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Effect of rmEGF combined with ELF-EMF on promoting wound healing in rats

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Abstract.
BACKGROUND: The process of wound healing is complex, and expediting it remains a challenge. The advantages of extremely low frequency electric and magnetic fields (ELF-EMF) are its non-invasive treatment, promotes healing and promotes myogenesis of C2C12 cells. Epidermal growth factor (EGF) is known to play a vital role in promoting wound healing, so a combination of ELF-EMF and EGF can have far-reaching significance.
OBJECTIVE: To study the effect of recombinant murine epidermal growth factor (rmEGF) combined with ELF-EMF on wound healing.
METHODS: Thirty-six rats were randomly divided into three groups: normal control group, EGF group, and ELF-EMF+EGF group, and a 20 mm × 20 mm dorsal wound was made. The wound healing rate of rats was calculated on the 3rd, 7th, 11th and 15th day. HE staining was used to observe the micro-morphological changes during the wound healing process.
RESULTS: The wound healing rate of EGF+ELF-EMF group was better than other groups. On the 15th day of wound healing, the wounds of each group were completely healed. On the 3rd, 7th, 11th and 15th day of HE staining, the early inflammatory cell infiltration, the arrangement of fibroblasts and the number of new capillaries in the wounds of EGF+ELF-EMF group were better than those of the other groups.
CONCLUSIONS: rmEGF combined with ELF-EMF significantly promotes wound healing in SD rats.

Keywords: Extremely low frequency electromagnetic field, ELF-EMF, recombinant murine epidermal growth factor, rmEGF, epidermal growth factor, EGF, epidermal growth factor receptor, EGFR, sprague-dawley rats, SD rats

1. Introduction

Due to a prolonged time in the healing of wounds, wound infection may get further aggravated, and osteomyelitis or sepsis may occur in severe cases [1,2,3,4]. Therefore, the rate of wound healing remains an important concern. Currently, there are many treatment methods, but they all have their own problems and shortcomings; hence, there is an urgent need for effective adjuvant treatment methods [5]. Epidermal growth factor is known to promote wound healing but in a steady manner. Literature studies have shown that ELF-EMF can produce positive biological effects on the body and is a non-toxic and non-invasive

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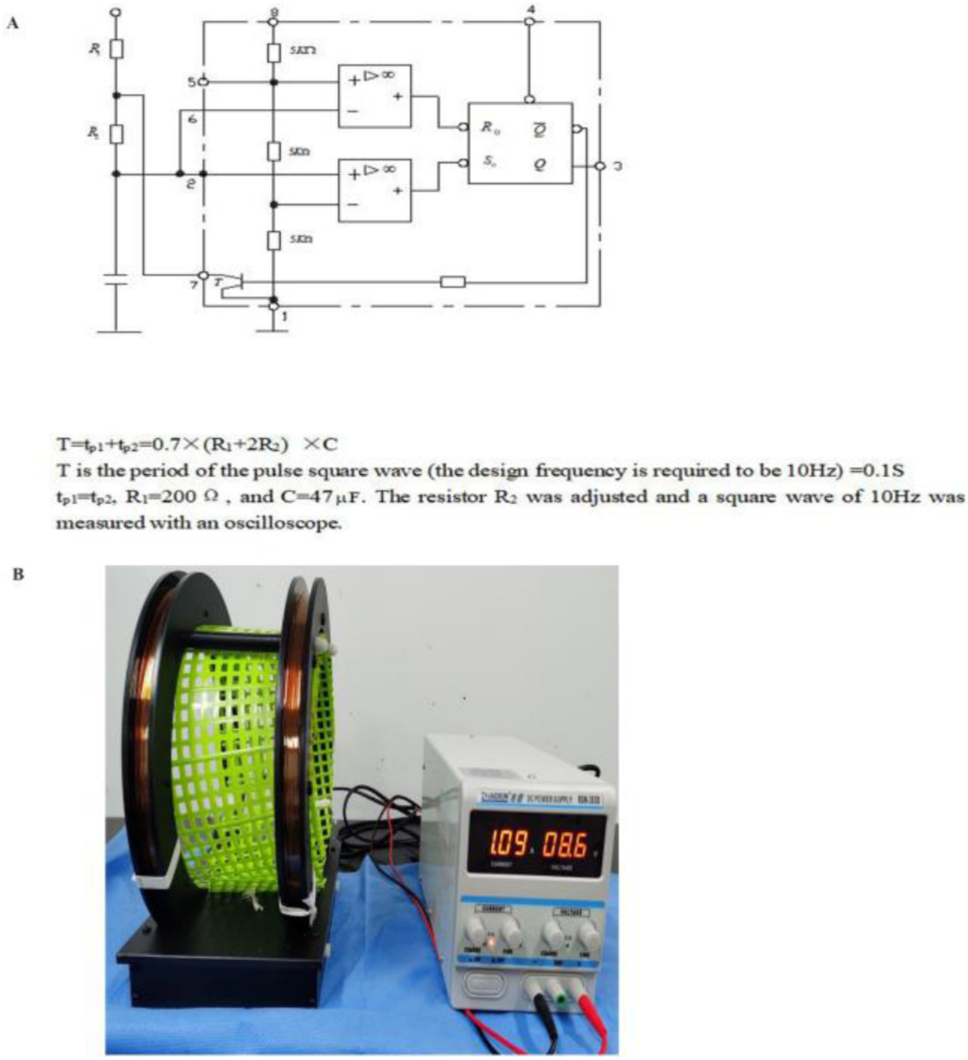

Fig. 1. Schematic representation of ELF-EMF equipment. A. Circuit diagram of ELF-EMF; B. ELF-EMF instrument with the intensity of 10 Hz/1.5 mT in operation.

method [6,7,8]. Therefore, this study envisaged the application of recombinant murine epidermal growth factor combined with ELF-EMF was used to treat the wound in SD rats. The wound healing was measured at different time points, and the morphological changes of each group were observed by HE staining, so as to study the effect of rmEGF combined with ELF-EMF on wound healing.

2. Material and methods

2.1. ELF-EMF equipment

The ELF-EMF equipment used in the study is shown in Fig. 1. The frequency of the magnetic field was 10 Hz, and a Helmholtz coil was used to ensure the uniformity of the magnetic field strength in the coil.

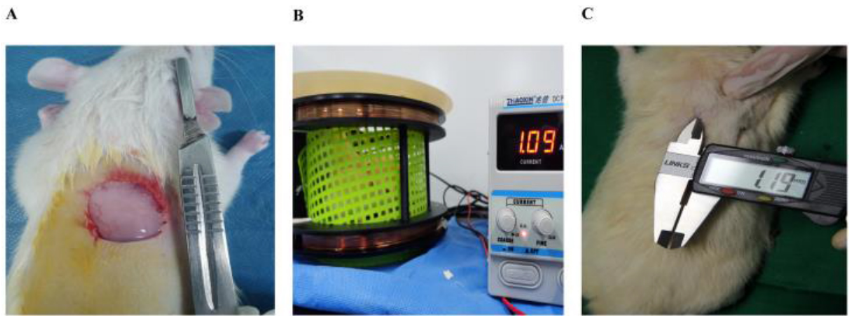

Fig. 2. Establishment of the animal model and the magnetic field intervention after grouping. A is wound establishment; B is SD rats exposed to 10 Hz/1.5 mT stimulation; C is measurement of wound healing.

2.2. *Animal model*

Anesthesia was induced by injecting 10 ml/kg of avertin (1.25%) intraperitoneally. After successful anesthesia, the rats were shaved, disinfected, and placed with bedding. A dorsal wound 20 mm $\times$ 20 mm was made on the back of the rats (Fig. 2-A), and then grouped: The SD rats in the normal control group were not treated with rmEGF and had no ELF-EMF intervention. SD rats in EGF alone group were treated with rmEGF with no ELF-EMF intervention. SD rats in the EGF+ELF-EMF group were treated with rmEGF and exposed to 10 Hz/1.5 mT ELF-EMF (Fig. 2-B).The rats received the 10 Hz/1.5 mT magnetic field intervention for 2 hours per day. The wound healing rate of rats was calculated and the samples were stained at different time intervals (3 d, 7 d, 11 d, and 15 d) (Fig. 2-C).

2.3. *HE staining of wound tissue*

The original wound on the back of the rats was harvested with a size of about 20 mm $\times$ 20 mm. Paraffin sections were fixed with 4% paraformaldehyde solution for 12 hours, rinsed with distilled water, dehydrated by alcohol using stepwise gradient and immersed in absolute ethanol xylene solution. The paraffin was removed overnight, the slices were subjected in warm water, the slices were dewaxed, stained, and finally sealed with neutral tree glue. They were observed and photographed under a microscope.

2.4. *Statistical analysis*

All experiments were repeated three or more times and analyzed by GraphPad Prism9.4.1(681) software. The data between multiple groups were tested for homogeneity of variance, and the data between groups were analyzed by one-way analysis of variance. If the homogeneity of variance test was not obtained, the rank-sum test was used to analyze the data between groups. $^{*}P < 0.05$, $^{**}P < 0.01$ indicates that the difference was statistically significant.

3. Results

3.1. *Calculation of wound healing rate*

On the third day of wound healing, the average wound healing rate was 20% in normal control group, 35% in EGF alone group, and 50% in EGF combined with ELF-EMF group. On the 7th day of wound

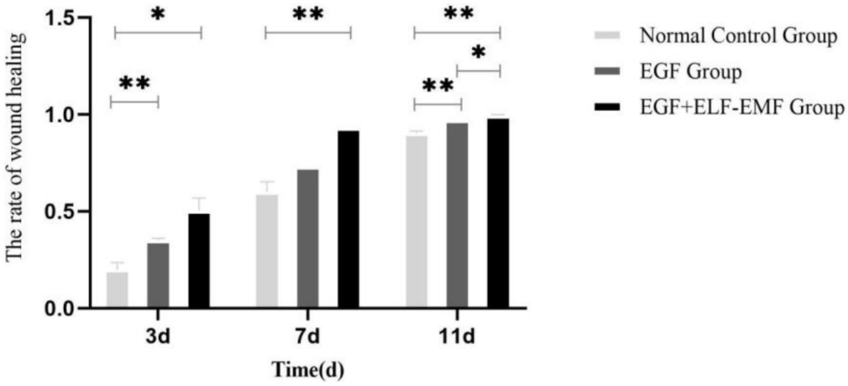

Fig. 3. Wound healing rate of different experimental groups.

healing, the average wound healing rate of normal control group was 60%, the average wound healing rate of EGF alone group was 73%, and the average wound healing rate of EGF+ELF-EMF group was 93%. On the 11th day of wound healing, the average wound healing rate of normal control group was 90%, the average wound healing rate of EGF alone group was 97%, and the average wound healing rate of EGF+ELF-EMF group was 99%. On the 15th day of wound healing, the wounds of all the groups were completely healed, and some wounds had scar formation. The results showed that on the 7th day of wound healing, the wound healing rate of the EGF combined with ELF-EMF group increased rapidly, compared with the control group, and the difference was statistically significant ($^{**}P < 0.01$). On the 11th day of wound healing, the wound healing rate of each group was higher, and the EGF combined with ELF-EMF group exhibited the best wound healing rate, compared with the control group, and the difference was statistically significant ($^{**}P < 0.01$; Fig. 3).

3.2. HE staining of wound tissue

On the third day of wound healing in the normal control group, an obvious inflammatory cell infiltration was noted with no evident endothelial cell proliferation. Further, the number of fibroblasts was less than other groups, and the arrangement was disordered (Fig. 4-3d-A). On the 3rd day after injury, there were proliferation of vascular endothelial cells, less capillary formation, more inflammatory cells, larger cell bodies of fibroblasts, and round and oval nuclei in granulation tissue in the EGF alone group, which were less than those in EGF combined with ELF-EMF group (Fig. 4-3d-B). At the same time, in the EGF combined with ELF-EMF group, the new granulation tissue showed proliferation of vascular endothelial cells, neovascularization with inflammatory cell infiltration, obvious proliferation of fibroblasts, and round or oval nuclei with deep staining (Fig. 4-3d-C).

On the 7th day of wound healing, in the normal control group, there were a few capillaries formed in the granulation tissue, the cells were mainly inflammatory cells, the fibroblasts were more than before, but less than other groups, and the capillaries were rare (Fig. 4-7d-A). In EGF alone group, there were more fibroblasts with darker color, active angiogenesis, and fewer inflammatory cells on the 7th day after injury (Fig. 4-7d-B). In the EGF combined with ELF-EMF group, a reduced inflammatory cell infiltration, a large number of fibroblasts, dark staining, and more capillary components were noted (Fig. 4-7d-C).

On the 11th day of wound healing, in the normal control group, the inflammatory cells were gradually reduced, the fibroblasts were significantly increased and arranged in a vortex disorderly shape (Fig. 4-11d-A). In the EGF alone group, the number of capillaries was reduced, the number of mature fibroblasts was

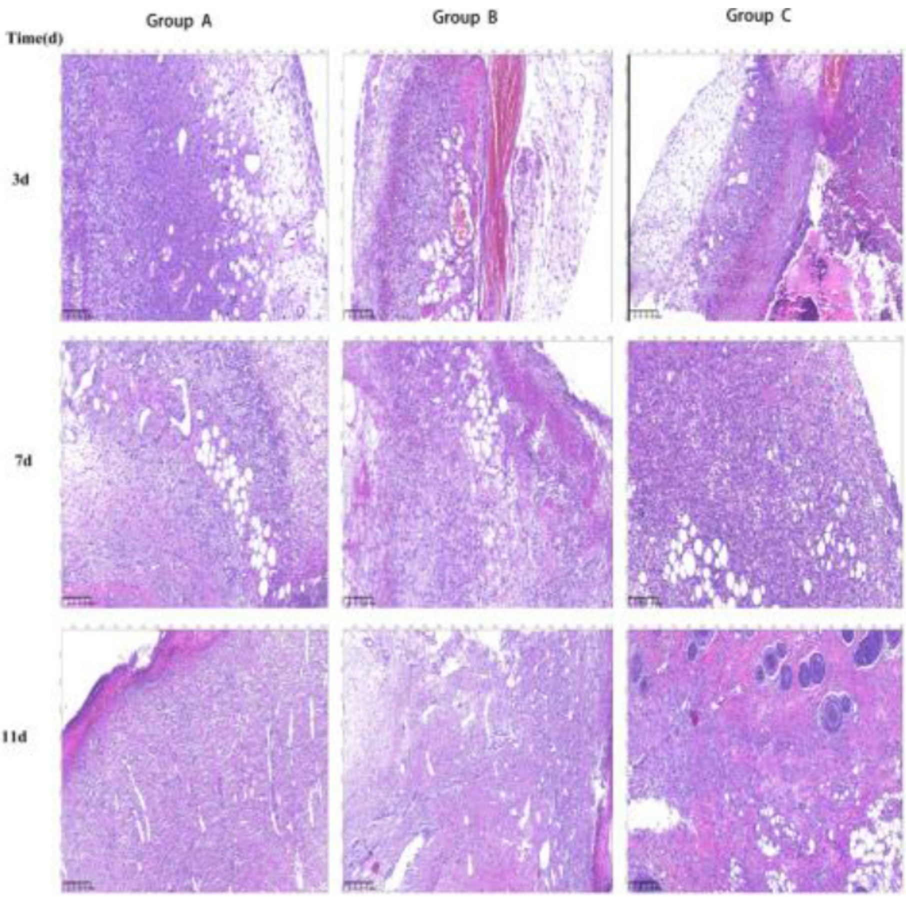

Fig. 4. Wound healing at different time intervals in different groups. Group A is the normal control group, Group B is the EGF group, and Group C is ELF-EMF+EGF group.

increased, cells were arranged in bundles, and the arrangement between bundles was disordered (Fig. 4-11d-B). In the EGF combined with ELF-EMF group, the number of new capillaries and inflammatory cells was reduced, the number of mature fibroblasts was increased, the cells were arranged in bundles, and the red matrix components were uniform (Fig. 4-11d-C).

3.3. *Observation of wound healing in each group*

After all the wounds healed (15th day), a large number of fibroblasts were observed in the wound specimens of EGF combined with ELF-EMF group. The cell body of fibroblasts was large, the nuclei were regular, the fibers were arranged in an orderly manner, and the skin epidermis was thickened. The skin appendages were significantly better than the other groups (Fig. 5-A/B/C).

4. Conclusion

This study was aimed to study the effect of recombinant murine epidermal growth factor (rmEGF) combined with ELF-EMF on wound healing in SD rats. The wound healing rate of EGF+ELF-EMF

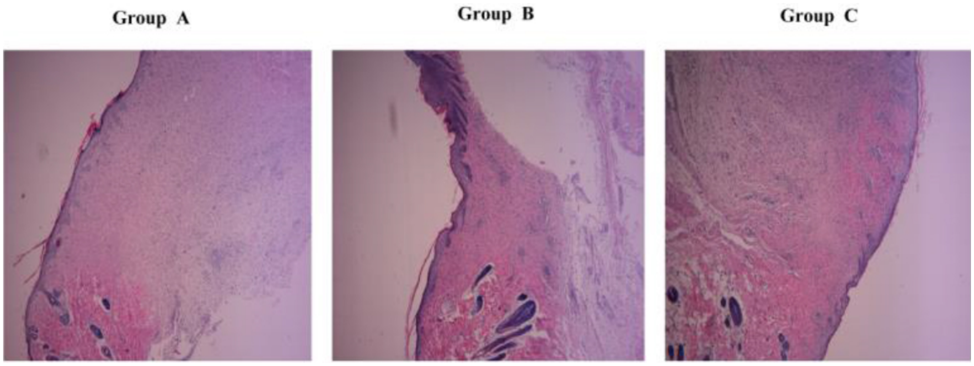

Fig. 5. Differences in HE staining of fibrous tissue within the healed wound on the 15th day.Group A is the normal control group, Group B is the EGF group, and Group C is ELF-EMF+EGF group.

group was the fastest compared with other groups.On the 3rd, 7th, 11th and 15th day of HE staining, the early inflammatory cell infiltration, the arrangement of fibroblasts and the number of new capillaries in the wounds of EGF+ELF-EMF group were better than those of the other groups. Thus, rmEGF combined with ELF-EMF can significantly promote the wound healing of SD rats.

5. Discussion

Wound healing is an extremely complex and orderly process [9]. EGFR is expressed in almost all kinds of cells, such as epidermal cells, fibroblasts, vascular endothelial cells, etc. [10]. EGF combines with EGFR to exert its biological activity, which can promote the formation of granulation tissue, proliferation of fibroblasts, and induce them to synthesize collagen fibers [11]. EGF acts on TGF-β to down-regulate the expression of fibroblasts and actin, inhibit the contraction of fibroblasts, and enable collagen to be arranged in an orderly manner to reduce scar formation [12,13]. Hence, exogenous EGF is generally added in the process of wound repair for faster wound healing [14]. Recombinant epidermal growth factor (rmEGF) is an exogenous epidermal growth factor prepared by gene recombination technology, which is very similar to the endogenous epidermal growth factor considering the biological activity, physiological function and structure [15]. Relevant studies have confirmed that there is a negative feedback regulation mechanism between the rmEGF and its receptor, so it does not cause complications such as excessive cell proliferation, allergy, and gene mutation [16,17].

There are exhaustive studies on the combination of recombinant epidermal growth factor with other drugs or treatment methods to accelerate wound healing; however, the synergistic effect was not significant. A large number of studies [18,19,20] have shown that extremely low frequency and low intensity electromagnetic fields can produce significant biological effects on organisms with little or no radiation, which lays the feasibility of this experimental study. Moreover, previous experiments have confirmed that 10 Hz/1.5 mT ELF-EMF has significant biological effects on proliferation and differentiation of C2C12 cells [21]. This is the reason for selecting the magnetic field parameter of 10 Hz/1.5 mT. Electromagnetic fields are known to regulate the inflammatory response of wounds and promote wound healing by regulating the activity of immune cells like neutrophils and macrophages [22]. It may also affect the wound healing process by up-regulating the expression of anti-inflammatory cytokines and down-regulating the expression of pro-inflammatory cytokines in the wound. In addition, the time of electromagnetic field exposure is also a hotly discussed issue. According to the literature [1,2,3], the irradiation time was different in different studies with different irradiation time, but the effect of 2-6 h irradiation was better, so 2 h was selected as the irradiation time in this experiment.

The combined application of rmEGF and ELF-EMF in wound healing has the advantages of a "non-thermal effect" of the ELF-EMF, along with fewer side effects on organisms due to the small radiation employed. At present, the mechanism of non-thermal effects is supported by the resonance theory, cyclotron resonance theory, etc. In this study, on the 7th day of wound healing, EGF combined with ELF-EMF group healed faster than other groups, and EGF combined with ELF-EMF group promoted the wound healing more rapidly. On the 11th day of wound healing, EGF combined with ELF-EMF group exhibited the best wound healing. The HE staining results also showed that the number of capillaries and fibroblasts in EGF combined with ELF-EMF group were better than those in other groups, and the cells were arranged in an orderly manner.

This study found that 10 Hz/1.5 mT combined with EGF could accelerate wound healing and exhibited a certain positive effect. However, the specific mechanism is not clear. It may possibly promote the migration and proliferation of keratinocytes and accelerate wound re-epithelialization, since electromagnetic fields are known to promote skin and soft tissue wound healing by promoting neovascularization and relaxation of local blood vessels [23,24]. This needs to be confirmed by subsequent studies. In addition, the effects of adding a simple magnetic field group or adding different intensities of 10 Hz on wound healing need to be considered, which can make this experimental study more convincing and reflect the rationality of the experimental design.

Acknowledgments

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Conflict of interest

None of the authors have any conflicts of interest to report.

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