Int J Clin Exp Med 2017;10(12):16319-16327
www.ijcem.com /ISSN:1940-5901/IJCEM0056347

Original Article

Collagen hydrogel dressing for wound healing and angiogenesis in diabetic rat models

Jing Lei[1,2,3], Peng Chen[2,3], Yiheng Li[2,3], Xiaoying Wang[1], Shunqing Tang[1,3]

[1]Department of Biomedical Engineering, College of Life Science and Technology Key Laboratory of Biomaterials of Guangdong Higher Education Institutes, Jinan University, Guangzhou, China; [2]Guangzhou Trauer Biotechnology Co., Ltd., Guangzhou, China; [3]Medical Collagen Engineering Technology Research and Development Center in Guangdong Province, Guangzhou, China

Received March 7, 2017; Accepted September 19, 2017; Epub December 15, 2017; Published December 30, 2017

Abstract: To evaluate the effect of collagen gel dressing upon the healing of skin defects and investigate its role in promoting capillary regeneration in diabetic rat models. In total, 160 Sprague-Dawley rats were selected in this study. At 9 weeks after model establishment, 120 rat models were established and randomly divided into the model (n=30), positive control (n=24) and experimental groups (n=25). Full-thickness skin wounds were made on the rat back. The rats in the normal control and model groups were externally treated with collagen gel dressing, and the recombinant human epidermal growth factor gel was administered in the positive control group. The area of wound healing and the quantity of new capillaries were measured. At 7 d after administration, the rats merely in the normal control group showed faster wound healing and became the fastest at 14 d. At 14 d, the non-healing area in the model group was the largest. At 21 d, the non-healing area in the normal control and experimental groups was smaller than those in other two groups, whereas those in the normal control and positive control groups were smaller than those in other two groups at 28 d. The healing area in the experimental group was larger than that in the positive control group at 14 and 21 d, and the quantity of new capillaries in the experimental group was the highest at 7-, 14- and 21-d. Collagen gel dressing can promote the rate and quality of full-thickness wound healing in diabetic rats.

Keywords: Collagen, hydrogel dressing, regeneration, angiogenesis, diabetes, excision wound

Introduction

Diabetes mellitus (DM) is one of the most prevalent chronic diseases worldwide [1]. Patients diagnosed with DM are likely to present with ulceration and multiple types of skin lesions. Wound healing is a complex process that involves with simultaneous actuation of soluble mediators, blood cells, extracellular matrix and parenchymal cells. DM is characterized by basement membrane thickening of blood arterioles and capillaries [2]. An ideal wound dressing should provide a moist environment, protect the wounds from secondary infection, remove wound exudates and promote tissue regeneration. However, no wound dressing currently fulfills all the requirements by diabetic ulcer therapy. The wound dressing technique should be selected based upon the wound type and staging, injury extension, patients' conditions and the affected tissues.

Collagen-based dressings have been of widespread interests in wound care. Besides as an inducer of cell signaling, collagen-based dressings, also acting as a sacrificial substrate, may improve the healing outcomes by deactivating excessive matrix metalloproteases [3]. Additionally, collagen-based dressings can recruit certain types of cells to the wound sites which facilitate the formation of granulation tissues. Furthermore, these collagen-based dressings are able to maintain the moist environment for the wounds by absorbing wound exudates [4, 5]. Much effort has been strengthened currently to optimize the composition and formulation of collagen dressings and enhance the clinical efficacy for treating skin lesions.

Recombinant human epidermal growth factor (EGF) has been proven to exert strong effect upon promoting cellular division, which is capable of promoting the differentiation and prolif-

eration of fibroblasts, facilitating the migration to the wound sites, stimulating the endothelial cell division and promoting epithelial cell regeneration [6]. Meantime, EGF can maintain granulation tissue moist, accelerate wound healing and shorten wound healing time by stimulating the collagen synthesis within the extracellular matrix. EGD can enhance the clinical efficacy for treating chronic ulcer by promoting vascular regeneration and improving local blood circulation.

Consequently, Sprague-Dawley rat models with diabetic skin defects were established and subsequently treated with collagen-based dressings to repair full-thickness skin lesions. The wound healing, tissue repairing and angiogenesis of these diabetic rat models were investigated to evaluate the feasibility and clinical efficacy of these collagen-based dressing therapies.

Materials and methods

Hydrogel preparation

Type I collagen extracted from bovine tendon was provided by Guangzhou Trauer Biotechnology Company (Guangzhou, China). Medicinal-grade gel matrix including hydroxy propyl methyl cellulose (HPMC, Hopetop pharmaceutical CO., Ltd., Huzhou, China) and polyvinyl alcohol (PVA) were purchased from Xi'an Yue Lai Medical Technology CO., Ltd, China. Collagen gel was prepared by mixing collagen with gel matrix and stirred well under the condition of 40 *r*/m at 25°C avoiding the incidence of collagen denaturation caused by bubbles. The concentration of collagen used was 3.0-3.5 mg/ml. The concentration of HPMC and PVA was adjusted to 2.5% and 0.25%.

Collagen characterization

SDS-PAGE was performed to characterize the type I collagen consisting of 4% concentrated gel and 7% separating gel and the amount of loading was ≥ 15 μg. The morphology of collagen hydrogel was observed under optical microscope (Nikon TE300, Japan) and microscopically analyzed by transmission electron microscope (Philips Tecnai 10, Holland).

Animal model establishment

A total of 120 Sprague Dawley rats were established as diabetic models by intra-peritoneal injection of streptozocin at a dosage of 55 mg/kg. At 72 h post-injection, the blood glucose level was monitored for consecutive 4 weeks. The diabetic animal models were successfully established when the blood glucose level reached ≥ 16.8 mmol/L for over 3 weeks and the symptoms of more drinking, more urine and more feeding were noted. Those rats with blood glucose level < 16.8 mmol/L for twice were excluded from subsequent analysis. The eligible rats were randomly divided into model (n=30), positive control (n=24) and experimental groups (n=25).

Wound preparation and treatment

At 9 weeks after model establishment, the rats were anesthetized by intra-peritoneal injection of chloral hydrate at a dose of 2.5 mL/kg. The dorsal region was shaved, and the skin was surgically prepared alternatively with 2% of chlorhexidine and 70% of alcohol scrubs. One full-thickness bi-pedicle skin flap measuring 3×3 cm was created on each animal. Full-thickness skin defects were created in the center of bi-pedicle ischemic skin flap on the back of rats. Collagen gel at a dosage of 0.1 mL/cm^2 was applied to the wounds of rats in the experimental group once daily. Commercially available recombinant human epidermal growth factor gel at a dose of 0.1 mL/cm^2 was used in the positive control group once daily. The rats in the normal control and model groups were treated with sterilized vaseline dressing once daily at a dosage of 0.1 mL/cm^2. The wounds were disinfected with iodine prior to administering medication.

Gross examination

The morphology of the wounds was observed by a digital camera (Super-500uZ Olympus, Japan). Scarring shape and physical properties were also observed at 7-, 14-, 21- and 28-d after repairing. The excision area was measured and the healing rate was calculated according to the formula as follows:

$$\text{Healing rate}=(S_0-S_1)/S_0\times100\%$$

In this equation, S_0 represents the original wound area and S_1 is defined as the wound area after collagen dressing treatment.

Hematoxylin and eosin staining

After treated with hydrogel dressing for 4 weeks, 6 rats in each group were euthanized at

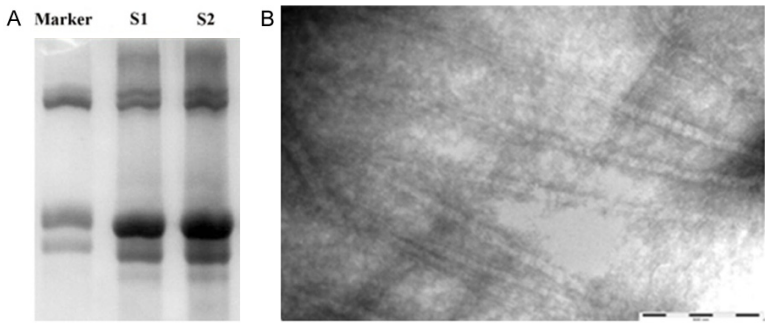

Figure 1. A. SDS-PAGE for characterizing the hydrogel collagen (Sigma collagen from calf skin as the marker, S1 is type I collagen (10 μg) for hydrogel dressing preparation and S2 is hydrogel containing 25 μg collagen. B. TEM imaging of collagen for hydrogel dressing (scale bar=500 nm).

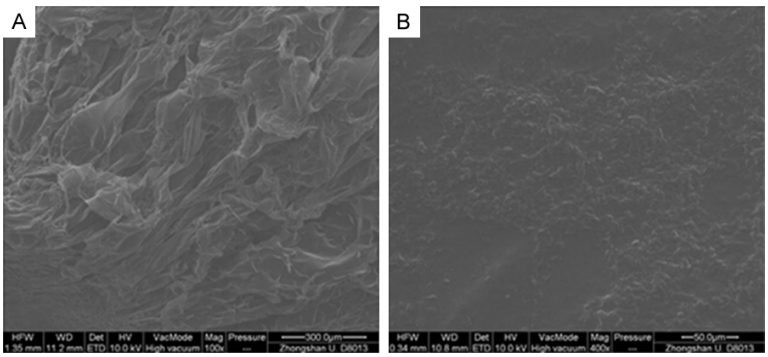

Figure 2. SEM imaging of collagen scaffolds and hydrogel membrane in the dry condition. A. Collagen scaffold (scale bar=300 μm; 100×). B. Collagen hydrogel membrane with a collagen concentration of 72%-75% (scale bar=50 μm; 400×).

postoperative 7-, 14-, 21- and 28-d. Wound margin and granulation tissue specimens were collected, fixed in 10% formaldehyde, dehydrated using conventional techniques, paraffin embedded and sliced into approximately 7 μm-thick sections and stained with hematoxylin and eosin (H.E). The sections were observed under biological microscope (Olympus CX31, Japan). The quantity of fibroblasts and new capillaries in the granulation tissue were counted in the center of the lesions.

Statistical analysis

SPSS 17.0 statistical software was utilized for data analysis (SPSS Inc. IL, USA). All data were statistically evaluated by analysis of variance (ANOVA) and post-hoc test with Bonferroni's correction. A *P* value of less than 0.05 was considered as a level of statistical significance. Single-factor ANOVA was performed by least significant difference (LSD) test.

Results

Characterization of collagen hydrogel dressing

As illustrated in **Figure 1A**, bovine tendon-derived collagen migrated between beta- and alpha1-collagen chains on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and electrophoretic behavior was not affected by HPMC or PVA. The thicker the gel membrane was, the smaller the water vapor transmittance was, but the thickness of gel membrane did not exert a significant effect upon water vapor transmittance. When the thickness of the film was doubled, the water vapor transmittance only changed by approximately 18.1 g/m^2·24 h. The aperture for pure collagen membrane was 40-150 μm and the average size was 100 μm, the film porosity was approximately 95.8% (**Figure 2A**). But the dry hydrogel film was relatively dense and the average aperture size ranged from 40 to 60 μm (**Figure 2B**). TEM results showed that the collagen fiber in hydrogel was disperse and the fiber diameter with a special periodic horizontal grain zone was approximately 200 to 300 nm (**Figure 1B**).

Stability of diabetic rat models

During the procedures of animal model establishment, 9 rats died from anesthesia or surgery. Eventually, 111 rats were included for subsequent diabetic model establishment including 32 rats for the normal control group, 30 rats for the model group, 24 rats for the positive control group and 25 for the experimental group. The remaining rats were found to have hyperglycemia during the experiment and DM-related symptoms were identified in addition to the normal control group. Statistical significance was noted between the normal control and three treatment groups, suggesting the successful and stable establishment of diabetic rats. The changes in the blood glucose level

Table 1. Comparison of blood glucose levels among four groups at different time points ($\bar{x}$±s, mmol/L)

Group	1 week	2 weeks	3 weeks	4 weeks
NC	7.37±0.78 (6)	6.52±0.98 (6)	6.42±0.38 (6)	6.38±0.44 (14)
MC	28.75±10.05** (6)	33.52±1.18** (6)	30.75±2.63** (6)	31.15±3.25** (12)
PC	28.58±8.10** (6)	33.65±0.86** (6)	30.42±2.63** (6)	29.33±3.58** (6)
EG	20.83±13.77* (6)	33.50±1.22** (6)	24.75±10.04** (6)	30.55±2.78** (7)

Note: *P < 0.05 and **P < 0.01 compared with NC (negative control group); the number of rats was present in the brackets.

Table 2. Comparison of body weight among four groups at different time points ($\bar{x}$±s, g)

Group	1 week	2 weeks	3 weeks	4 weeks
NC	262.00±12.01 (6)	297.83±24.96 (6)	353.00±27.27 (6)	388.57±30.95 (14)
MC	171.08±20.57* (6)	210.00±24.28* (6)	234.00±13.80* (6)	296.67±61.48* (12)
PC	146.83±23.59* (6)	220.58±29.61* (6)	233.58±37.20* (6)	217.42±29.40* (6)
EG	167.67±53.22* (6)	198.83±16.63* (6)	220.00±30.41* (6)	255.79±51.42* (7)

Note: *P < 0.01 compared with NC (negative control group); the number of rats was present in the brackets.

and body weight for all groups are illustrated in **Tables 1** and **2**.

Morphology and repairing of diabetic wounds

All wounds were found to be irregular, drying and ruddy with elevated wound margin. Most wound scab and became very hard at 3 d after collagen hydrogel dressing or other treatments. No statistical significance was noted in macroscopic observation among all groups within 7 d after treatment (P>0.05). Except the model group, the wounds in all other groups were dry and ruddy and apparent contraction was documented in the wound margin at 14 d after surgery. The wounds in the model control were healed at the slowest rate among all groups.

Except the negative control group, the wounds were slowly repaired and the healing rate at 1 week after treatment did not significantly differ among the other three groups (P>0.05). However, at 14 d after treatment, the area of non-healed wounds for animals treated with collagen hydrogel and EGF gel was significantly smaller compared with that in the model group (both P < 0.05). At 21 d after corresponding treatment, the area of non-healed wounds in the negative control and collagen dressing groups was considerably smaller than those in the other two groups (all P < 0.05). The area of non-healed wounds in the negative control and EGF gel treatment groups was significantly smaller than those in the model control and collagen dressing groups, significantly differing from that in the model group (all P < 0.05). A downward trend was found for the healing rate in the collagen hydrogel group, but there was no significant difference between the two groups when treated for 4 weeks, as shown in **Figure 3**.

The effect of collagen hydrogel upon promoting wound healing was significantly higher than that in the control group (**Table 3**). Moreover, topical use of EGF gel for 4 weeks could rapidly reduce the wound area. At 14 d, the wound healing rate achieved 65.50%, significantly differing from that in the model group (P < 0.05), and reached up to 96.87% at 28 d, significantly differing from that in the model group (P < 0.01). The wound healing rate was 69.02% at 14 d and reached 93.93% at 21 d, significantly differing compared with the model group (all P < 0.05).

Histological examination

Topical tissue inflammation was observed by histological examination, as revealed in **Figure 4**. The granulation tissue and a large number of invasive neutrophils were found under the necrotic tissue in the negative group. Grassy-like granulation tissues were found after 21 d and skin attachments were visible during the skin repairing after 28 d. In the model group, wound scab formed after 7 d, a dense layer of granulation tissues and a large number of invasive inflammatory cells were observed in the lesions. After 14 d, loose granulation tissue accompanied with inflammatory cell invasion was found. A small amount of new blood vessels and a large number of invasive inflammatory cells were visible in scabby subcutaneous lesions after 3 weeks. A high quantity of neutrophils and tissue fluid migrated into the scabby tissues at 28 d. At 7 d, loose granulation tissues, neutrophils and monocytes were found in

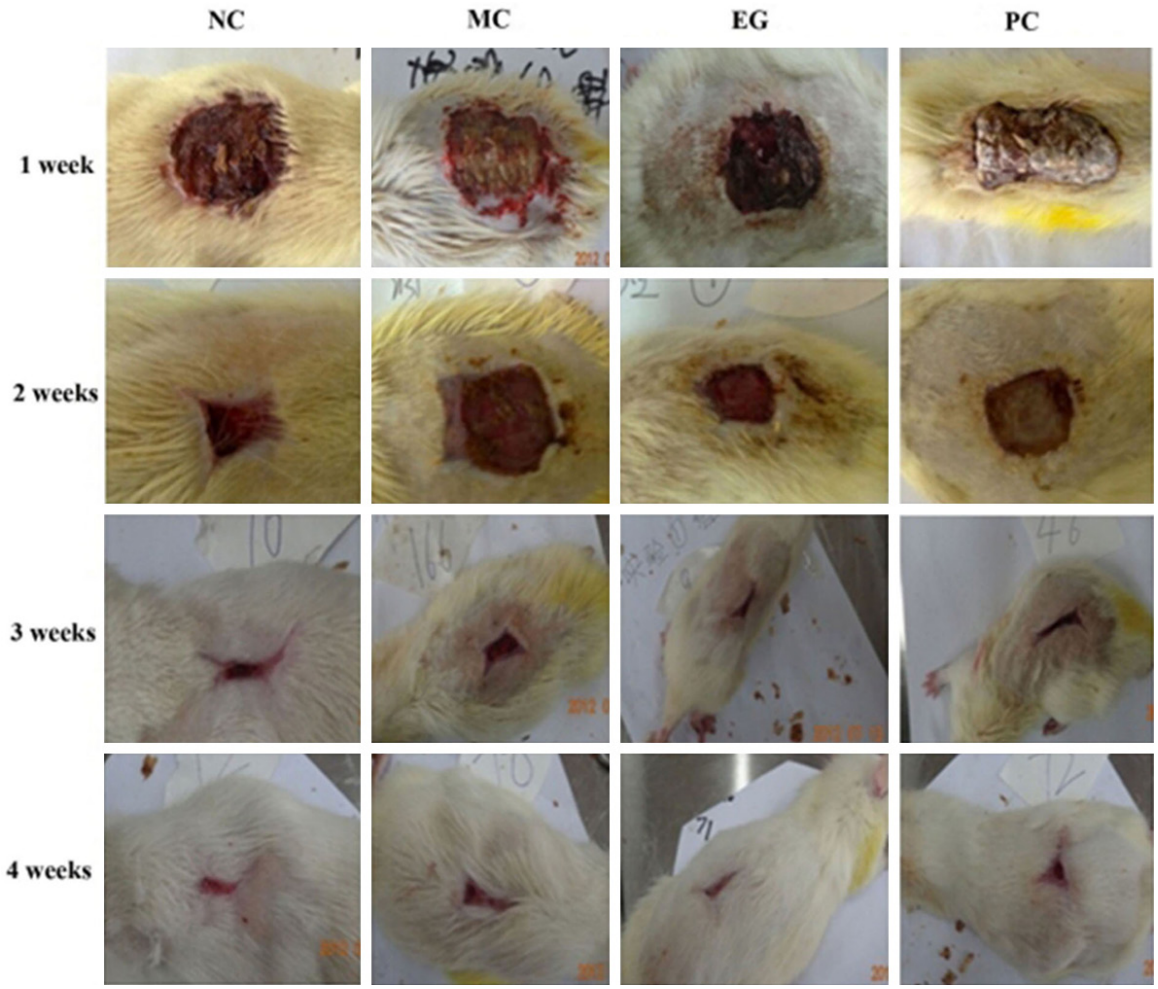

Figure 3. Gross examination among four groups during 4 weeks. Note: EG, PC, MC and NC refer to the experimental (collagen hydrogel), positive control (EGF gel), model and normal control groups.

Table 3. Comparison of the area of non-healed wounds among four groups at different time points ($\bar{x}\pm s$, cm^2)

Group	1 week	2 weeks	3 weeks	4 weeks
NC	7.69±0.97 (6)	1.68±0.87 (6)	0.40±0.20 (6)	0.15±0.15 (14)
MC	8.30±0.99 (6)	5.20±2.64[b] (6)	1.23±0.75[b] (6)	0.73±0.36[b] (12)
PC	8.77±0.57[a] (6)	3.11±0.47[c] (6)	1.35±0.67[b] (6)	0.28±0.17[d] (6)
EG	8.87±0.27[a] (6)	2.79±0.87[c] (6)	0.55±0.26[c] (6)	0.43±0.33[a] (7)

Note: [a]$P < 0.05$, [b]$P < 0.01$ compared with NC (negative control group); [c]$P < 0.05$ and [d]$P < 0.01$ compared with MC (model control group); the number of rats was present in the brackets.

the wound area of rats treated with collagen hydrogel. During the next 14 d, the granulation tissues became dense, and inflammatory cells invasion and angiogenesis could be observed in the wound area. Most of the epidermis was scabby at 28 d after collagen hydrogel treatment. Compared with the collagen dressing group, more obvious inflammatory cells invasion was found at 28 d and angiogenesis was also observed at 21 d in the EGF treatment group (**Table 4**).

Changes in the fibroblast quantity

The quantity of fibroblasts in the experimental group was significantly higher compared with that of the other treatment groups. As revealed in **Table 5**, in the model control group, the proliferation rate of fibroblasts at 7 d was significantly higher compared with that in the EGF treatment group ($P < 0.05$), and the proliferation rate remained relatively stable during the next three weeks in the EGF group. By contrast, the proliferation rate in the EGF treated

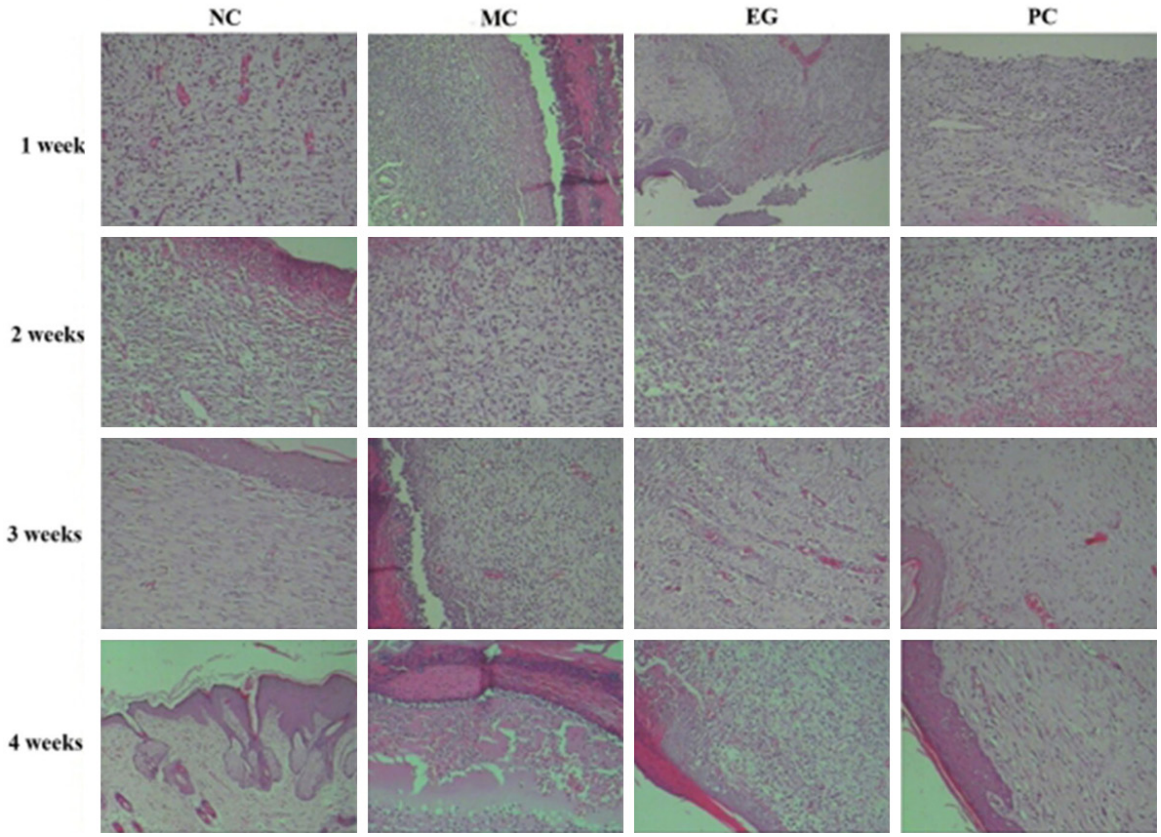

Figure 4. Histological examination of the collagen (10×). Note: NC, MC, EG and PC refer to the normal control, model, experimental and positive control groups.

Table 4. Comparison of the wound healing rate among four groups at different time points ($\bar{x}\pm s$, %)

Group	1 week	2 weeks	3 weeks	4 weeks
NC	14.5±10.79 (6)	81.36±9.64 (6)	95.60±2.20 (6)	98.37±.1.66 (14)
MC	7.76±10.94 (6)	42.20±29.38[b] (6)	86.30±8.31[b] (6)	91.87±4.02[b] (12)
PC	2.59±6.35[a] (6)	65.50±5.17[c] (6)	84.98±7.44[b] (6)	96.87±1.84[d] (6)
EG	1.41±2.98 (6)	69.02±9.66[c] (6)	93.93±2.88[c] (6)	95.23±3.70[c] (7)

Note: [a]$P < 0.05$ and [b]$P < 0.01$ compared with NC (negative control group); [c]$P < 0.05$ and [d]$P < 0.01$ compared with MC (model control group); the number of rats was present in the brackets.

group was slower than that of collagen dressing group, but there was no significant difference compared with the model control at 3 and 4 weeks. The results showed that collagen hydrogel played a certain role in promoting the proliferation of fibroblasts and the occurrence of angiogenesis, eventually accelerating the repairing and healing of diabetic wounds.

Changes in the angiogenesis

In terms of angiogenesis, the count of new capillaries in the collagen hydrogel treatment group was significantly higher than those in the other groups at 1 week (all $P < 0.05$), significantly differing from that in the model group ($P < 0.05$), as illustrated in **Table 6**. During the first three weeks, the number of new capillaries in the collagen hydrogel group was higher compared with that in the EGF group, suggesting that collagen gel could more effectively promote the angiogenesis for diabetic wound healing compared with EGF.

Discussion

Many researches have concluded that acetic acid and pepsin could be used for type I collagen extraction from animal tendon tissues with a high purity. Electrophoresis spectrum for type I collagen typically includes 4 or 6 bands. The presence of beta- and gamma-chains and higher aggregates in pepsin-solubilized collagen

Table 5. Comparison of the fibroblast count among four groups at different time points ($\bar{x}\pm s$, n=6, cells)

Group	1 week	2 weeks	3 weeks	4 weeks
NC	116.00±19.40	108.67±20.88	123.17±34.83	165.21±36.30
MC	69.33±18.20	203.83±37.93[a]	123.00±45.52	173.67±71.18
PC	82.00±14.42	120.17±36.54[c]	135.33±56.67	163.17±38.83
EG	128.17±59.26[b]	150.17±25.43[a,c]	114.67±19.82	137.14±20.06

Note: [a]$P < 0.01$ compared with NC (negative control group); [b]$P < 0.05$ and [c]$P < 0.01$ compared with MC (model control group).

Table 6. Comparison of the number of capillaries among four groups at different time points ($\bar{x}\pm s$, n=6, vessels)

Group	1 week	2 weeks	3 weeks	4 weeks
NC	34.17±4.30	35.17±2.86	40.17±8.84	34.29±17.84
MC	30.50±4.85	43.17±13.88	68.83±30.51[a]	46.00±16.50
PC	38.83±7.63	45.00±20.27	36.50±11.36[c]	41.33±24.56
EG	44.33±16.50[b]	49.17±22.02	56.00±12.88	32.71±7.78

Note: [a]$P < 0.05$ compared with NC (negative control group); [b]$P < 0.05$ and [c]$P < 0.01$ compared with MC (model control group).

indicated that these collagens were highly cross-linked and suggested that some of these cross-links was involved in triple-helical regions of the molecule [7].

HPMC and PVA have been widely used as drug coating material inert materials. The obvious advantage was that they could blend with collagen in aqueous solution without any physical and chemical reaction. Collagen solution could form a dry film with high concentration. HPMC could increase the viscosity and elasticity of the gel in favor of adhesion and moist environment for the wound healing. PVA could enhance the flexibility of collagen membrane and keep the exchange of water vapor in the trauma. The water vapor transmission rate for normal skin was (204.0±12.0) g/m^2·24 h and (278.4±26.4) g/m^2·24 h for skin of grade I burns. In fact, we have found in previous studies that the collagen hydrogel dry membrane water vapor transmittance could reach 401.66 g/m^2·24 h.

Collagen hydrogel could promote the healing of excision wound and there was almost the same effect with EGF. The wound healing promoting effect of collagen hydrogel was better than the control group. The faster healing rate in the negative control group suggested that the immune function has not been destroyed. Continuous topical application of EGF to granulation tissue has been demonstrated to increase the rate of collagen accumulation in wounds. Studies have indicated that a single topical application of EGF would lead to a short period of elevated wound collagen content, followed by a rapid breakdown of the newly acquired collagen. Epidermal growth factor receptor (EGF-R) signaling was essential for growth of pancreatic islets. Even a modest attenuation of EGF-R signaling could lead to a severe defect in postnatal growth of the beta-cells, which leads to the development of DM [8, 9].

In this investigation, we found that bovine collagen gel dressings could promote the repair of chronic wounds mainly by stimulating and inducing local fibroblast proliferation, accelerating endogenous collagen synthesis in the wound area. Some researchers believed that bFGF could promote wound healing mainly because of the Akt phosphatidyl inositol 3 kinase and rho kinase signaling pathways involved in the instant activation process of Rac and rho proteins induced by bFGF, so as to activate the muscle fiber cells and fibroblasts and induce fibroblast apoptosis in the later for promoting wound healing and reducing the scar formation [10].

Angiogenesis, fibroplasia and new collagen synthesis could collectively lead to formation of visible, vascularized scar tissue. Analysis of the data and the literature supports the conclusions that activated macrophages in the wound could release substances to stimulate fibroplasia, collagen synthesis and angiogenesis *in vivo* [11]. Scholars have found some evidence proving that collagen could induce the angiogenesis for the wound by promoting cell chemotaxis and promote blood vessels and tissue regeneration for patients with gingival recession in root canal therapy [12]. Collagen-chitosan sponge scaffold encapsulated with thymosin beta 4 (CCSS-eTbeta4), an angiogenic factor had been use for treating the cutaneous wounds of diabetic rats with hind limb ischemia [13]. Collagen scaffold was used mainly for drugs or stem cells and cytokines carrier and control the

release of drugs in wound healing treatment [14-18]. Clinical studies have confirmed that the activation of platelet growth factor was very important for the repair of 38 patients with chronic nutritional ulcer [19]. A swine model of chronic ischemic wounds was repaired with a modified collagen gel dressing, and more macrophages appeared in the wound than ischemic wound after 7 days repair. In this study, we also found that newborn blood capillary number was obviously more than the control group and EGF-treated group in the first two weeks, and in the 3rd week, there was a significantly difference compared with the negative group. It could be speculated that gel dressings could up-regulate the expression of Mrc-1 (macrophages biomarkers) and induce the expression of anti-inflammatory cytokine, including IL-10 and b-FGF. There was a different up-regulated expression of TGF-beta, vascular endothelial growth factor (VEGF), Von Willebrand factor and type I collagen after 7 days wound healing. A large number of mature endothelial cells were recruited and it was rich in blood flow in the wound area in 21 days. Fibroblasts count showed that collagen gel dressings participated in the new angiogenesis and reconstruction of the new organization, promoting the wound repair by involved in the regulation of wound inflammation [20, 21]. This study investigated the effect of collagen/HPMC/PVA hydrogel to accelerate cutaneous wound healing and angiogenesis in streptozotocin-induced diabetic rats. The collagen hydrogel with HPMC and PVA has a promising value for clinical treatment of chronic ischemia wound or ulcer.

Collagen hydrogel dressing could effectively improve the microenvironment for chronic diabetic wounds, accelerate the proliferation of fibroblasts and promote the angiogenesis, thereby promoting the healing of diabetic defects and improving the quality of wound healing. Moreover, collagen hydrogel dressing yields no wound stimulation, hypersensitive reaction, infection or alternative adverse responses. Both collagen hydrogel dressing and recombinant growth factors share the similar role in the process of wound repairing and healing.

Acknowledgements

This project is supported by the Small and Mid-sized Enterprise Innovation Fund and Science and Technology Plan Projects of Guangdong Province. The authors highly appreciate the Center of Guangdong Medical Collagen Engineering Technology and the Laboratory Animal Center of Guangzhou University of Chinese Medicine for free use of experimental instrument and equipment. This study was supported by Marine Economy Innovation and Development Project (GD2012-B03-002), Science and Technology Plan Projects of Guangzhou (201508020126), Small and Mid-sized Enterprise Innovation Fund (11C2621441-3212).

Disclosure of conflict of interest

None.

Address correspondence to: Shunqing Tang, Department of Biomedical Engineering, College of Life Science and Technology Key Laboratory of Biomaterials of Guangdong Higher Education Institutes, Jinan University, Room 233, 2nd Floor, The 2nd Institute of Building, 601 Huangpu Road West, Tianhe District, Guangzhou 510632, Guangdong, China. Tel: +8620-85220469; Fax: +86208522-0469; E-mail: shunqing_tang@sina.com

References

[1] Harris MI. Epidemiology of diabetes mellitus among the elderly in the United States. Clin Geriatr Med 1990; 6: 703-19.

[2] Galkowska H, Wojewodzka U, Olszewski WL. Low recruitment of immune cells with increased expression of endothelial adhesion molecules in margins of the chronic diabetic foot ulcers. Wound Repair Regen 2005; 13: 248-54.

[3] Metzmacher I, Ruth P, Abel M, Friess W. In vitro binding of matrix metalloproteinase-2 (MMP-2), MMP-9, and bacterial collagenase on collagenous wound dressings. Wound Repair Regen 2007; 15: 549-55.

[4] Brett D. A review of collagen and collagen-based wound dressings. Wounds 2008; 20: 347-56.

[5] Holmes C, Wrobel JS, Maceachern MP, Boles BR. Collagen-based wound dressings for the treatment of diabetes-related foot ulcers: a systematic review. Diabetes Metab Syndr Obes 2013; 6: 17-29.

[6] Singh V, Devgan L, Bhat S, Milner SM. The pathogenesis of burn wound conversion. Ann Plast Surg 2007; 59: 109-15.

[7] Bashey RI, Bashey HM, Jimenez SA. Characterization of pepsin-solubilized bovine heart-valve collagen. Biochem J 1978; 173: 885-94.

[8] Miettinen PJ, Ustinov J, Ormio P, Gao R, Palgi J, Hakonen E, Juntti-Berggren L, Berggren PO, Otonkoski T. Downregulation of EGF receptor signaling in pancreatic islets causes diabetes due to impaired postnatal beta-cell growth. Diabetes 2006; 55: 3299-308.

[9] Hennessey PJ, Nirgiotis JG, Shinn MN, Andrassy RJ. Continuous EGF application impairs long-term collagen accumulation during wound healing in rats. J Pediatr Surg 1991; 26: 362-5; discussion 365-6.

[10] Abe M, Yokoyama Y, Ishikawa O. A possible mechanism of basic fibroblast growth factor-promoted scarless wound healing: the induction of myofibroblast apoptosis. Eur J Dermatol 2012; 22: 46-53.

[11] Hunt TK, Knighton DR, Thakral KK, Goodson WH 3rd, Andrews WS. Studies on inflammation and wound healing: angiogenesis and collagen synthesis stimulated in vivo by resident and activated wound macrophages. Surgery 1984; 96: 48-54.

[12] Wang HL, Modarressi M, Fu JH. Utilizing collagen membranes for guided tissue regeneration-based root coverage. Periodontol 2012; 2000: 140-57.

[13] Ti D, Hao H, Xia L, Tong C, Liu J, Dong L, Xu S, Zhao Y, Liu H, Fu X, Han W. Controlled release of thymosin beta 4 using a collagen-chitosan sponge scaffold augments cutaneous wound healing and increases angiogenesis in diabetic rats with hindlimb ischemia. Tissue Eng Part A 2015; 21: 541-49.

[14] Greenhalgh DG. Treating a collagen scaffold with a low concentration of nicotine-promoted angiogenesis and wound healing. J Surg Res 2013; 185: 543-44.

[15] Ichioka S, Kouraba S, Sekiya N, Ohura N, Nakatsuka T. Bone marrow-impregnated collagen matrix for wound healing: experimental evaluation in a microcirculatory model of angiogenesis, and clinical experience. Br J Plast Surg 2005; 58: 1124-30.

[16] O'Loughlin A, Kulkarni M, Creane M, Vaughan EE, Mooney E, Shaw G, Murphy M, Dockery P, Pandit A, O'Brien T. Topical administration of allogeneic mesenchymal stromal cells seeded in a collagen scaffold augments wound healing and increases angiogenesis in the diabetic rabbit ulcer. Diabetes 2013; 62: 2588-94.

[17] Hou C, Shen L, Huang Q, Mi J, Wu Y, Yang M, Zeng W, Li L, Chen W, Zhu C. The effect of heme oxygenase-1 complexed with collagen on MSC performance in the treatment of diabetic ischemic ulcer. Biomaterials 2013; 34: 112-20.

[18] Sun W, Lin H, Xie H, Chen B, Zhao W, Han Q, Zhao Y, Xiao Z, Dai J. Collagen membranes loaded with collagen-binding human PDGF-BB accelerate wound healing in a rabbit dermal ischemic ulcer model. Growth Factors 2007; 25: 309-18.

[19] Obolenskii VN, Ermolova DA. The use of thrombocytic growth factors and collagen-containing substances in treatment of the chronic wounds of various etiology. Khirurgiia (Mosk) 2012; 5: 42-7.

[20] Elgharably H, Ganesh K, Dickerson J, Khanna S, Abas M, Ghatak PD, Dixit S, Bergdall V, Roy S, Sen CK. A modified collagen gel dressing promotes angiogenesis in a preclinical swine model of chronic ischemic wounds. Wound Repair Regen 2014; 22: 720-29.

[21] Liem PH, Morimoto N, Ito R, Kawai K, Suzuki S. Treating a collagen scaffold with a low concentration of nicotine promoted angiogenesis and wound healing. J Surg Res 2013; 182: 353-61.