Construction of Injectable Tetra-Polyethylene Glycol Hydrogel Adhesive for Wound Healing

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Abstract. Hydrogel wound dressings are medical materials that accelerate wound healing by maintaining a moist environment, promoting tissue repair, and absorbing exudate. Its core efficacy is based on the material's high-water content (typically 60%-90%) and biocompatibility, which can provide ideal repair conditions for wounds while reducing the risk of complications. In this work, we constructed a class of tetra-polyethylene glycol hydrogel systems based on rapid chemical reactions, with a fast gelation rate, significant mechanical strength, and sufficient adhesion. Cytotoxicity tests demonstrated that the hydrogels had good biocompatibility, the hydrogel adhesives did not cause secondary damage to the surrounding tissues, and could promote tissue repair and regeneration, providing ideas for the development of a new generation of bioadhesives.

Keywords: Hydrogel, strong tissue adhesion, microstructure, mechanical properties, tissue binder.

1. Introduction

Soft tissue injuries are most common in areas such as skin and mucous membranes, and their repair mainly relies on suturing with stitches or staplers to bring the two ends of the wound closer and close. However, the mechanical modulus of sutures and nails is far greater than that of human soft tissues, and secondary damage to the surrounding tissues and additional pain are inevitable. In addition, the gaps between the sutures or nails can still be weak points of the wound, and some patients have severe scarring due to obvious rejection reactions [1]. For softer or more vulnerable organ injuries such as liver, spleen, kidney, etc., sutures or staplers cannot be used; instead, dressings can be applied for a short period of time to stop the bleeding. Dressings are currently one of the most common means of treating chronic trauma in clinical practice, providing temporary protection of the wound, stopping bleeding, and preventing contamination [2]. The most commonly used and longest-lasting dressings to date are still gauze products, but these traditional dressings have many significant drawbacks, such as hindering the epithelialization of tissue cells and delaying wound healing. The fibers of the dressing are prone to shedding, causing foreign body reactions and affecting healing. The granulation tissue of the wound is prone to grow into the mesh of the dressing, causing pain during dressing changes and secondary injury [3]. Poor bacteriostatic effect and easy passage of pathogens causing infection, etc. When the injury is combined with other special circumstances, existing treatments often lack effective approaches, especially for bleeding and infection. Gauze compression for hemostasis is currently the most common trauma hemostasis method. Although it has some hemostasis effect, it also poses the risk of tissue damage and difficulty in removal. For injuries with a higher risk of infection, the internal fixation material may provide a favorable environment for microbial colonization [4-6]. At present, the incidence of injuries with severe bleeding or infection risk remains high, and existing treatments are difficult to integrate hemostasis, antibacterial, healing promotion and other functions for the treatment of complex wounds.

Tissue adhesives have been extensively studied and developed as an effective approach to addressing these clinical challenges. The tissue adhesives currently used in clinical practice mainly include fibrin adhesives and cyanoacrylate products. These tissue adhesives are easy to operate, avoid secondary damage, relieve patient pain, and partially replace the function of internal fixation materials. However, there are still many flaws in the existing medical tissue adhesives that need to be addressed [7]. Due to the close contact with the human body, medical adhesive materials need to have good biocompatibility and degradability, be completely degradable or the degradation products can be

excreted from the body through metabolism, avoid immune rejection reactions, and ensure the safety of use [8]. In addition to the above-mentioned properties, the production cost should not be too high in order to improve the feasibility of clinical use, and the ability to achieve mass production is also a key point of research and development.

An ideal wound repair material must have a series of remarkable features, such as rapid coagulation ability, maintaining a suitable moist environment for the wound, allowing for oxygen exchange, adsorbing wound exudate, accelerating wound closure, reducing pain, and preventing infection, etc. Currently, in addition to traditional sterile gauze and absorbent cotton, many functional materials have been developed for wound repair, Such as nano-silver, chitosan, alginate, collagen, polyurethane, etc. Although these materials play some positive role in wound repair, the application of these dressing products is subject to many limitations such as single function, complex preparation process, high cost, poor physicochemical properties, and potential toxicity. Therefore, the exploration and development of ideal wound repair materials have always been a hot and difficult point in the field.

Hydrogels, with their three-dimensional reticular matrix structure similar to ECM and mechanical properties similar to the elasticity of skin and soft tissue, can induce cell adhesion, migration and ECM deposition through mechanical action, thereby promoting wound repair. Microstructural parameters of hydrogels, such as water content, crosslinking density, mesh size, etc., can directly affect the biological functions and behaviors of cells, thereby influencing skin wound repair. Because hydrogels are hydrophilic polymers that form a stable cross-linked structure network after absorbing a large amount of water, physical isolation and moisture retention are the most fundamental and important functions for them to become wound dressings. The moist environment of the wound can reduce the dehydration of tissue cells, maintain cell proliferation and migration activity, maintain a good microenvironment for wound healing, slow the progressive deepening of the wound, etc. One of the basic requirements approved by the U.S. Food and Drug Administration for wound repair hydrogel products is good moisture retention and hydration capacity [9, 10]. The commonly used hydrogel dressings in clinical practice (such as IntraSite®Gel, Flamigel®, Purilon®Gel, etc.) have significant effects in maintaining the wet balance of wound healing, promoting autolytic debridement and wound healing, and reducing dressing change pain compared to traditional dressings (such as gauze, absorbent cotton, etc.). Studies have shown that protein-based polymers (such as gelatin, collagen, oligopeptides, etc.) have limited solubility, which reduces the ability of their hydrogel scaffolds to retain moisture for a long time, while water-soluble polysaccharides (such as hyaluronic acid, chitosan, sodium alginate, etc.) and their derived polymers are more widely used in wound repair due to their excellent long-term moisture retention ability. The moisturizing effect of wound dressings is determined by the rate at which water evaporates in the local environment of the wound, that is, the water vapor transmission rate [11,12]. Therefore, the moisture retention effect of the hydrogel can be regulated by adjusting its water vapor transmission rate, but a hydrogel with an appropriate water vapor transmission rate should be selected based on the type of wound, the amount of exudate from the wound, and the stage of repair, in order to better promote the healing effect of the wound. Some scholars have directly applied an unshaped hydrogel prepared from hydroxy-methyl cellulose and propylene glycol to the dermabrasion scabs after deep second-degree burns in clinical practice and observed that the hydrogel has a significant moisturizing effect, can significantly reduce the adhesion of the dressing to the wound and patient pain during dressing changes, increase the wound healing rate, reduce the wound infection rate and surgical skin grafting rate, and reduce the number of dressing changes. Shorten the complete healing time of the wound and reduce scar hyperplasia.

The most important function of tissue adhesives is to provide sufficient bonding strength between tissue interfaces, allowing tissues to close and heal without the need for internal fixation materials. The greater challenge lies in the fact that the environment to be bonded is often accompanied by bleeding or exudation during actual use, which requires that the bonding process of medical adhesives can be carried out on wet surfaces or in a water environment, that is, with wet availability [13]. Although many studies are focused on developing materials that meet these functional requirements, there is currently no tissue adhesive that can simultaneously have good biocompatibility

and wet surface adhesion, and the development of such materials is extremely urgent [14]. Therefore, based on the physiological characteristics of skin damage, this project constructed tissue adhesive systems through chemical modification methods and designed and prepared a class of tetra-polyethylene glycol (Tetra-PEG) hydrogels with injectable and strong tissue adhesion properties (Figure 1). Due to the rapid cross- linking reaction of the amines and active esters of the tetra-polyethylene glycol, when the gel precursor is in contact with the tissue surface simultaneously, the active esters at the end of the tetra-polyethylene glycol can easily chemically bond with the amines on the tissue surface, thus having excellent mechanical strength, strong tissue adhesion and biocompatibility, which can meet the requirements of rapid adhesion and effective repair of animal skin injury wounds.

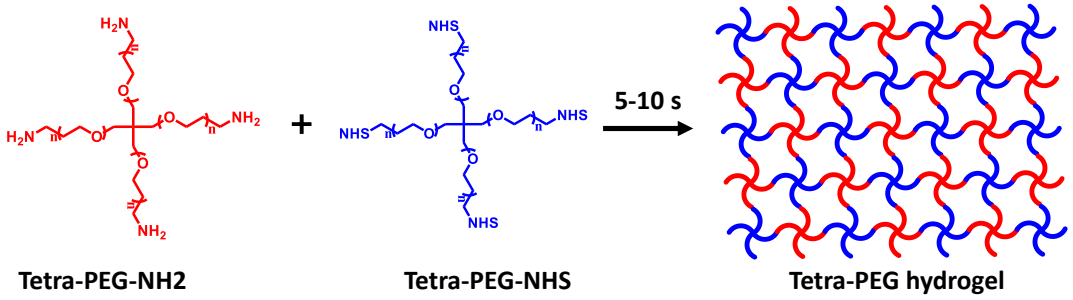

Figure 1. Preparation route of the medical hydrogel adhesive

2. Experimental Section

2.1. Main ingredients and reagents

Tetra-polyethylene glycol active esters (Tetra- PEG-NHS, M_w=10 kDa, M_w/M_n = 1.03) and tetra-polyethylene glycol amines (Tetra-PEG-NH_2, M_w=10 kDa, M_w/M_n = 1.02) were purchased from Xiamen Sinobangge Biotechnology Co., LTD., China.

2.2. Preparation of Tetra-PEG hydrogel

A series of tetra-polyethylene glycol hydrogels with strong water absorption, good histocompatibility and strong tissue adhesion can be constructed by mixing two different functionalized polyethylene glycols (tetra-polyethylene glycol amines and tetra-polyethylene glycol active esters) in equal molar ratios.

2.3. Scanning Electron Microscopy

Scanning electron microscopy (SEM) tests were conducted on a JSM-6700F microscope (JEOL Corporation, Japan) at an accelerated voltage of 5kV. The prepared Tetra-PEG hydrogels were freeze-dried in a freeze dryer at -80 °C for 3 days, and the freeze-dried gels were sputtered with Pt layers in an E-1010 ion sputtering instrument before testing for 120 s.

2.4. Compression performance

Compression tests were conducted on a universal tensile testing machine (3365 Instron, USA) to prepare Tetra-PEG hydrogels in a cylindrical mold (10 mm in diameter, 4 mm in height). The compression test rate was set at 3 mm/min until the sample broke (n=3). The compressive stress is calculated by dividing the force applied by the cross- sectional area.

2.5. Adhesion performanc

Tetra-PEG hydrogels were prepared in situ on pigskin samples and stained with Rhodamine B. After waiting for 2 min, the pigskin samples were subjected to operations such as twisting and squeezing to test the gel's adhesion to the skin.

To quantitatively determine the adhesion strength of the Tetra-PEG hydrogel to the skin, two 2 cm wide pigskin samples were selected. Tetra-PEG hydrogel was prepared in situ in the 2×2 cm area at the front end of the samples, and the two samples were overlapped with each other, with the overlapping area being the same as the gluing area. After 10 min of light pressing, the test was conducted using a universal tensile testing machine (3365 Instron, USA). All tests were conducted at a constant tensile rate of 50 mm/min. Adhesion strength is determined by the maximum force value divided by the adhesion area. Use commercially available fibrin glue as a control group for comparison.

2.6. Cytotoxicity

Cytotoxicity of Tetra-PEG hydrogel degradation products was evaluated using mouse fibroblasts (NIH-3T3) by CCK-8 assay. NIH-3T3 cells were cultured at 37 °C in DMEM medium containing 10% fetal bovine serum with a 5% concentration of CO_2 in the incubator. After inoculating the cells into 96-well plates at a density of 10,000 cells per well and culturing for 24 h, the medium in each well was replaced with 200 μL of fresh medium and 20 μL of degradation solution was added to achieve final concentrations of 0.01, 0.1, and 1 mg/mL of degradation solution. After culturing again for 24 hours, aspirate the supernatant, add 100 μL of fresh medium and 10 μL of CCK- 8 to each well, and then culture for another 3 hours. Measure the absorbance of the medium in each well at 450 nm. Cell viability was calculated by comparing absorbance with that of the control group and untreated cells.

The survival rate of NIH-3T3 cells was also visually characterized by life-and- death staining. Cells were stained two days after inoculation using the Calcein-AM/PI (propidium iodide) assay kit. Morphology of the cells was observed using an inverted fluorescence microscope.

3. Results and Discussion

3.1. Preparation of hydrogels

A simple mixture of the two components can form a Tetra-PEG hydrogel in a very short time, which is injectable, easy to use, and convenient for in-situ implantation or spraying, as shown in Figure 2. This gel-forming method is simple and easy to operate, which greatly saves preparation time and is beneficial for wound sealing in emergency situations.

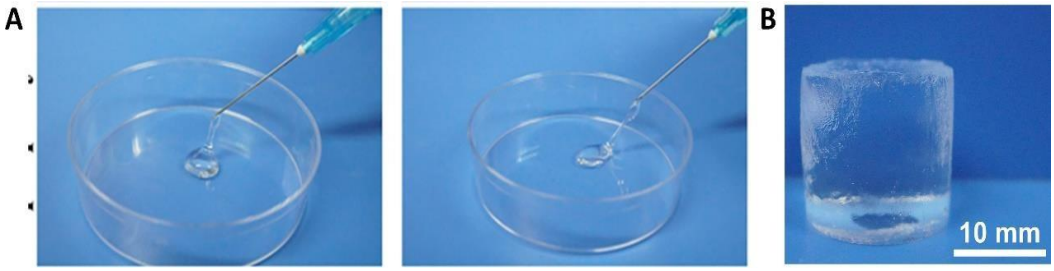

Figure 2. Injectability and formability of the hydrogel

3.2. Structural and mechanical characterization of hydrogels

Tetra-PEG hydrogels exhibit uniform porous structures and excellent mechanical properties due to their ability to form ideal network structures. The Tetra-PEG hydrogel was freeze-dried and the micromorphology of the freeze-dried gel was observed under a scanning electron microscope. As shown in Figure 3A, the freeze-dried Tetra-PEG presented a loose and porous structure with a relatively uniform pore structure; Secondly, compression tests were used to evaluate the mechanical strength of the Tetra-PEG hydrogel, as shown in Figure 3B, with a compressive strength of up to 11.3 MPa, demonstrating excellent for a hydrogel to act as a precursor to a binder is that it has sufficient tissue adhesion capacity. We prepared Tetra-PEG hydrogels in situ on the surface of pigskin. Theoretically, the active ester groups in the gel could chemically bond with the amino groups on the skin surface to form an effective adhesion. As shown in Figure 4, the Tetra-PEG hydrogel adheres tightly to the pigskin and does not detach when bent or twisted.

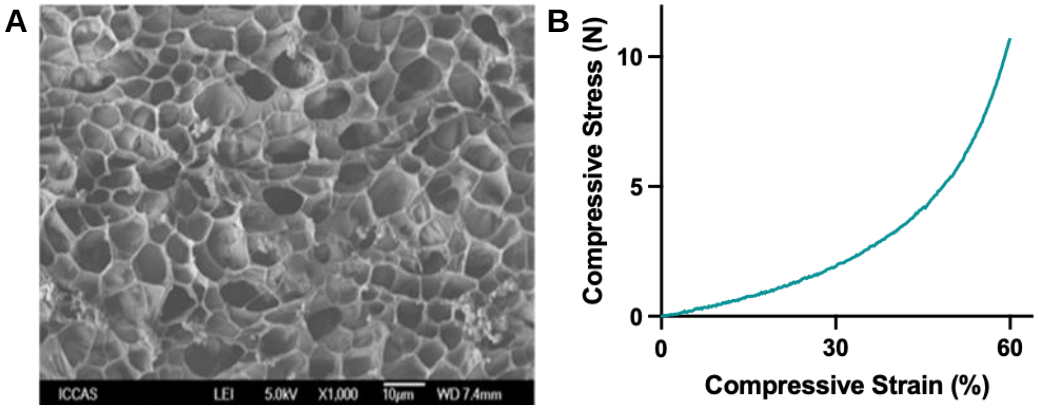

Figure 3. Microstructure and mechanical compression properties of hydrogels the condition

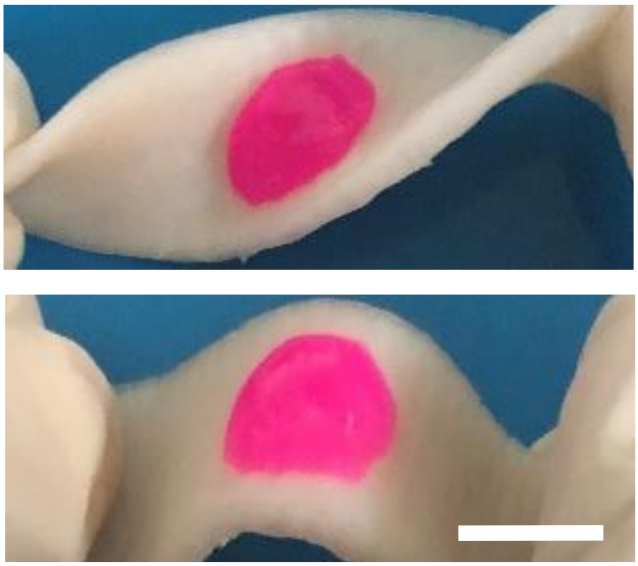

Figure 4. Tetra-PEG hydrogel adhesion to pigskin, scale =1 cm

In addition, the adhesion strength of Tetra-PEG hydrogel was quantitatively evaluated using a universal tensile testing machine. First, we selected two pigskin splines, as shown in Figure 5A, and prepared the Tetra-PEG hydrogel in situ in the overlapping area of the spline lap. After waiting for 10 minutes, we stretched the splines up and down with a universal tensile testing machine until the splines separated. As shown in Figure 5B, Tetra-PEG hydrogel has superior adhesion strength to the surface of pigskin compared to commercially available fibrin gel, which is conducive to subsequent wound adhesion experiments.

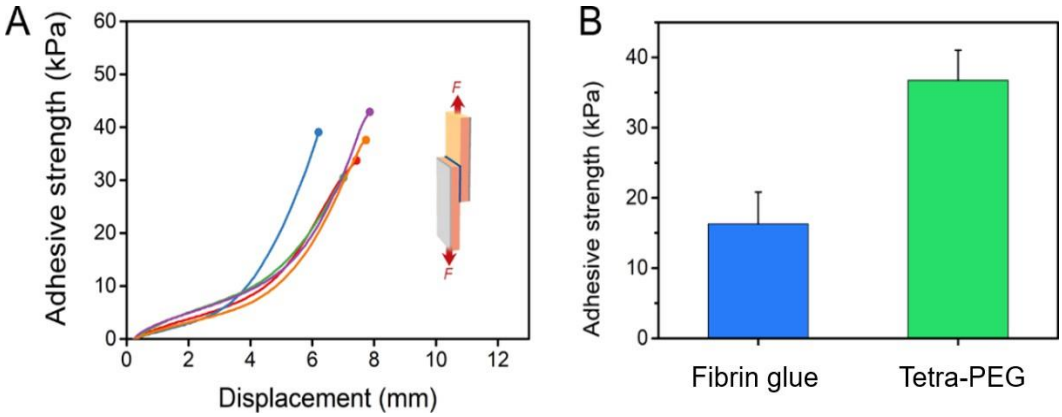

Figure 5. (A) Tetra-PEG hydrogel adhesion curve to pigskin. (B) The adhesion comparison of Tetra-PEG hydrogel and fibrin gel.

3.3. Cellular safety of the hydrogel

Safety and efficacy are important indicators for evaluating biomaterials. Before conducting experiments on the effectiveness of wound adhesion, we verified the safety of Tetra-PEG hydrogel from the dimensions of cell compatibility, tissue compatibility, etc. We first co-cultured the self-degrading products of the Tetra-PEG hydrogel with mouse fibroblasts (NIH-3T3) cells. After 24 hours of culture, cell viability was still above 90% when the degradation product concentration was up to 10 mg/mL compared with the blank PBS solution (Figure 6A). In addition, we also demonstrated the excellent cytocompatibility of the Tetra-PEG hydrogel using live/dead staining experiments (Figure 6B).

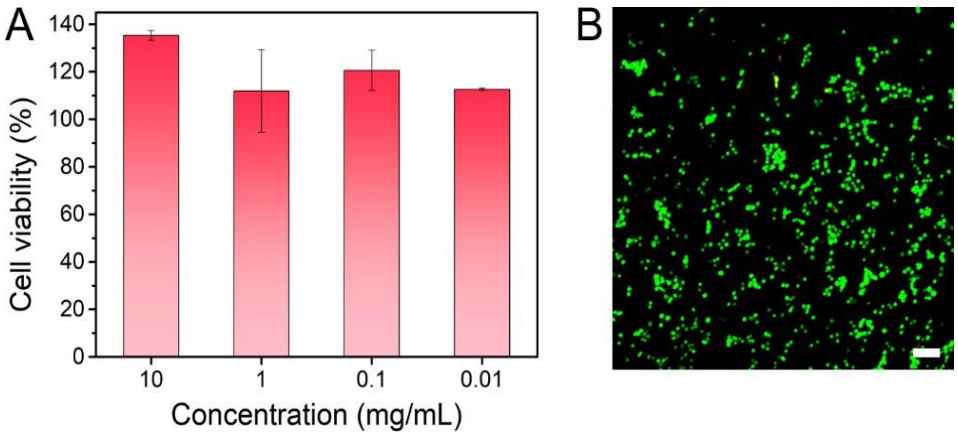

Figure 6. (A) CCK8 detection of cytotoxicity of Tetra-PEG hydrogel degradation products. (B) Live/dead staining photographs of cells co-cultured with Tetra-PEG hydrogel degradation products for 24 hours, scale =100 μm

4. Conclusion

This work developed an injectable medical hydrogel adhesive that can be used for the sealing and healing of skin wounds. The main research results are as follows: 1) A class of Tetra-PEG hydrogels with injectable and strong tissue adhesion properties was prepared by constructing a tissue adhesive system through chemical modification. Due to the rapid cross-linking reaction of the amino groups and active esters of the tetra-polyethylene glycol, when the gel precursor comes into contact with the tissue surface, the active esters at the end of the tetra-polyethylene glycol can simultaneously chemically bond with the amino groups on the tissue surface, thus achieving rapid and effective tissue bonding; 2) The Tetra-PEG hydrogel has a fast gelation rate, significant mechanical strength and sufficient adhesion, which makes the hydrogel conducive to rapid wound closure; 3) Cytotoxicity tests demonstrated excellent biocompatibility of Tetra-PEG hydrogel. Therefore, these injectable Tetra-PEG hydrogels, which are easier to handle than surgical sutures and do not cause secondary damage to the surrounding tissues, will develop into a class of highly promising medical adhesives.

References

[1] Hoekstra MJ, Hermans MHE, Richters CD, Dutrieux RP. A histological comparison of acute inflammatory responses with a hydrofibre or tulle gauze dressing. J Wound Care, 2002, 11(3): 113-117.

[2] Khunmanee S, Jeong Y, Park H. Crosslinking method of hyaluronic-based hydrogel for biomedical applications. J Tissue Eng, 2017, 8: 1-16.

[3] Wang C, Niu H, Ma X, Hong H, Yuan Y, Liu C. Bioinspired, Injectable, quaternized hydroxyethyl cellulose composite hydrogel coordinated by mesocellular silica foam for rapid, noncompressible hemostasis and wound healing. ACS Appl Mater Interfaces, 2019, 11(38): 34595-34608.

[4] Matsuda T, Kawakami R, Namba R, Nakajima T, Gong JP. Mechanoresponsive self-growing hydrogels inspired by muscle training. Science, 2019, 363(6426): 504- 508.

[5] Vakalopoulos KA, Wu Z, Kroese LF, et al. Clinical, mechanical, and immunohistopathological effects of tissue adhesives on the colon: An in-vivo study. J Biomed Mater Res B Appl Biomater, 2017, 105(4): 846-854.

[6] Xue H, Hu L, Xiong Y, et al. Quaternized chitosan-matrigel-polyacrylamide hydrogels as wound dressing for wound repair and regeneration. Carbohydr Polym, 2019, 226: 115302.

[7] Hashemnejad SM, Kundu S. Rheological properties and failure of alginate hydrogels with ionic and covalent crosslinks. Soft Matter, 2019, 15(39): 7852-7862.

[8] Li J, Celiz AD, Yang J, et al. Tough adhesives for diverse wet surfaces. Science, 2017, 357(6349): 378-381.

[9] Sun G, Shen YI, Harmon JW. Engineering pro-regenerative hydrogels for scarless wound healing. Adv Healthc Mater, 2018, 7(14): e1800016.

[10] Nuutila K, Eriksson E. Moist wound healing with commonly available dressings. Adv Wound Care, 2021, 10(12): 685-698.

[11] Xu R, Xia H, He W, et al. Controlled water vapor transmission rate promotes wound-healing via wound re-epithelialization and contraction enhancement. Sci Rep, 2016, 6: 24596.

[12] Xu R, Luo G, Xia H, et al. Novel bilayer wound dressing composed of silicone rubber with particular micropores enhanced wound re-epithelialization and contraction. Biomaterials, 2015, 40: 1-11.

[13] Rao P, Sun TL, Chen L, et al. Tough hydrogels with fast, strong, and reversible underwater adhesion based on a multiscale design. Adv Mater, 2018, 30(32): e1801884.

[14] Zhu W, Chuah YJ, Wang D-A. Bioadhesives for internal medical applications: A review. Acta Biomaterialia, 2018, 74: 1 to 16.