficial for wound healing because it can accelerate wound healing, cause little damage to cells, and promote cell migration. Further research has shown that kaempferol hydrogel also has antibacterial effects and can significantly inhibit the growth of E.coli. In addition, kaempferol hydrogel has the advantages of being less susceptible to temperature fluctuations and having high plasticity.

6. Conclusion

This paper introduces a hydrogel prepared from polyvinyl alcohol (PVA). The hydrogel has good biocompatibility and physical and chemical properties. It is suitable for moist conditions and provides wound covering protection. Moreover, it can also function as a carrier for medications, allowing for a gradual release of drugs. We introduced the natural plant ingredient kaempferol to improve the wound environment and accelerate healing. Kaempferol has various biological activities, such as anti-inflammatory, anti-cancer, antibacterial, and antioxidant, but it has not yet been used in trauma treatment.

This research prepared and characterized the properties of kaempferol composite hydrogel through experiments, verified its antibacterial properties, and evaluated its effect on repairing full-thickness skin defects in mice. Research results indicate that kaempferol composite hydrogel positively affects cells and possesses antibacterial properties, promoting the healing of full-thickness skin defects. In experiments to assess the properties of kaempferol hydrogel, we confirmed its rheological and mechanical characteristics, demonstrating its durability and strong plasticity.

In conclusion, this paper presents a promising new wound treatment method. By creating a hydrogel composed of polyvinyl alcohol and kaempferol, we have achieved the dual benefits of moist protection and drug release, potentially accelerating wound healing. The research findings open up exciting possibilities for enhancing wound treatment and healing speed. They also lay a solid foundation for future research and clinical application.

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