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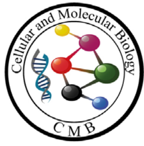

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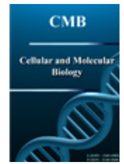

Original Article

Effect of collagen sponge combined with epidermal growth factor in repairing maxillofacial head and neck wounds in rats

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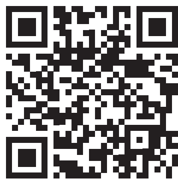

Abstract

Collagen sponge and epidermal growth factor (EGF) promote wound healing. However, the effect of collagen sponge combined with EGF in repairing maxillofacial head and neck wounds remains unclear. The rats were divided into 3 groups, including experimental group 1 (Vaseline gauze+EGF), experimental group 2 (collagen sponge+EGF) with control group (Vaseline+normal saline), and maxillofacial head and neck wounds were simulated. Wound pathological morphology was detected by HE staining; wound EGF, IL-1β, IL-6 along with TNF-α contents by ELISA and MMP1 level by western blot. At 7 and 14 days after treatment, wound healing rate of two experimental groups was higher than that of control group, and that of experimental group 2 presented higher than that of experimental group 1. Compared with control group, experimental group 1 had significantly fewer inflammatory cells in the wound tissue, local erythrocyte spillage outside the vascular walls, more collagen deposition and more granulation tissue. Compared with experimental group 1, inflammatory cells in wound tissues of experimental group 2 were significantly reduced, the collagen tissues were visible and arranged, and the growth of the wound granulation tissue was obvious. IL-1β, IL-6 along with TNF-α levels in two experimental groups presented lower than control group, and EGF level was higher. More importantly, in contrast to experimental group 1, IL-1β, IL-6 along with TNF-α in experimental group 2 presented lower, and EGF level presented higher. At 14 days after treatment, MMP1 level in two experimental groups was lower than control group. In contrast to experimental group 1, MMP1 level in experimental group 2 was lower. In summary, collagen sponge combined with EGF for the first time significantly improved the healing speed of maxillofacial head and neck wounds and reduced the scar left after wound healing.

Keywords: Collagen sponge, Epidermal growth factor, Maxillofacial head and neck wounds, MMP1, TNF-α.

1. Introduction

Oral and maxillofacial head and neck are not only the exposed part of the human body but also the concentrated reflection area of human aesthetics, the damage of this part has a great impact on the appearance and function [1]. Data have shown that maxillofacial head and neck injuries account for about 30%-40% of total body injuries, of which skin and soft tissue injuries account for about 60%-70% of maxillofacial injuries [2]. With the rapid development of social economy along with the continuous improvement of residents' living standards, people pay more attention to their appearance. When maxillofacial head and neck trauma, patients often have strong recovery aesthetic requirements, especially children, poor treatment often brings physical and psychological damage and blows to patients, coupled with the family's high expectations, easy to cause doctor-patient disputes [3]. Therefore, the treatment of maxillofacial head and neck trauma must take into account the principle of plastic surgery to reduce the scar left after wound healing as much as possible. At present, healing methods for scars have become the focus of attention and research by many scholars, but so far, the therapeutic effect is still unable to meet people's pursuit of beauty [4].

Collagen sponge is made of beef tendon as raw material. After selecting and removing impurities, fat and fascia tissue, it is cleaned and disinfected, purified type I collagen is prepared by enzymatic hydrolysis, acid, alkali and organic solvent treatment, and then cross-linked, frozen and dried into spongy form [5]. Collagen sponge has the following functions [6, 7]: (1) It can be degraded, has certain water absorption, and expands after contact with blood; (2) It can induce fibroblast derivation, and aggregation, stimulate collagen production and arrangement, stimulate capillary formation, ensure blood supply and oxygen supply required for gross tissue formation, and promote wound healing; (3) It can induce mineral deposition, promote the formation of new bone, and induce osteogenesis; (4) It can induce chemotactic monocytes, macrophages and white blood cells to enhance local resistance; (5) It has anti-fibri-

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nolytic effect and significantly strengthens the hemostatic process. At present, collagen sponge has been widely used in wound repair, tissue filling and other fields in clinical work, and has achieved good curative effects.

It has been found that growth factors have a crucial role in wound healing, and the growth factors involved in wound repair contain PDGF, TGF-β, IGF-1, bFGF, KGF and EGF [8]. In the process of wound repair, the functions of growth factors include [9, 10]: (1) Guide neutrophils and macrophages into the wound area to clear the necrotic tissue; (2) Promote proliferation of fibronuclear epidermal cells; (3) Promote the synthesis and release of the body's growth factors; (4) Elevate the formation of granulation tissue; (5) Promote the synthesis and accumulation of extracellular interstitial molecules; (6) Inhibit scar tissue hyperplasia. Among them, EGF has a strong ability to promote wound healing, promote cell division, promote the synthesis of extracellular matrix such as hyaluronic acid, fibulnexin, glycoprotein and hydroxyproline acid, and thus reduce scar hyperplasia [11].

In view of this, our study selected rats to prepare maxillofacial head and neck skin and soft tissue trauma models, applied molecular biology technology, through histomorphology and immunohistochemical system observation, and studied the effect and mechanism of collagen sponge combined with EGF in maxillofacial head and neck trauma repair through animal experiments, so as to offer scientific basis for its clinical application and promotion.

2. Materials and methods

2.1. Animals

Thirty healthy adult Wistar rats without special pathogen grades were selected, both male and female, weighing 160~210 g. Before the experiment, all the rats fasted for 12 h, but did not restrict their water intake. The study met the ethical standards established by the unit and was approved by the Committee of our hospital.

2.2. Animal model of maxillofacial head and neck wound

Wistar rats were treated with peritoneal anesthesia of 3% pentobarbital sodium (150 mg/kg) to prepare full-layer skin defect wounds on the maxillofacial head and neck of rats. In other words, the full-layer skin of 0.5 cm × 0.5 cm was randomly cut by scalpel in 3 places on the maxillofacial head and neck of rats. The wound should be wet and applied with saline gauze.

2.3. Debridement of animal maxillofacial head and neck trauma

Physical methods were used to completely debridement, and after hemostasis, the wound was repeatedly rinsed with normal saline and dilute iodophor. After the wound was cleaned, the wound was covered with normal saline gauze for use.

2.4. Animal grouping

This study was conducted using the same body self-control. A maxillofacial head and neck wound was randomly selected and treated with Vaseline gauze of the same size, and EGF was sprayed once every 6 hours (experimental group 1). A maxillofacial head and neck wound was randomly selected to be filled with collagen sponge of the same size as the wound, and EGF was sprayed once every 6 hours (experimental group 2). A maxillofacial head and neck wound was randomly treated with Vaseline gauze of the same size, and normal saline was sprayed once every 6 hours (control group). The experimental work in this study was carried out by three project participants.

2.5. Observation indicators

The wound healing rate in two experimental groups and the control group were observed. Wound healing rate refers to the percentage of the area of the postoperative wound shrinkage to the original wound area. The wound area of each group was calculated by using the method of film grid number. Wound healing rate = (original surface of the wound - actual measured area) ÷ original area of the wound ×100%.

The histological changes of rats in two experimental groups and the control group were observed. After wound healing, samples were taken from 2 experimental groups and the control group, and the specimens were dehydrated and transparent by routine after fixation, impregnated with wax, and then prepared into 4 μm thick continuous sections, which were adhered to the slide coated with polylysine, and the histopathological structure was observed under an optical microscope after hematoxylin-eosin (HE) staining. The thickness of newborn epithelium was measured once in each field and averaged in 5 fields under a 40 × optical microscope.

The serum levels of epidermal growth factor (EGF), interleukin-1β (IL-1β), interleukin-6 (IL-6) along with tumor necrosis factor α (TNF-α) were compared between 2 experimental groups and the control group. 0.5 ml venous blood was collected 1 d before surgery and 5 d after surgery on an empty stomach. After anticoagulation, centrifuge was used for 3000 r/min for 15 min. After standing for 10 min, upper serum was taken and stored at -20° ice. Serum levels of EGF, IL-1β, IL-6 along with TNF-α were detected by enzyme-linked immunosorbent assay (ELISA).

Fibrocollagenase-1 (MMP1) was detected by western blot. 200 μL of protein lysate was added to per 100 mg of tissue and lysed on ice for 30 min. Followed by centrifugation at 12000 rpm at 4°C for 15 min the supernatant was collected. The protein concentration was determined with the BCA protein concentration detection kit, and the sample was boiled with SDS-PAGE loading buffer for 5 min. Then, 20 μg protein was subjected to 10% SDS-polyacrylamide gel electrophoresis, the membrane was transferred in a 100 V constant pressure ice bath for 2 h, and then closed with 5% skim milk at room temperature for 1 h, followed by incubating with primary antibody including anti-MMP1 (1:500) and anti-β-actin (1:500) overnight at 4°C. Next, the membrane was cultivated with secondary antibody (1:3000) at room temperature for 1 h. The membrane was subjected to ECL luminescent solution for reaction, and exposed to the gel imaging system. Image lab 3.0 software was used to obtain and analyze the images. The expression level of the MMP1 was determined by the ratio of the gray value of the target protein band to the gray value of the internal reference β-actin band.

2.6. Statistical analysis

SPSS 23.0 statistical software was used for analysis, and the measurement data were tested normality, expressed as mean ± standard deviation (x±s), and t-test was adopted for comparison. Counting data was presented as [n (%)],

and chi-square test or Fisher exact probability method was adopted for comparison. P<0.05 meant significant difference.

3. Results

3.1. Wound healing rate in 3 groups

On the 3rd day after treatment, no significant difference was seen in wound healing rate among the 3 groups (P>0.05). On the 7th and 14th day after treatment, the wound healing rate of two experimental groups was higher than that of control group (P<0.05), and that of experimental group 2 presented higher than that of experimental group 1 (P <0.05, Fig. 1).

3.2. Pathological morphology of wound tissue in 3 groups

On the 14th day after treatment, a large number of inflammatory cells gathered and infiltrated, a small amount of fiber and collagen were deposited, and granulation tissue was formed on the wound of rats in control group. In contrast to control group, the inflammatory cells in the wound tissue of rats in experimental group 1 were significantly reduced, local red blood cells could be seen to overflow outside the blood vessel wall, a large amount of collagen was deposited, and granulation tissue was increased. Compared with experimental group 1, the inflammatory cells of the wound tissue in experimental group 2 were significantly reduced, the collagen tissues were clearly visible and arranged, and the growth of the wound granulation tissue was obvious (Fig. 2).

3.3. Serum levels of EGF, IL-1β, IL-6 and TNF-α in 3 groups

On the 14th day after treatment, the serum levels of IL-1β, IL-6 along with TNF-α in two experimental groups were lower than control group, and EGF level in two experimental groups was higher (P<0.05). More importantly, in contrast to experimental group 1, the serum levels of IL-1β, IL-6 along with TNF-α in experimental group 2 were lower, and EGF level in experimental group 2 was higher (P<0.05, Fig. 3).

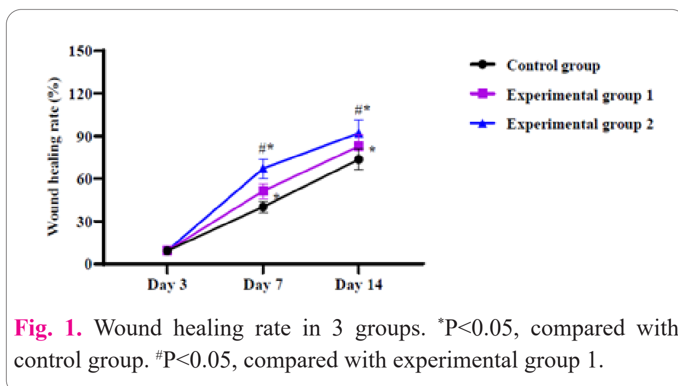

Fig. 1. Wound healing rate in 3 groups. *P<0.05, compared with control group. #P<0.05, compared with experimental group 1.

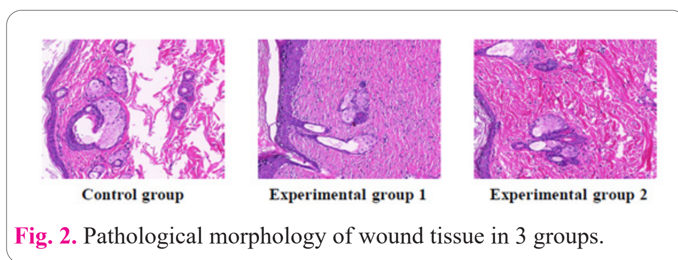

Fig. 2. Pathological morphology of wound tissue in 3 groups.

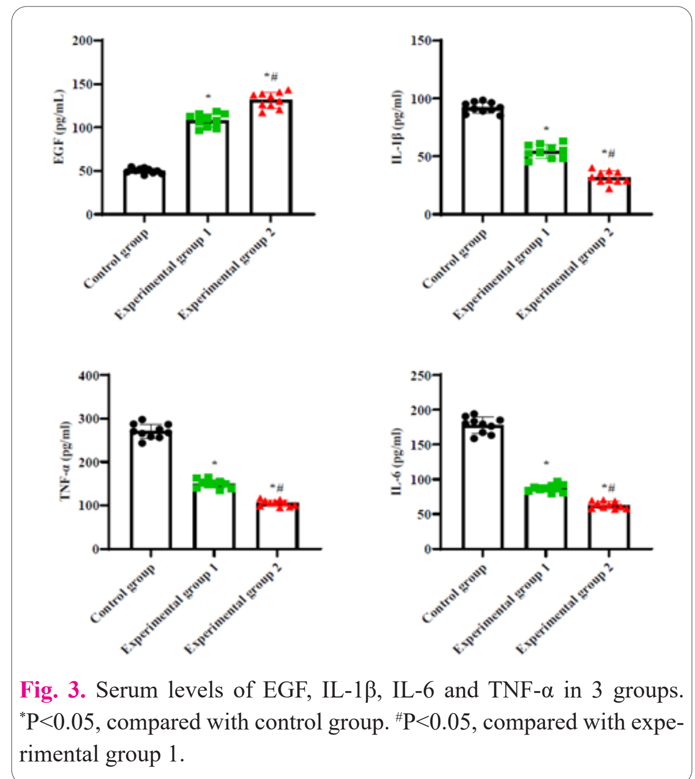

Fig. 3. Serum levels of EGF, IL-1β, IL-6 and TNF-α in 3 groups. *P<0.05, compared with control group. #P<0.05, compared with experimental group 1.

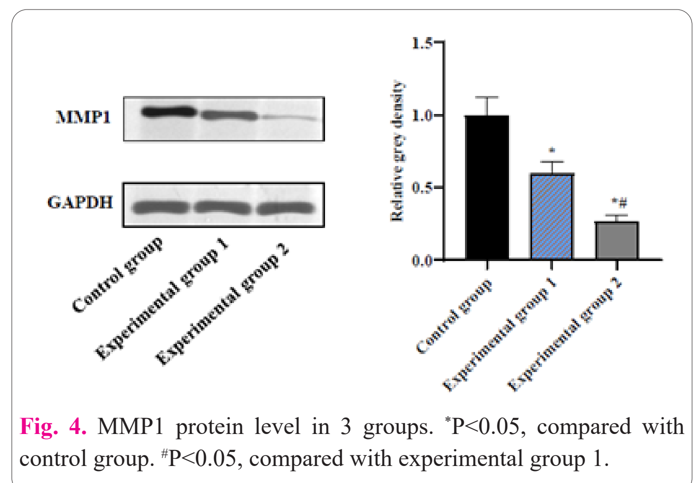

Fig. 4. MMP1 protein level in 3 groups. *P<0.05, compared with control group. #P<0.05, compared with experimental group 1.

3.4. MMP1 protein level in 3 groups

On the 14th day after treatment, the protein level of MMP1 in two experimental groups presented lower than control group. In contrast to experimental group 1, the protein level of MMP1 in experimental group 2 was lower (Fig. 4).

4. Discussion

Maxillofacial head and neck wounds are mostly caused by trauma, infection, tumor surgery and many other reasons, such as untimely wound repair and improper treatment, which often cause scar healing, seriously affect the appearance and function, and then cause physical and mental harm to the patient to leave a psychological shadow. Therefore, wound healing without scar as much as possible has always been one of the urgent problems to be solved in aesthetic oral surgery.

For maxillofacial head and neck trauma, the traditional method is timely debridement and hemostasis according to the depth and degree of trauma, and cosmetic suture or flap repair if necessary [12]. After the wound healed completely, anti-scar treatment is performed with pressure method and anti-scar drugs. Recently, with the continuous

development of laser technology, the current application of laser and photon for anti-scar treatment has also obtained a certain effect [13]. Traditional anti-scar therapy has a long treatment time and poor comfort, mainly due to poor patient compliance, especially in children, and the treatment effect is not satisfactory [14]. Laser therapy also requires multiple courses of continuous treatment, and the treatment cost is high, and cannot be widely promoted in the clinic [15].

Maxillofacial head and neck skin is thin, which is easy to cause dermal or even full-layer skin injury after trauma, and the wound repair is slow, and obvious scar tissue is often left after healing [16]. In addition, the maxillofacial head and neck are rich in blood flow, the amount of wound bleeding and exudation after trauma is more, and there are physiological spaces in this part, which is difficult to bandage and drainage, and easy for secondary wound infection, resulting in poor wound healing [17]. All these are the main causes of scar hyperplasia after maxillofacial head and neck trauma. According to studies on scarless healing after skin trauma, the expression of scar healing gene loci can be induced by inhibiting the expression of scar healing gene loci, promoting the orderly arrangement of collagen, and thus achieving scarless healing [18].

Collagen sponge is a kind of biomedical material with a structure similar to human collagen. It has a reticular porous structure and can induce the infiltration and proliferation of repair cells in the dressing [19]. The collagen content is up to 98%, which has the effects of hemostasis, promoting wound healing and sustained release in small doses [20]. Collagen sponge also has good biocompatibility, biodegradability, and weak antigenicity, and can be degraded and absorbed by collagenase in the healing process. After covering the wound, it can be degraded by the body and eventually produce various amino acids that can be absorbed by the body to provide nutrients for wound repair [21].

Many scholars at home and abroad have confirmed that growth factors can significantly promote wound repair and tissue regeneration, such as nerve, inflammation, ulcer, and tissue transplantation repair [22]. Some scholars have also found that growth factors can accelerate the clearance of necrotic tissue in the wound, improve the formation of capillaries and the regeneration ability of epithelial tissue, and significantly shorten the healing time of the wound [23]. As for the principle of reducing scar, relevant studies have suggested that growth factors can reduce the proportion of type I/III collagen, which has a positive effect on inhibiting scar formation [24]. Other studies have shown that EGF has a strong ability to promote wound healing, promote cell division, promote the synthesis of extracellular matrix such as hyaluronic acid, fibronectin, glycoprotein and hydroxyproline acid, and thus reduce scar hyperplasia [25]. In clinic, EGF is widely used in the repair of acute and chronic wounds such as burns and wounds, and has a certain effect [26].

In our study, the results suggested that on the 7th and 14th day after treatment, the wound healing rate of two experimental groups was significantly higher than that of control group, and that of experimental group 2 presented higher than that of experimental group 1. All these results suggested that collagen sponge combined with EGF could accelerate the healing of maxillofacial head and neck wounds in rats, which was consistent with previous studies [27].

The wound-healing process is very complex and depends on the participation of various collagen, soluble mediators, cytokines and inflammatory cells [28]. TNF-α is a multifunctional Th1 cytokine mainly produced by activated mononuclear macrophages [29]. When the body is injured and stimulated, TNF-α will be released rapidly to stimulate the host defense mechanism and chemotactic other immune cells to clear wound infection [30]. IL-1β is synthesized and secreted by a variety of cells and can mediate the immune response in the early stage, participate in local anti-inflammatory, phagocytosis and clearance of necrotic tissue [31]. IL-6 is an inflammatory factor synthesized and secreted by a variety of cells, which has a key role in the body's immune regulation and is a key inflammatory transmitter and regulatory factor in the body [32]. At the same time, MMPs play a very important role in repairing the growth of tissue cells and inflammatory cells and promoting wound epithelialization and neovascularization [33]. MMP1 is a crucial member of the MMPs family, and most of its hydrolyzed products are the main components of collagen after denatured and in the extracellular matrix [34]. Under physiological conditions, MMP1 and other factors complement each other to regulate the body and promote embryo formation, organ growth, cell renewal, wound repair, healing, etc [35,36].

In our study, the results suggested that on the 14th day after treatment, the serum levels of IL-1β, IL-6, TNF-α and MMP1 in two experimental groups presented lower than control group, and EGF levels in two experimental groups was higher. More importantly, in contrast to experimental group 1, the serum levels of IL-1β, IL-6, TNF-α and MMP1 in experimental group 2 presented lower, and EGF level in experimental group 2 was higher. All these results suggested that collagen sponge combined with EGF could promote the healing of maxillofacial head and neck wounds in rats via inhibiting inflammatory response and MMP1 expression, which was in line with previous report [21].

5. Conclusion

In summary, collagen sponge and EGF have incomparable advantages over other materials in promoting wound healing and reducing scar tissue hyperplasia. In this project, collagen sponge combined with EGF for the first time can significantly improve the healing speed of maxillofacial head and neck wounds and reduce the scar left after wound healing. More importantly, the operation is simple and the treatment cost is low, which is expected to provide a new idea for wound repair.

Conflict of Interests

The authors declare no competing interests.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

We have received approval from the Experimental Animal Ethics Committee of Quanzhou First Hospital.

Informed Consent

Not applicable.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

WZ contributed to the study conception and design. Experimental operation, data collection and analysis were performed by WQ, LT and HY. The first draft of the manuscript was written by WQ and all authors commented on previous versions of the manuscript.

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