ORIGINAL ARTICLE

Efficacy of Iranian Hydrogel on Wound Healing in Rat as an Animal Model

MT Noorbala[1], MH Dashti Rahmatabadi[2]*, F Binesh[3], A Morshedi[2]

[1]Department of Dermatology, [2]Department of Physiology, [3]Department of Pathology, Shaheed Sadoughi University of Medical Sciences, Yazd, Iran

Abstract

Background: Hydrogels are one of the commonly used dressings for chronic ulcers. Nowadays, much therapeutics can be delivered to the wound or circulation by the gels. This study was conducted to evaluate the efficacy of Iranian hydrogel in wound healing in rat as an animal model.

Methods: Thirty four Wistar male rats were anaesthetized and a full thickness circular skin wound was created by excising the dorsum of the rats. The animals were divided into two groups. In the experimental Ir gel group, the wounds were dressed by hydrogel and in the control NS group by conventional moist normal saline gauze. The wound areas were measured on days 1, 3, 7 and 12 for comparing the wound healing rate in the two groups. Laboratory investigation, skin tensile strength and histopathology of the healed tissues were also considered for evaluation.

Results: Mean percentages of wound contraction on the 3rd, 7th and 12th days were not significantly different between the two groups. The differences in skin tensile strength, in the two groups, on days 15 and 30 were not significant. Histopathologically, wound repair was excellent in 6 (60%), good in 3 (30%) and poor in 1 (10%) of specimens in control NS group while these figures were 6 (60%), 4 (40%) and 0 (0%) in the Ir gel group respectively and the difference was not statistically significant.

Conclusion: The effect of Iranian hydrogel effect on wound healing in rats was identical to moist gauzes. As Iranian hydrogel did not have any adverse effects and was easily removed from the wound, it can be safely used for dressing of wounds.

Keywords: Hydrogel; Dressing; Wound healing; Rat

Introduction

Since skin repair in wounds plays an important role in homeostasis, oozing of water, electrolyte and prevention of invasion by microorganisms, the skin generally needs to be covered with a dressing immediately after it is injured.[1] It is widely accepted that a moist environment encourages rapid healing and most modern wound care products are designed to provide these conditions.[2,3] Three categories of wound dressings were reported including biologic, synthetic and biologic–synthetic ones. Alloskin and pigskin are biologic dressings commonly used clinically, but they have some disadvantages including limited supplies, high antigenicity, poor adhesiveness and risk of cross-contamination. Synthetic dressings have a long shelf life, inducing minimal inflammatory reaction and carry almost no risk of pathogen transmission.[4] In recent years, researchers have focused on biologic–synthetic dressings, which are bilayered and consist of high polymer and biologic materials.[5-7] These three categories of wound dressing are all used frequently in the clinical settings, but none is without disadvantages. An ideal dressing should maintain a moist environment at the wound interface, allow gaseous exchange, act as a barrier to microorganisms, and remove excess exudates. It should also be non-toxic, non-allergenic, non-adherent and be easily removed without trauma. Furthermore, it should be made from

***Correspondence:** Mohammad Hossein Dashti-Rahmatabadi, PhD, Department of Physiology, Shaheed Sadoughi University of Medical Sciences, Yazd, Iran. Tel: +98-351-8241751-5, Fax: +98-351-8247084, e-mail: dashti-r@ssu.ac.ir
Received: January 16, 2009 Accepted: May 17, 2009

a readily available biomaterial that requires minimal processing, possesses antimicrobial properties and promotes wound healing. A large number of reports denote to new, improved wound dressings by synthesizing and modifying biocompatible materials.[8-10]

Recently, Yazd Radiation Application Research School in Iran has made a cross-linked polymerized hydrogel dressing composed of distilled water, Poly N-vinyl pyrrolidone, Poly ethylene glycol and agar, and shaped into sheets for wound dressing.[11] This study was conducted to evaluate the wound healing efficacy of this Iranian hydrogel in experimental rats.

Materials and Methods

In this study, 34 male Wistar rats aged 3 months and weighing 200–250 g were obtained from the Shaheed Sadoughi Medical University Animal House. For experimental procedures, permission was provided from the Animal Ethics Committee of Shahid Sadughi University of Medical Sciences inYazd, Iran, in accordance with the internationally accepted principles for laboratory animal use and care mentioned by the European Community guidelines. The animals were anesthetized with diethyl ether and their dorsal hairs were shaved with an electric razor. The area was sterilized with 70% ethanol. A full thickness circular skin wound of 1 cm in diameter was created by excising the dorsum of the rats and it was disinfected, using 70% ethanol.

For wound dressing, the animals were randomly and equally divided into 2 groups (17 rats per group). In the control group, the wounds were covered with conventional sterile gauze humidified by sterile normal saline (NS group), while in the Experimental group, the wounds were dressed with Iranian hydrogel sheet (Ir gel group). All the dressing specimens were of equal size, i.e. 1.5 cm × 1.5 cm. In order to fix the dressings, a piece of elastic adhesive bandage was applied on the top of the wound dressings and the treated rats were placed in individual cages. All the dressings were changed daily in the morning. The rectal temperature and wound area were measured in the 1st, 3rd, 7th and 12th days of the experimental procedures. The degree of healing was expressed as the wound contraction ratio (WCR):

$$\mathrm{WCR} = \frac{A_0 - A_t}{A_0} \times 100\%$$

Where A_0 and A_t are respectively the initial area and the wound area after the application of the dressings (e.g. 3rd, 7th or 12th days after wound dressing period).[12-14] At the end of the experiment, when the wound healing was completed, on days 15th and 30th, five rats in each group were randomly selected and deeply anesthetized by excess diethyl ether and a blood sample was taken. Then, the animals were scarified under diethyl ether overdose. The cured wound tissue (1 cm × 3 cm) including the entire wound area with adjacent normal skin was excised for tensile strength measurements and histopathological examinations.

Fifteen and 30 days after wound creation, each time, five rats in each group were randomly selected for scarification under deep anesthesia and rectangular skin pieces measuring 1 cm × 3 cm were provided. We incized scar lines transversely at the middle of the longitudinal axis and sectioned them in clamps for a mechanical testing device (LRX Material Testing Machine, Lloyd Instruments, 1987, Fareham-Hants, UK). Distraction was performed at a rate of 10 mm/min. The breakdown length was recorded in mm.[15] All tests were performed by the same investigator and monitored by a second investigator to avoid any false negative results from minor inaccuracies in the placement of the skin pieces.

All the broken specimens derived from the tensile strength tests were fixed in a 10% formalin-saline solution for 24 h. Formalin-fixed biopsies were dehydrated in a graded alcohol series, using an overnight tissue processing schedule (Hypercenter XP, Shandon Inc., Pittsburgh, PA, USA). All of the tissues were embedded in paraffin and sectioned at a thickness of 4–6 μm (M1R rotary microtome, Shandon Inc.). After tissue processing, the sections were stained with hematoxylin and eosin (Varistain 24-4, Shandon Inc.). The stained sections were examined semi-quantitatively under a light microscope (Olympus BX50, Japan). The slides were examined and the following positive indices of wound healing parameters were assessed including the integrity of the epithelial surface (partial or complete), presence of inflammation (slight, mild, severe), fibrosis (presence or absence), and granulation tissue (presence or absence). Wound repair was evaluated, using these parameters and each slide was rated as "excellent" "good" or "weak" repair.[15,16]

Blood samples were taken from the heart and sent to YAZD Central Lab for routine laboratory tests. To compare the histopathologic findings, the chi-square test was performed. The t Student test was used for the statistical analysis of wound contraction ratio, body temperature and wound tensile strength. Two-tailed probabilities $\leq$ 0.05 were considered significant. The data were analyzed, using a statistical software package (SPSS 11.0 for Windows, Chicago, IL, USA).

Results

During the experimental procedure, two rats in the NS group and one in Ir gel group died due to anesthesia complications but there were no differences among the groups in terms of the survival rate. There were no significant changes in the mean body temperature among the NS and Ir gel groups (37.0±0.4 vs. 36.9±0.45, *p*=0.842). The mean percentage of wound contraction on the 3rd, 7th and 12th days in the NS and Ir gel groups were 25.2±22.0 vs. 22.4±12.8, 70.9±13.8 vs. 70.7±14.6 and 97.9±2.4 vs. 95.5±9.7, respectively and there was no significant difference among the two groups (*p*=0.517, *p*=0.977, and *p*=0.491, respectively.

The WCR of Ir gel was lower than that of NS after 3 and 12 days, but this difference was not statistically significant. The mean maximum possible tensile length of the skin specimens in the region of the healed wound (breakdown length) as an index for wound tensile strength on day 15 was 33.4±10.8 mm in the NS group and 27.8±9.8 mm in the Ir gel group and the difference was not significant (*p*=0.421). On day 30, there was also no significant deference in the index of tensile strength among the NS and Ir gel groups (37.4±7.2 vs. 35.6±10.2, respectively, *p*=0.956).

Wound repair was excellent in 6 (60%), good in 3 (30%) and poor in 1 (10%) of specimens in NS group while these figures were 6 (60%), 4 (40%) and 0 (0%) in the Ir group respectively. Our results showed that after 12 days, most of the wounds healed and after 15 days, the WCR was 100% in all animals. In contrast to Ir gel group, hemorrhage on the skin wound was observed on the third day and scab was present on the 7th day in some NS group, indicating that their adhesive property on wound surface was more than Ir gel group.

Statistical analysis did not show any significant differences in wound healing pattern between the two groups (*p*=0.912). Blood parameters examined in all blood samples were within the normal limits in both groups.

Discussion

According to Huang *et al.*, epithelialization is retarded by the dry scab and can be accelerated if the wound is kept moist.[17] However, the WCR of Ir gel was lower than that of NS after 3 and 12 days, but this difference was not statistically significant. Hydrogel dressings are cross-linked polymer gels that provide and maintain a moist wound environment which prevent the secondary damage when dressings are changed and also accelerate epithelialization and more easily keratinocytes migration.[18] In this study, we used moist gazes in NS group, so wound healing rate in the 2 groups was nearly identical in terms of the moisture factor. Another important characteristic of wound dressings is the absorption property for wound exudates.[1] Since the WCR in Ir gel group was the same as that in the NS group, it seems that Ir gel which is mainly composed of distilled water could further absorb a moderate amount of wound exudates by swelling. In the present study, there was no elevation in the body temperature or any abnormality in blood examinations, indicating that Ir gel is as safe as moist gauze with normal saline.

According to Eisenbud *et al*.[19], several experimental studies showed beneficial effects of hydrogel dressings, especially in chronic human ulcers (such as diabetic ulcers and bed sores) and they have supplanted saline-moistened gauze for many applications. An additional useful characteristic of hydrogels is gradual delivery of some drugs or growth factors to the injured tissue which facilitates the healing.[20,21]

In this study, we also evaluated the tensile strength and histopathology of the healed tissues. Wound tensile strength is largely dependent upon normal fibroblast function and collagen formation and accumulation.[22,23] Fibroblast dysfunction during wound management as a result of dressing materials will lead to delayed wound contraction and weak tensile strength.[24]

In the present study, there was no statistically significant difference between the two groups concerning the histological pattern and skin tensile strength. As Iranian hydrogel did not have any adverse effects and was easily removed from the wound, it can be safely used for dressing of wounds.

Conclusion

According to our findings, Iranian hydrogel (Ir gel) did not show any adverse effect on wound healing in rats and its efficacy was at least comparable to moist gauzes. But it was non-adherent and could be removed without trauma to the wound. Additionally, this hydrogel did not have any toxicity, allergic effect or pyrogenicity in this study. So, we recommend further studies regarding its effect on wound healing in human skin, especially burns and chronic ulcers such as diabetic ulcers or bed sores.

Acknowledgements

The authors are grateful to Yazd Radiation Application Research School, for the free supply of Ir gel. This study was financially supported by research vice chancellor of Shaheed Sadoughi Medical School, Yazd, Iran. We would also like to thank A.S. Salami for assisting the experimental procedure.

Conflict of interest: None declared.

References

1 Obara K, Ishihara M, Ishizuka T, Fujita M, Ozeki Y, Maehara T, Saito Y, Yura H, Matsui T, Hattori H, Kikuchi M, Kurita A. Photocrosslinkable chitosan hydrogel containing fibroblast growth factor-2 stimulates wound healing in healing-impaired db/db mice. *Biomaterials* 2003;**24**: 3437-44. [12809772] [doi:10.1016/S0142-9612(03)00220-5]

2 Winter GD. Formation of the scab and the rate of epithelialization of superficial wounds in the skin of the young domestic pig. *Nature* 1962; **193**:293-4. [14007593] [doi:10.1038/193293a0]

3 Barnett SE, Irving SJ. Studies of wound healing and the effect of dressings. In: M. Szycher, Editor, High performance biomaterials, Technonic, Lancaster; 1991, pp. 583-620.

4 Lu S, Gao W, Gu HY. Construction, application and biosafety of silver nanocrystalline chitosan wound dressing. *Burns* 2008;**34**:623-8. [18226459] [doi:10.1016/j.burns.2007.08.020]

5 Bruin P, Jonkman MF, Meijer HJ, Pennings AJ. A new porous polyetherurethane wound covering. *J Biomed Mater Res* 1990;**24**:217-26. [2329116] [doi:10.1002/jbm.820240208]

6 Suzuki S, Matsuda K, Isshiki N, Tamada Y, Ikada Y. Experimental study of newly developed bilayer artificial skin. *Biomaterials* 1990;**11**: 356-60. [2119236] [doi:10.1016/0142-9612(90)90114-6]

7 Matsuda K, Suzuki S, Isshiki N, Yoshioka K, Okada T, Ikada Y. Influence of glycosaminoglycans on the collagen sponges component of a bilayer artificial skin. *Biomaterials* 1990;**11**:351-5. [2400801] [doi:10.1016/0142-9612(90)90113-5]

8 Draye JP, Delaey B, Van de Voorde A, Van Den Bulcke A, De Reu B, Schacht E. In vitro and in vivo biocompatibility of dextran dialdehyde cross-linked gelatin hydrogel films. *Biomaterials* 1998;**19**:1677-87. [9840003] [doi:10.1016/S0142-9612(98)00049-0]

9 Shibata H, Shioya N, Kuroyangi Y. Development of new wound dressing composed of spongy collagen sheet containing dibutyryl cyclic AMP. *J Biomater Sci Polym Ed* 1997;**8**:601-21. [9241584] [doi:10.1163/156856297X00209]

10 Ulubayram K, Nur Cakar A, Korkusuz P, Ertan C, Hasirci N. EGF containing gelatin-based wound dressings. *Biomaterials* 2001;**22**:1345-56. [11336307] [doi:10.1016/S0142-9612(00)00287-8]

11 Ajji Z, Mirjalili G, Alkhatab A, DadaH. Use of electron beam for production of hydrogel dressings. *Radiation Phys Chem* 2008;**77**:200-2. [doi:10.1016/j.radphyschem.2007.05.016]

12 Balakrishnan B, Mohanty M, Fernandez AC, Mohanan PV, Jayakrishnan A. Evaluation of the effect of incorporation of dibutyryl cyclic adenosine monophosphate in an in situ-forming hydrogel wound dressing based on oxidized alginate and gelatin. *Biomaterials* 2006;**27**:1355-61. [16146648] [doi:10.1016/j.biomaterials.2005.08.021]

13 Mi FL, Shyu SS, Wu YB, Lee ST, Shyong JY, Huang RN. Fabrication and characterization of a sponge-like asymmetric chitosan membrane as a wound dressing. *Biomaterials* 2001;**22**:165-73. [11101160] [doi:10.1016/S0142-9612(00)00167-8]

14 Balakrishnan B, Mohanty M, Umashankar PR, Jayakrishnan A. Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin. *Biomaterials* 2005;**26**:6335-42. [15919113] [doi:10.1016/j.biomaterials.2005.04.012]

15 Ozbek N, Guneren E, Yildiz L, Meydan D, Cakir S, Coskun M. The effect of pre-operative conventional and hyperfractionated radiotherapy schedules on wound healing and tensile strength in rats: an experimental study. *Int J Oral Maxillofac Surg* 2005;**34**:185-92. [15695049] [doi:10.1016/j.ijom.2004.05.005]

16 Cotran RS, Kumar V, Robbins SL. Robbins Basic Pathology, 7th ed., WB Saunders, Philadelphia; 2003, pp. 61-78.

17 Huang MH, Yang MC. Evaluation of glucan (polyvinyl alcohol) blend wound dressing using rat models. *Int J Pharm* 2008;**346**:38-46. [17662545] [doi:10.1016/j.ijpharm.2007.06.021]

18 Kim JO, Park JK, Kim JH, Jin SG, Yong CS, Li DX, Choi JY, Woo JS, Yoo BK, Lyoo WS, Kim JA, Choi HG. Development of polyvinyl alcohol-sodium alginate gel-matrix-based wound dressing system containing nitrofurazone. *Int J Pharm* 2008; **359**:79-86. [18440737] [doi:10.1016/j.ijpharm.2008.03.021]

19 Eisenbud D, Hunter H, Kessler L, Zulkowski K. Hydrogel wound dressings: where do we stand in 2003? *Ostomy Wound Manage* 2003; **49**:52-7. [14652421]

20 Fujita M, Ishihara M, Morimoto Y, Simizu M, Saito Y, Yura H, Matsui T, Takase B, Hattori H, Kanatani Y, Kikuchi M, Maehara T. Efficacy of Photocrosslinkable Chitosan Hydrogel Containing Fibroblast Growth Factor-2 in a Rabbit Model of Chronic Myocardial Infarction. *J Surg Res* 2005;**126**:27-33. [15916971] [doi:10.1016/j.jss.2004.12.025]

21 Geer DJ, Swartz DD, Andreadis ST. Biomimetic Delivery of Keratinocyte Growth Factorupon Cellular Demand for Accelerated Wound Healing in Vitro and in Vivo. Am J Pathol 2005;**167**:1575-86. [16314471]

22 Stephens FO, Hunt TK. Effect of changes in inspired oxygen and carbon dioxide tensions on wound tensile strength: an experimental study. *Ann Surg* 1971;**173**:515-9. [5573643] [doi:10.1097/00000658-197104000-00006]

23 Stadler I, Lanzafame RJ, Evans R, Narayan V, Dailey B, Buehner N, Naim JO. 830-nm irradiation increases the wound tensile strength in a diabetic murine model. *Lasers Surg Med* 2001;**28**:220-6. [11295756] [doi:10.1002/lsm.1042]

24 Busti AJ, Hooper JS, Amaya CJ, Kazi S. Effects of Perioperative Antiinflammatory and Immunomodulating Therapy on Surgical Wound Healing. *Pharmacotherapy* 2005;**25**: 1566-91. [16232020] [doi:10.1592/phco.2005.25.11.1566]