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Liposomal gel loaded with pro-xylane intermediate promotes chronic wound healing

Bowen Wu[1], Maomao Wang[1], Ruyan Zhang[1] Jinli Xiao[1] and Yan Xie[2,3*]

[1]College of Clinical Medicine, Ningxia Medical University, Yinchuan, China
[2]Tissue Organ Bank & Tissue Engineering Centre, General Hospital of Ningxia Medical University, Yinchuan, China
[3]School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Brisbane, Australia

Abstract: Chronic wounds are a common and difficult problem in clinics. It is of great significance to develop effective and economical methods to treat chronic wounds. The present study aimed to investigate the effects of liposomal gel loaded with Pro-xylane intermediate on chronic wounds. Rat models of chronic wounds were created and verified. The pro-xylane intermediate (C-β-D-xylopyranoside-n-propan-2-one, PXYI) were encapsulated with liposomes and the liposomes containing PXYI were mixed with Pluronic F-127 gel to obtain PXYI liposomal gel (PXYI-LG). PXYI-LG has been applied to the rat's chronic wounds. The therapeutic effects were evaluated by wound healing rate and wound healing time. Additionally, Alamar Blue was used to detect the effect of PXYI on the proliferation of human skin fibroblasts (HF) and human immortalized epidermal cells (HaCaT). It was not found that PXYI alone could promote the proliferation of HF and HaCaT. From the 12th to the 32nd day, the wound healing rate of PXYI-LG group were significantly higher than that of the normal saline (NS) group ($P<0.05$). The number of days when the wound healing rate reached 90% was significantly shorter in the PXYI-LG group (21.8±1.8) than in the NS group (28.4±1.6) ($P< 0.01$). In summary, the results demonstrate that the PXYI-LG can promote chronic wound healing.

Keywords: Chronic wounds, liposomal gel, pro-xylane intermediate, rat models of chronic wounds, wound healing.

INTRODUCTION

When the normal healing process is affected or interrupted, chronic wounds form. Wounds that are not repaired according to the normal process within 4-8 weeks are considered as chronic wounds (Izadi and Ganchi, 2005). Statistically, 1%-2% of the world's population has been affected by chronic wounds (Sen *et al*., 2009). Chronic wounds can affect the quality of life as severely as kidney and heart diseases. The mortality of some patients with chronic wounds is now comparable to that of cancer patients (Armstrong *et al*., 2007). Chronic wounds mostly occur in the elderly population, which deprive patients of labor and consume a lot of medical resources, causing a huge burden on society (Olsson *et al*., 2019). The treatments of chronic wounds mainly include surgical treatment, traditional drug therapy, biological therapy, physical therapy, growth factor therapy, coverage with novel dressings, stem cell therapy, gene therapy, transplantation of tissue-engineered skin, etc. Due to the high cost or side effects, the current treatments for chronic burn wounds are not satisfactory. The treatment of chronic wounds with high efficiency, economy and minimal side effects needs to be continuously explored.

C-xylopyranoside derivatives are a class of synthetic compounds. Studies have shown that C-xylopyranoside derivatives promote the synthesis of glycosaminoglycans (GAGs), enhance the connection between the dermis and epidermis, and increase the content of collagen in the dermis (Pineau *et al*., 2011, Sok *et al*., 2008). The Pro-xylane intermediate (PXYI) is one of the C-xylopyranoside derivatives. Based on the existing studies on C-xylopyranoside derivatives, we hypothesized that PXYI may promote wound healing of chronic wounds.

Liposomes are widely used as drug carriers that have a similar bilayer molecular structure to human cell membranes. The liposome-based drug delivery system has good biocompatibility, also excellent skin penetration, and it is nontoxic and biodegradable (Hua, 2015, Pierre and Dos Santos Miranda Costa, 2011, Wang *et al*., 2020). Liposomes usually exist in the form of suspension. Liposomal gel is prepared by mixing liposome suspension with a gel matrix, which is able to prolong the residence time of the drug in skin (Dawoud *et al*., 2019, Elnaggar *et al*., 2014). In addition, liposomal gel is able to achieve sustained release of drugs locally and improve the bioavailability of drugs (Dawoud *et al*., 2019). Pluronic F-127 (poloxamer 407) is one of the thermosensitive polymers often used in drug delivery systems (Brunet-Maheu *et al*., 2009). Aqueous solutions of Pluronic F-127 of 20-30% (w/w) are liquid at low temperatures (4-5^{o}C), but becomes gels at room temperature (Diniz *et al*., 2015, Kant *et al*., 2014). Therefore, Pluronic F-127 gel can be used as a gel matrix and can be mixed with liposomes to obtain a liposomal gel.

The aim of this study is to investigate the effects of liposomal gel loaded with pro-xylane intermediate on

**Corresponding authors:* e-mails: yanxie.yx@outlook.com

chronic wounds. This study provides a new horizon and idea for treating chronic wounds in the clinic.

MATERIALS AND METHODS

Ethical approval

All experiments involved in this study were approved by the medical research ethics committee of the General Hospital of Ningxia Medical University (KYLL-2021-1005).

Animals

All animals used were treated humanely, and the experimental process complied with the relevant provisions of the "animal health and protection law" and the "code of practice for animal care and use for scientific research purposes". The female SD rats used in the experiment, weighing 250-300g, were purchased from the animal experiment center of Ningxia Medical University. All rats were free to drink water and eat standard diet. The rats were kept in the individual ventilated cages (IVC) at the animal experiment center of Ningxia Medical University.

Preparation of liposome dispersions

Liposome dispersions were prepared by way of a film technique (Bangham *et al*., 1965). Briefly, soy lecithin (Lequan Biotechnology Co., Ltd, Hebei, China) and cholesterol (Sigma, USA) were placed in a flask and chloroform was added to dissolve them.

The chloroform solvent was then removed under reduced pressure using a rotary evaporator, forming a lipid film on the inside wall of the flask. The lipid film was then hydrated using phosphate buffer containing PXYI (Puripharm Co., Ltd, Huzhou, China). The obtained suspension was passed through 1.2μm pore size nylon filter 1 time, 0.45μm pore size nylon filter 1 time, 0.22μm pore size nylon filter 3 times on the extruder, respectively, to obtain the liposome dispersions loaded with PXYI. In the hydration process of above steps, the addition of phosphate buffer without PXYI can obtain the blank liposome dispersions (PXYI free).

Preparation of liposomal gel

Briefly, Pluronic F-127 (Biorab, Beijing, China) was added to distilled water and dissolved by heating and stirring to obtain gel matrix (Kant *et al*., 2014). The liposome dispersion (PXYI free and loaded) was added to the gel matrix and stirred evenly to obtain the liposomal gel (PXYI free and loaded).

Establishment of rat chronic wound models

The animal model of chronic wounds was made by full-thickness burn combined with local injection of Adriamycin.

First, determined the scald time and temperature that can cause full-thickness burns in rats. Rats were anesthetized by intraperitoneal injection of 3% pentobarbital sodium (Jinyao Amino Acid Co., Ltd., Tianjin, China). After skin preparation, four scalded areas were marked on the back of each rat, which were round with an area of 2 cm^2, distributed along both sides of the spine. The rats' skin was scalded for 10s, 15s, 20s, 25s, 30s, 35s, respectively, at 97°C using a scald apparatus (Yiyan Technology Development Co., Ltd, Jinan, China). At each scald time, three wounds were repeated in d6ifferent skin areas of different rats. Twenty-four hours after scalding, rats were euthanized by excessive anesthesia, and skin samples from the scald area were taken and fixed in 10% paraformaldehyde (Servicebio Co., Ltd, Wuhan, China), followed by paraffin embedding and hematoxylin and eosin (H & E) staining was performed to observe the histological morphology of the skin.

Then, verified the validity of the modeling method. After determining the scald time and temperature, six SD rats were randomly divided into two groups (the chronic wound model group and the control group), with 3 rats in each group (4 wounds per rat, a total of 12 wounds). After scalding, rats in the model group were injected with Adriamycin (Meilunbio, Dalian, China) solution (2mg/ml) into scalded wounds at five injection points (wound center, upper edge, lower edge, left edge and right edge), 0.06 ml at each point. The rats in the control group were not treated with Adriamycin solution. Each rat was kept in a separate cage. Each wound was taken photos per week and the wound healing rate was calculated as this formula: (initial wound area - residual wound area)/initial wound area). After 4 weeks (28 days), the rats were euthanized by excessive anesthesia and the wound samples were fixed in 10% paraformaldehyde, followed by paraffin embedding and immunohistochemical staining was performed to observe the expression of Matrix metalloproteinase 2 (MMP-2) and Matrix metalloproteinase 9 (MMP-9) in the wounds of different groups. The scald time and scald temperature for creating acute wounds were consistent with the chronic wound model group and skin samples from acute wounds were taken 24 hours after scalding.

Image J software (NIH, USA) was used to measure the wound area of rats and quantify the immunohistochemical staining of samples.

The effect of liposomal gel loaded with pro-xylane intermediate on chronic wound healing

In pre-experiments observed that PXYI is able to promote the healing of chronic wounds in rats. Considering the previously mentioned advantages of liposomal gels as drug carriers, we decided to investigate the effect of liposomal gel loaded with PXYI on chronic wounds in rats.

Twenty SD rats were randomly divided into five groups with four rats (16 wounds) in each group after modeling. The wounds of the rats in the five groups were treated with Pro-xylane intermediate (PXYI), liposomal gel loaded with Pro-xylane intermediate (PXYI-LG), liposomal gel (LG), normal saline (NS) and epidermal growth factor (EGF) respectively once every other day. The wounds were photographed before each application, and the wound healing rate ((initial wound area - residual wound area) / initial wound area) was calculated. All rats were euthanized on Day 32 and skin samples were obtained for subsequent experiments.

Cell culture

Human fibroblasts (HF) were isolated from the discarded skin of surgical patients. The skin was obtained with the informed consent of the patient. Isolation of HF was followed by the method previously described by Mohamad *et al.* (2019). HF were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) containing 10% Fetal bovine serum (FBS, Sigma, USA) at 37°C in an incubator with 5% CO_2. Human immortalized epidermal cells (HaCaT) were purchased from the national biomedical experimental cell resource bank (China). HaCaT were cultured in Minimum Essential Medium (MEM, Gibco, USA) containing 10% FBS at 37°C in an incubator with 5% CO_2.

Cell proliferation assay

Alamar Blue was used to detect the effect of PXYI on the proliferation of HF and HaCaT (Chen *et al*., 2017). In brief, HF (2×10^3 cells/well) and HaCaT (4×10^3 cells/well) were seeded into 96-well plates (Thermo Fisher Scientific, New York, USA) respectively. After 24 hours of incubation, the supernatant was removed and different concentrations of PXYI (1:9, 1:27, 1:81) were added in HF and HaCaT, respectively. In addition, DMEM containing 10%FBS and MEM containing 10% FBS were added to HF and HaCaT respectively as positive controls. DMEM and MEM were added to HF and HaCaT respectively as negative control. After 48 hours of incubation at 37°C in an incubator with 5% CO_2, the drug and culture medium in the wells were removed, and 100μL of Alamar Blue was added to each well. After 4 hours of incubation, Fluorescence (FI) value was determined at λex 560 nm and λem 590 nm in a Microplate Reader (Tecan, Infinite 200 Pro, Austria). Use (FI value of drug group/FI value of negative control group) × 100% to indicate the effect of drugs on cell proliferation.

STATISTICAL ANALYSIS

One-way ANOVA and Tukey's post hoc test were used to identify statistically significant differences. P values of <0.05 were considered statistically significant. All values are expressed as the mean ± standard error of mean (SEM).

RESULTS

Characteristics of liposomal gel

The blank liposomal gel is white (fig. 1A) and the liposomal gel loaded with pro-xylane intermediate (PXYI) is yellow (fig. 1B). They are all soft semi-solid. The average particle size of liposome loaded with PXYI is 83.49 nm and the Zeta potential of liposome loaded with PXYI is -25.8067 mV.

Establishment of chronic wound model in rats

The animal model of chronic wound was made by full-thickness burn combined with local injection of Adriamycin. The modeling process is shown in fig. 2A. First, determined the scald time and temperature that can cause full-thickness burns in rats (fig. 2B). After scalding the rat skin at 97°C for 35 seconds, the epidermis, dermis and skin accessories of the rat skin were all necrotic (fig. 2Bf). Therefore, determined that the scalding temperature was 97°C and the scalding time was 35 seconds.

Then, verified the validity of the modeling method. We found that if scald factors alone were administered (control group, fig. 2Cb), wounds were nearly completely healed at 28 days with a healing rate of 98.2±1%. When doxorubicin was locally injected into scald wounds (chronic wound model group, fig. 2Ca), the healing rate of wounds at 28 days was 32.6±3.8%, which was much lower than that of the control group, $P<0.01$ (fig. 2Cc). Meanwhile, studies have shown that high levels of matrix metalloproteinases (MMPs) are associated with chronic wound formation and high levels of MMPs exist in most chronic wounds (Tardaguila-Garcia *et al*., 2019). The results of immunohistochemical staining showed that the expressions of MMP-2 and MMP-9 in wounds of chronic wound model were significantly higher than those in acute wounds, $P<0.01$ (fig. 2D).

In summary, the method of locally injecting doxorubicin in full-thickness burn wounds to establish an animal model of chronic wounds is effective.

Effect of liposomal gel loaded with Pro-xylane intermediate on chronic wound healing

The wound healing process of rats was observed in different groups after treatment, and took photos to calculate the wound healing rate (fig. 3A, table 1). From the 16th to the 32nd day, the wound healing rates of PXYI, PXYI-LG and EGF groups were significantly higher than that of NS group ($P<0.01$), but there was no statistical difference in the wound healing rates among these three groups (fig. 3B). On the 28th and 32nd days, the wound healing rate of LG Group was also significantly higher than that of NS group ($P<0.01$).

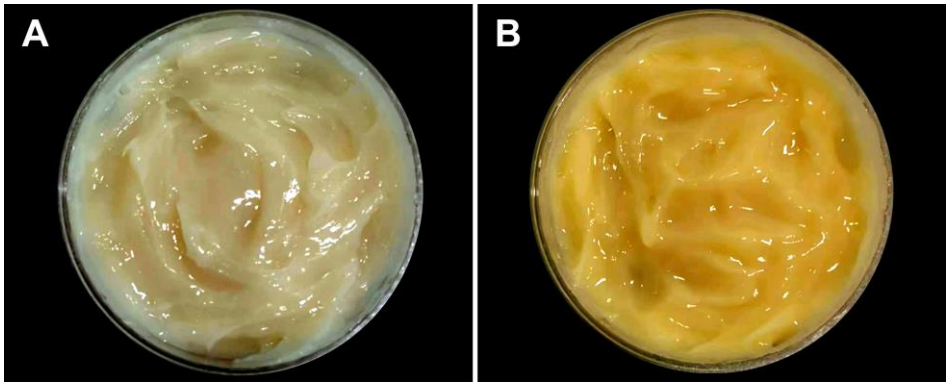

Fig. 1: Liposomal gel. (A) The blank liposomal gel. (B) The liposomal gel loaded with PXYI.

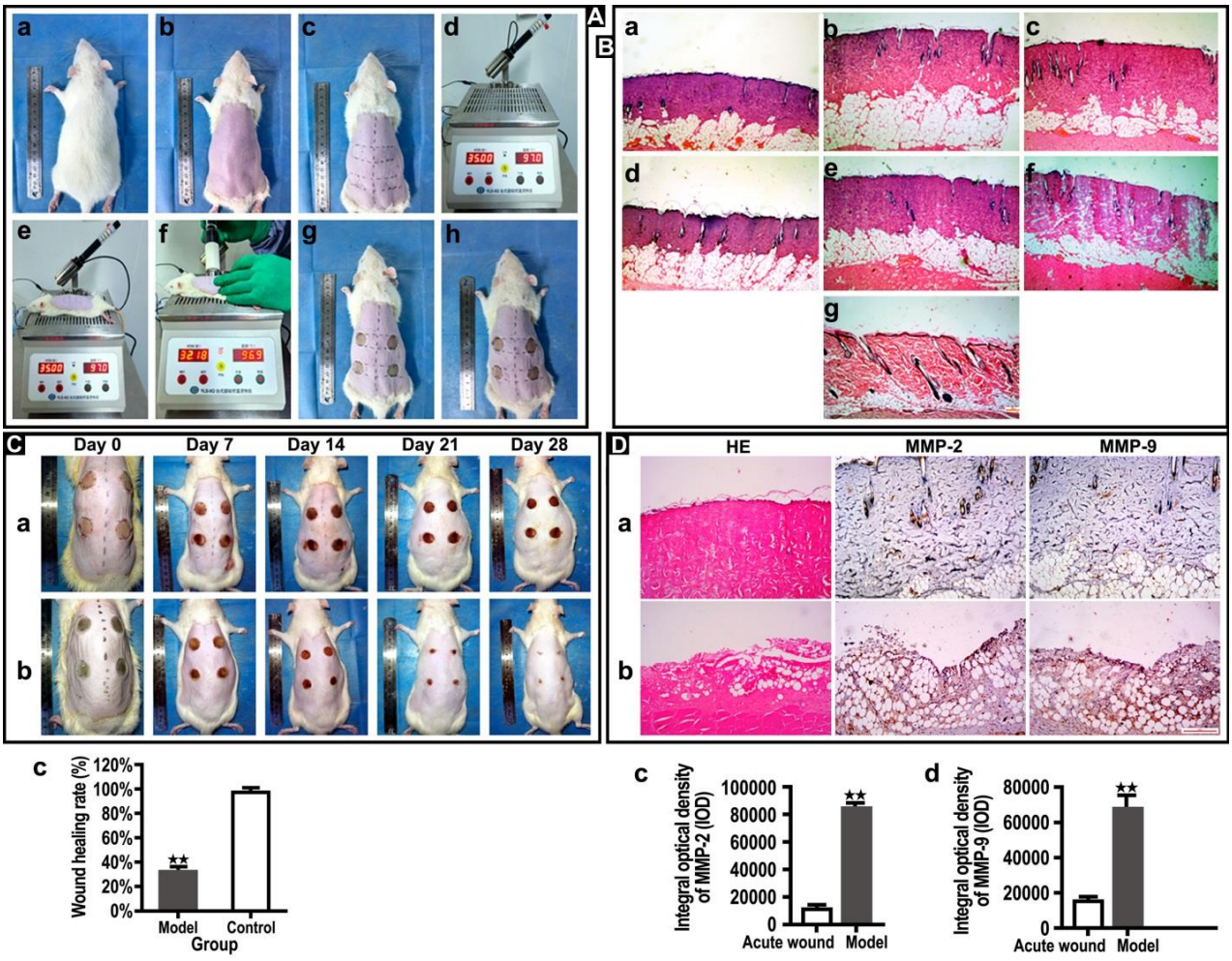

Fig. 2: Establishment of chronic wounds in rats. (A) Process of chronic wound modeling in rats. a: anesthesia, b: skin preparation, c: marking the area to be scalded, d: setting the parameters of the scald apparatus, e~g: scalding 4 wounds along both sides of the spine of the rat back, h: injecting Adriamycin into the scalded wound of the rat. (B) Histological morphology at different scald times. a, b, c, d, e and f correspond to skin with scald time of 10 s, 15 s, 20 s, 25 s, 30 s and 35 s respectively and g is normal skin. (C) Wound healing in chronic wound model group and control group. a: Chronic wound model group. After scalding, Adriamycin was injected into the scalded wound. b: Control group. Only scald factors were given to rat skin. c: The wound healing rate of the model group and the control group. The data were expressed as the mean ± SEM (n=7 in each group). Compared with the control group, **p<0.01. (D) Expression of MMP-2 and MMP-9 in wounds of chronic wound model and acute wounds. a: Acute wounds. b: Chronic wounds. c: Differences in the expression of MMP-2. d: Differences in the expression of MMP-9. The data were expressed as the mean ± SEM (n=9 in each group). Compared with the acute wound group, **p<0.01. Scale bar = 200μm.

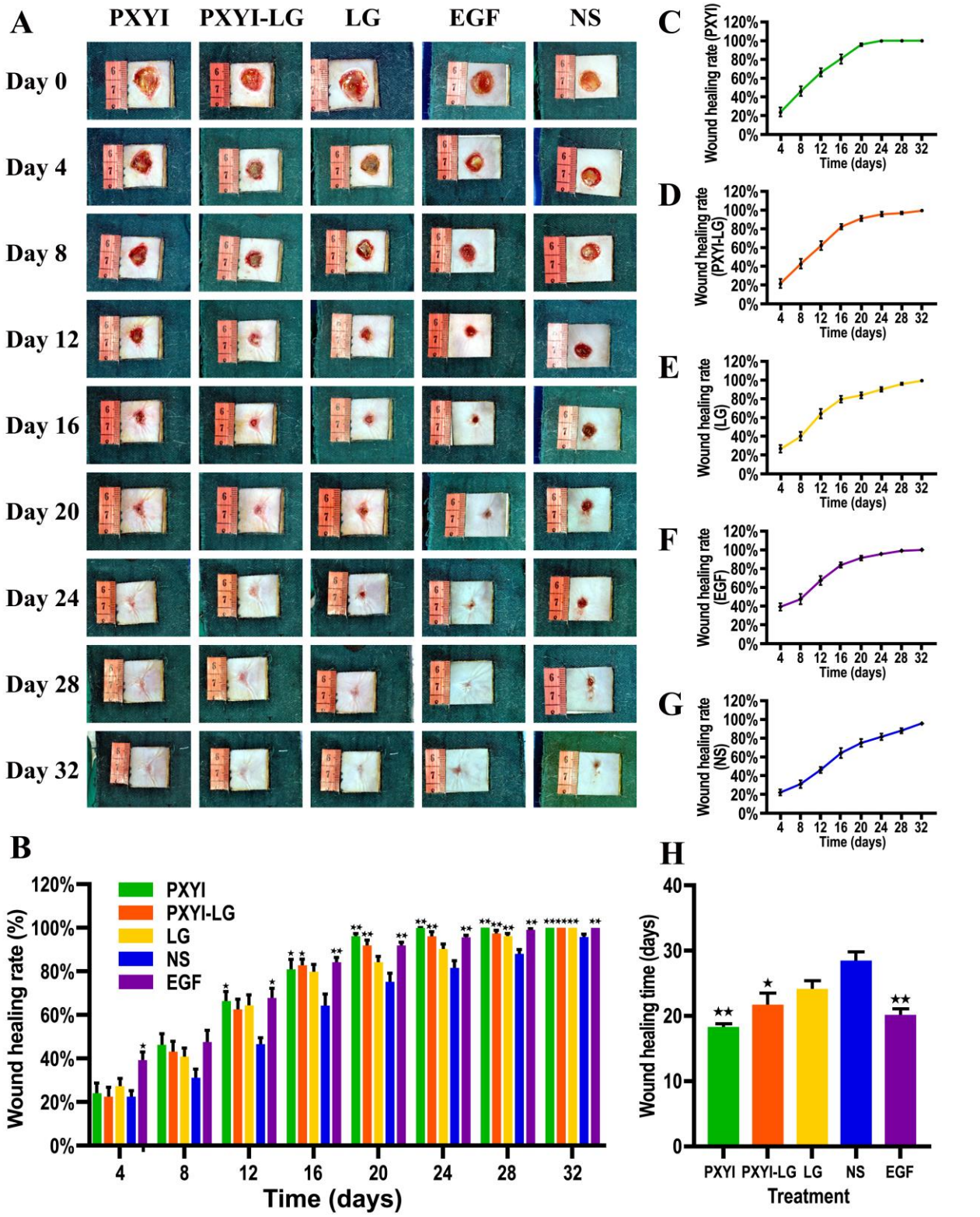

Fig. 3: Wound healing assessment. (A) Gross appearance of wounds. Wounds were treated with PXYI, PXYI-LG, LG, EGF and NS. (B) Wound healing rates at day 4, 8, 12, 16, 20, 24, 28 and 32. The data were expressed as the mean ± SEM (n=9 in each group). Compared with the NS group, *p<0.05, **p<0.01. (C-G) Healing trends of wounds in different groups. (H) The number of days when the wound healing rate reached 90% in different groups. The data were expressed as the mean ± SEM (n=9 in each group). Compared with the NS group, *p<0.05, **p<0.01.

Table 1: Wound healing rate (n = 9)

Days	PXYI	PXYI-LG	LG	NS	EGF
Day4	23.7 ± 4.9%	22.1 ± 4.7%	26.8 ± 3.9%	22.2 ± 3.0%	39.1 ± 3.8%
Day8	46.0 ± 5.3%	43.0 ± 4.9%	40.4 ± 4.3%	31.0 ± 4.0%	47.5 ± 5.5%
Day12	66.3 ± 4.3%	62.3 ± 4.9%	64.2 ± 5.0%	46.3 ± 3.3%	67.6 ± 4.6%
Day16	80.8 ± 4.7%	82.7 ± 2.9%	79.7 ± 3.4%	64.2 ± 5.4%	83.9 ± 2.5%
Day20	96.0 ± 1.4%	91.7 ± 2.5%	83.7 ± 3.2%	75.0 ± 4.1%	91.7 ± 1.7%
Day24	99.8 ± 0.2%	96.1 ± 2.1%	90.1 ± 2.4%	81.5 ± 3.5%	95.6 ± 1.0%
Day28	100.0 ± 0.0%	97.3 ± 1.4%	96.0 ± 1.2%	87.9 ± 2.2%	99.1 ± 0.5%
Day32	100.0 ± 0.0%	100.0 ± 0.0%	99.4 ± 0.4%	95.8 ± 1.2%	100.0 ± 0.0%

Note: The data in the table are expressed as mean ± SEM

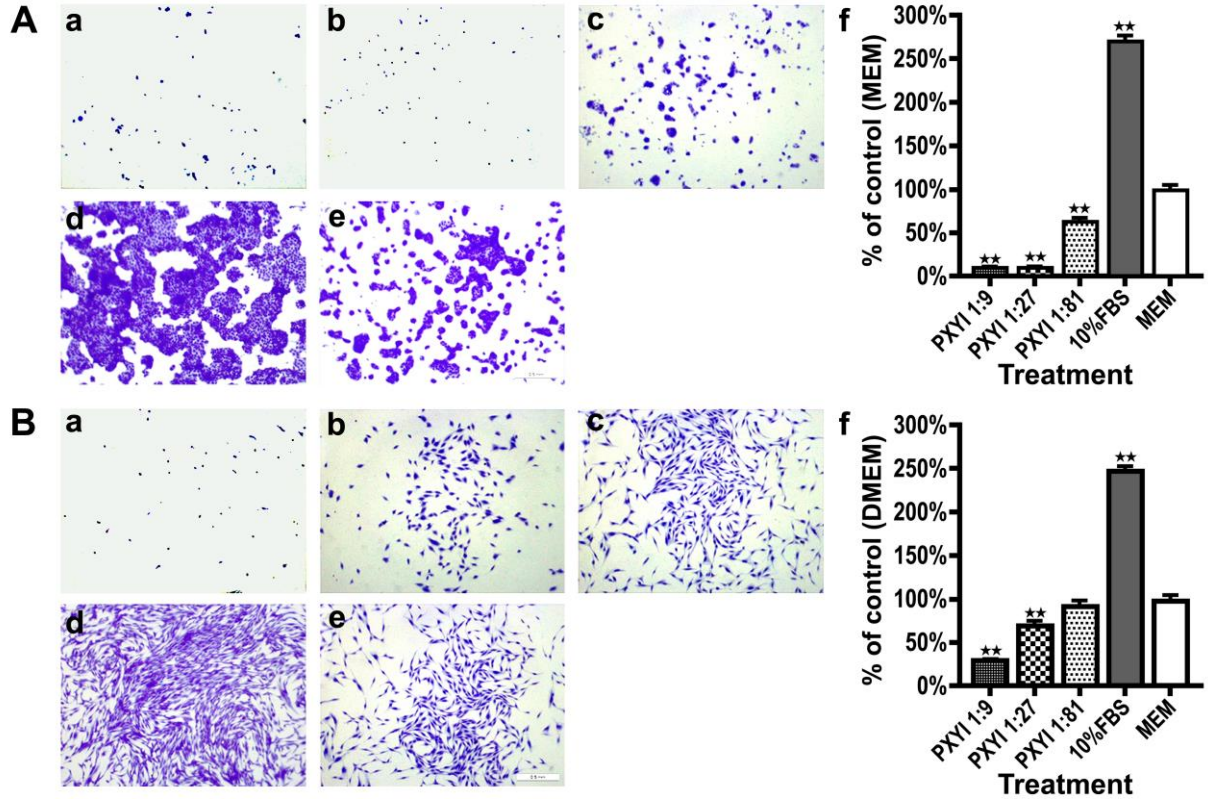

Fig. 4: Cell proliferation assay. (A) Effect of PXYI on HaCaT proliferation. a-c: The dilution ratios of PXYI are 1:9, 1:27 and 1:81 respectively. d: Positive control (MEM with 10%FBS). e: Negative control (MEM). f: Quantification of the effect of PXYI on HaCaT proliferation. The data were expressed as the mean ± SEM (n=9 in each group). Compared with the Negative control, **p<0.01. Scale bar =500μm. (B) Effect of PXYI on HF proliferation. a-c: The dilution ratios of PXYI are 1:9, 1:27 and 1:81 respectively. d: Positive control (DMEM with 10%FBS). e: Negative control (DMEM). f: Quantification of the effect of PXYI on HF proliferation. The data were expressed as the mean ± SEM (n=9 in each group). Compared with the Negative control, **p<0.01. Scale bar = 500μm.

In addition, we compared the number of days when the wound healing rate reached 90% in different groups. The time required to achieve 90% wound healing was 18.2 ± 0.7 days in the PXYI group, 21.8±1.8 days in the PXYI-LG group, 24.0±1.0 days in the LG group, 20.0±1.0 days in the EGF group and 28.4±1.6 days in the NS group. The number of days when the wound healing rate reached 90% was significantly shorter in the PXYI and EGF groups than in the NS group (P<0.01) and it was significantly shorter in the PXYI-LG group than in the NS group (P<0.05). However, there was no significant difference in PXYI, PXYI-LG and EGF groups (fig. 3H).

Effect of PXYI on HaCaT proliferation

When the dilution ratio of PXYI was 1:9, 1:27 and 1:81, it exhibited inhibitory effects on the proliferation of HaCaT, P<0.01 (fig. 4A). However, with the decrease of PXYI concentration, the inhibitory effect of PXYI on HaCaT gradually weakened.

Effect of PXYI on HF proliferation

The results showed that when the dilution ratio of PXYI was 1:9 and 1:27, it inhibited the proliferation of HF, $P<0.01$. When the dilution ratio of PXYI was 1:81, its effect on HF proliferation was not significantly different from that of the negative control (fig. 4B).

DISCUSSION

Although there are many therapies for chronic wounds, they are not completely satisfactory to patients due to expensive or side effects. It is very important to continue to develop treatments that are more beneficial to patients.

According to the results of previous studies, we found that C-xylopyranoside derivatives have the potential to repair skin wound. Therefore, we speculate that PXYI, as one of C-xylopyranoside derivatives, may promote the healing of chronic wounds. In order to test the effect of PXYI on chronic wounds, we established an animal model of chronic wound in rats and verified the model. We detected higher MMP-2 and MMP-9 in the model of chronic wounds than in the acute wounds, which is consistent with the study reported by Wysocki *et al* (1993). This result shows that our chronic wound model, at least from the perspective of wound microenvironment, is consistent with the standard of chronic wounds.

We observed in the pre-experiment that PXYI is able to promote the healing of chronic wounds. However, PXYI is liquid at normal temperature and it is not easy to retain in the wound for a long time. We therefore considered the use of liposomal gel for the delivery of PXYI. The results showed that, similar to PXYI, PXYI -LG could increase the wound healing rate and shorten the wound healing time, thus promoting the healing of chronic wounds. The concentration of PXYI in PXYI -LG is relatively low, so the use of liposomal gel to deliver PXYI may reduce the potential side effects of PXYI (Hou *et al*., 2022). In addition, in animal experiments, we found that PXYI -LG, which was previously applied, remained on the wound surface before each repeated application. This indicates that using liposomal gel to deliver PXYI can indeed prolong the residence time of PXYI in the wound.

In order to explore the potential mechanism of PXYI promoting chronic wound healing, we investigated the effect of PXYI on the proliferation of HF and HaCaT. We observed that high concentrations of PXYI inhibited the proliferation of HF and HaCaT. The reason may be that the high concentration of drugs causes the change of osmotic pressure around the cells, thus affecting the cells. It may also affect cells through some ways that we haven't found yet, which may be related to the side effects of PXYI. We will further explore the possible mechanism for this phenomenon in subsequent experiments. We have not observed that PXYI alone can promote the proliferation of HF or HaCaT. Jun Muto *et al* showed that C-xylopyranoside derivatives are able to promote the secretion of GAGs from keratinocytes and promote fibroblast growth factor-10 (FGF-10) dependent proliferation and migration of keratinocyte (Muto *et al*., 2011). Therefore, we speculate that the presence of FGF-10 may be a necessary condition for PXYI to promote the proliferation of HF or HaCaT. The role of PXYI in promoting wound healing in animals may be partly due to the presence of FGF-10 in animal tissues, which makes PXYI promote the proliferation or migration of fibroblasts and keratinocytes. We will verify this possible mechanism in future experiments. In addition, there are quite a few drugs that need to be metabolized *in vivo* to exert their pharmacological effects, which require the participation of specific cells (Almazroo *et al*., 2017, Vermeir *et al*., 2005). The mechanism of PXYI promoting wound healing may also involve specific cell-mediated drug metabolism.

In the context of existing literature, there are few studies on chronic wound models and potential drugs for the treatment of chronic wounds. Our study provides a validated and stable method for chronic wound modeling. The stable method of chronic wound modeling can contribute to the in-depth study of related drugs and related mechanisms. In addition, this study proved the promoting effect of PXYI on chronic wounds provide potential new drugs for the treatment of chronic wounds. Of course, there are also some deficiencies in this study. First, the causes of chronic wounds are extremely complex. For different types of chronic wounds, there is a lack of a comprehensive model that can completely simulate human chronic wounds. We need a variety of models to study chronic wounds. Although the animal model of chronic wound established in this study can be used as a method to study chronic wounds, it can not represent all types of chronic wounds. Secondly, there are obvious differences between rat skin and human skin. Compared with the epithelization in the process of human cutaneous wound healing, there is obvious skin contraction in the process of rat cutaneous wound healing, which may have some impact on the reliability of the experimental results. In the future research, we need to select animals (such as pigs) whose skin is closer to human beings, and establish various types of chronic wound animal models, so as to simulate the real situation of clinical chronic wounds as much as possible. In addition, we have not conducted a more in-depth study on the molecular mechanism of PXYI promoting chronic wound healing. As a 2D experiment, monolayer cell experiment can only reflect whether there is a direct relationship between drugs and specific cells. While the animal organism, as a complex whole, in the process of wound healing occurs very complex interactions of cells and molecules, drugs may have a direct or indirect effect on the organism through many different pathways. We need to apply more 3D culture systems (Sami, Heiba *et*

al., 2018) that have advantages over monolayer cell experiments to help us obtain more reasonable and reliable results.

CONCLUSION

In conclusion, we successfully established and verified an animal model of chronic wounds. And we demonstrated that the PXYI liposomal gel can enhance the wound healing rate and shorten the wound healing time of chronic wounds.

REFERENCES

Almazroo OA, Miah MK and Venkataramanan R (2017). Drug metabolism in the liver. *Clin. Liver Dis,* **21**(1): 1-20.

Armstrong DG, Wrobel J and Robbins JM (2007). Guest Editorial: Are diabetes-related wounds and amputations worse than cancer? *Int. Wound J.*, **4**(4): 286-287.

Bangham AD, Standish MM and Watkins JC (1965). Diffusion of univalent ions across the lamellae of swollen phospholipids. *J. Mol. Biol.*, **13**(1): 238-252.

Brunet-Maheu JM, Fernandes JC, de Lacerda CA, Shi Q, Benderdour M and Lavigne P (2009). Pluronic F-127 as a cell carrier for bone tissue engineering. *J. Biomater. Appl.*, **24**(3) 275-287.

Chen Q, Zhou H, Yang Y, Chi M, Xie N, Zhang H, Deng X, Leavesley D, Shi H and Xie Y (2017). Investigating the potential of Oxymatrine as a psoriasis therapy. *Chem. Biol. Interact.*, **271**: 59-66.

Dawoud MHS, Yassin GE, Ghorab DM and Morsi NM (2019). Insulin mucoadhesive liposomal gel for wound healing: A formulation with sustained release and extended stability using quality by design approach. *AAPS Pharm. Sci. Tech.*, **20**(4): 158.

Diniz IM, Chen C, Xu X, Ansari S, Zadeh HH, Marques MM, Shi S and Moshaverinia A (2015). Pluronic F-127 hydrogel as a promising scaffold for encapsulation of dental-derived mesenchymal stem cells. *J. Mater. Sci. Mater. Med.*, **26**(3): 153.

Elnaggar YS, El-Refaie WM, El-Massik MA and Abdallah OY (2014). Lecithin-based nanostructured gels for skin delivery: An update on state of art and recent applications. *J. Control. Release*, **180**: 10-24.

Hou Y, Meng X, Zhang S, Sun F and Liu W (2022). Near-infrared triggered ropivacaine liposomal gel for adjustable and prolonged local anaesthesia. *Int. J. Pharm.*, **611**: 121315.

Hua S (2015). Lipid-based nano-delivery systems for skin delivery of drugs and bioactives. *Front. Pharmacol.*, **6**: 219.

Izadi K and Ganchi P (2005). Chronic wounds. *Clin. Plast. Surg.*, **32**(2): 209-222.

Kant V, Gopal A, Kumar D, Gopalkrishnan A, Pathak NN, Kurade NP, Tandan SK and Kumar D (2014). Topical pluronic F-127 gel application enhances cutaneous wound healing in rats. *Acta Histochem.*, **116**(1): 5-13.

Mohamad N, Loh EYX, Fauzi MB, Ng MH and Mohd Amin MCI (2019). *In vivo* evaluation of bacterial cellulose/acrylic acid wound dressing hydrogel containing keratinocytes and fibroblasts for burn wounds. *Drug Deliv Transl Res*, **9**(2): 444-452.

Muto J, Naidu NN, Yamasaki K, Pineau N, Breton L and Gallo RL (2011). Exogenous addition of a C-xylopyranoside derivative stimulates keratinocyte dermatan sulfate synthesis and promotes migration. *PLoS One*, **6**(10): e25480.

Olsson M, Jarbrink K, Divakar U, Bajpai R, Upton Z, Schmidtchen A and Car J (2019). The humanistic and economic burden of chronic wounds: A systematic review. *Wound Repair Regen.*, **27**(1): 114-125.

Pierre MB and Dos Santos Miranda Costa I (2011). Liposomal systems as drug delivery vehicles for dermal and transdermal applications. *Arch. Dermatol. Res.*, **303**(9) 607-621.

Pineau N, Carrino DA, Caplan AI and Breton L (2011). Biological evaluation of a new C-xylopyranoside derivative (C-Xyloside) and its role in glycosaminoglycan biosynthesis. *Eur. J. Dermatol.*, **21**(3): 359-370.

Sami DG, Heiba H and Abdellatif A (2018). Wound healing models: A systematic review of animal and non-animal models. *Wound Medicine*, **24**(1): 8-17.

Sen CK, Gordillo GM, Roy S, Kirsner R, Lambert L, Hunt TK, Gottrup F, Gurtner GC and Longaker MT (2009). Human skin wounds: A major and snowballing threat to public health and the economy. *Wound Repair Regen.*, **17**(6): 763-771.

Sok J, Pineau N, Dalko-Csiba M, Breton L and Bernerd F (2008). Improvement of the dermal epidermal junction in human reconstructed skin by a new c-xylopyranoside derivative. *Eur. J. Dermatol.*, **18**(3): 297-302.

Tardaguila-Garcia A, Garcia-Morales E, Garcia-Alamino JM, Alvaro-Afonso FJ, Molines-Barroso RJ and Lazaro-Martinez JL (2019). Metalloproteinases in chronic and acute wounds: A systematic review and meta-analysis. *Wound Repair Regen.*, **27**(4): 415-420.

Vermeir M, Annaert P, Mamidi RN, Roymans D, Meuldermans W and Mannens G (2005). Cell-based models to study hepatic drug metabolism and enzyme induction in humans. *Expert Opin. Drug Metab. Toxicol.*, **1**(1): 75-90.

Wang W, Shu GF, Lu KJ, Xu XL, Sun MC, Qi J, Huang QL, Tan WQ and Du YZ (2020). Flexible liposomal gel dual-loaded with all-trans retinoic acid and betamethasone for enhanced therapeutic efficiency of psoriasis. *J. Nanobiotechnology*, **18**(1): 80.

Wysocki A, Staiano-Coico L and Grinnell F (1993). Wound fluid from chronic leg ulcers contains elevated levels of metalloproteinases MMP-2 and MMP-9. *J. Invest. Dermatol.*, **101**(1): 64-68.