Authentic Human Epidermal Growth Factor: A Panacea for Wound Healing

WKR Wong*, KL Ng and Xiuhua Hu

Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China

*Corresponding Author: WKR Wong, Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China.

Received: July 26, 2018; Published: August 20, 2018

Abstract

Timely and effective treatment for injuries/wounds could greatly reduce the risks of deterioration, infections, and more seriously, undergoing major surgical operations. Authentic human epidermal growth factor (aEGF; 53 amino acids) and human basic fibroblast growth factor (abFGF; 146 amino acids), which are functionally versatile skin growth factors, have been shown to be capable of promoting wound healing effectively. However, due to the confusions caused by unauthentic EGF and bFGF isoforms or derivatives, in conjunction with their high prices, the applications of aEGF and abFGF have been underrated. In this communication, we present the results of the application of aEGF to the effective treatments of various wounds, which included diabetic foot ulcers, Steven-Johnson Syndrome and bedsores, employing a facile topical method easily administered by even a layman. In addition, as demonstrated by a professional cosmetologist, when aEGF was working in conjunction with abFGF, an efficient healing effect on damaged skin resulting from micro-needling was observed. Further research studies would help to elucidate whether the two skin growth factors might work co-operatively to provide effective treatments for other serious skin disorders/problems including deep wounds and hard-to-heal chronic wounds.

Keywords: Authentic Primary Structure; EGF; bFGF; Diabetic Foot Ulcers; Bedsores; Steven-Johnson Syndrome; Micro-Needling; Restoration

Abbreviations

EGF: Human Epidermal Growth Factor; bFGF: Human Basic Fibroblast Growth Factor; aEGF: Authentic EGF; abFGF: Authentic bFGF; DFU: Diabetic Foot Ulcers; SJS: Steven-Johnson Syndrome; HA: Hyaluronic Acid

Introduction

Being the largest organ in the human body, the skin is subjected to a wide variety of insults to result in injuries or wounds. Very often, delays in providing the wounds with timely and proper treatments could result in deteriorating conditions, infections, and more seriously, the requirement of surgical operations.

Despite the availability of a large number of substances or compounds for wound healing, our group has been interested in the application of two functionally versatile skin growth factors: human epidermal growth factor (EGF) and human basic fibroblast growth factor (bFGF) to the treatments of various wounds. However, whether the two factors possess their authentic structures: EGF is a 53-residue polypeptide [1], whereas bFGF is composed of 146 amino acids [2], is an important consideration determining the performance of the two proteins. There are two major concerns about the use of both factors as active ingredients. First, both factors are very expensive; commercially available EGF may cost as high as US$ 0.250 million g⁻¹ [3], whereas bFGF is even much more expensive, costing US$ 1.2 million g⁻¹ [4]. The second concern is that EGF and/or bFGF procured from commercial sources commonly possess unauthentic structures [5-9], thus appearing as isoforms or derivatives. Growth factor derivatives might not only be less bioactive and stable than their authentic counterparts [10], but they have also been speculated to exhibit better affinities with receptor mutants, thus resulting potentially in malignant transformation [11]. As far as EGF is concerned, the 53-residue authentic protein has been shown to be highly stable and potent [12]. Moreover, it is the mature form being produced and circulated in our body to exercise various physiological functions, and thus, it is expected to be safe for applications.

In view of the practicalities of authentic skin growth factors, we determined to engineer host systems to express recombinant EGF and bFGF possessing the native structures, which comprise 53 and 146 amino acids, respectively [12-15]. Employing an extracellular production strategy, an E. coli excretion system was engineered to successfully express authentic recombinant EGF (aEGF) [12,13,15]. With a refined fed-batch fermentation process, excretion of aEGF from the new expression system was efficiently enhanced to a high level of 325 mg liter⁻¹ [13]. The cost-effectively produced aEGF has been applied for the treatments of scalds [16] as well as other skin disorders or problems [17-19]. In all treatments, aEGF has been shown to be highly effective, safe and stable as reported previously [10,20].

Recently, with the use of inteins or protein introns [21] to mediate expression and auto-catalytic cleavages of fusion proteins, our group has demonstrated, for the first time ever, the successful production of recombinant authentic bFGF (abFGF) using both E. coli and B. subtilis as hosts [14,22,23]. Through the application of genetic enhancement and a refined fermentative protocol, the production of abFGF has been largely enhanced to an impressive yield of 610 mg liter⁻¹ [22]. The cost-effective availability of abFGF has enabled our study of its application to skincare and wound healing. In this communication, the results of our use of aEGF, either alone or in conjunction with abFGF, for the treatments of various wounds are reported. All the treatments were achieved at home, supporting the convenience and effectiveness of the treatment protocol.

Materials and Methods

**Sources of aEGF and abFGF**

Expression of aEGF and abFGF in E. coli JM101 (pWKW2) and JM101 (pWK311RomPAd) transformants, respectively [13,15,22], was achieved in fermentors as described previously [13,22]. Details of purification and characterization of aEGF and abFGF were reported previously [15,24].

**Preparation of media containing growth factors**

The “Platinum cream” containing 0.04% (w/w) aEGF was purchased from a Hong Kong company: Gene-viante Limited. The cream base, Watsons Aqueous Cream [25], was purchased from Watsons Hong Kong. For the medium prepared to treat Steven-Johnson syndrome, 0.1% (v/v) glycerol (as aEGF carrier) and 0.01% (w/w) aEGF were supplemented to sterile normal saline solution. In micro-needling, the hyaluronic acid (HA) solution introduced into skin contained 0.5% (w/v) sodium hyaluronate (pharmaceutical grade) and 0.0003% (w/w) aEGF. An enhanced version of the HA solution was also supplemented with 0.0003% (w/w) abFGF. All of the preparations were carried out under aseptic conditions.

**Treatments of diabetic foot ulcers and bedsores**

For treating diabetic foot ulcers (DFU) or bedsores at home, an affected area was first cleansed thoroughly with Dettol [16,26] diluted as recommended by the manufacturer. The disinfected area was then topically applied with a slight film of Platinum cream according to the protocol described previously [26].

**Treatments of wounds resulting from micro-needling**

The micro-needling machine (Model: SH-4; Beijing Sea Heart International Science and Technology Co., Ltd.) contained a 9-pin head for introducing HA solution into the skin. After the process, the face was cleaned with sterile wipes, followed by gently adding sufficient HA solution to cover up all the wounds on the face. Afterwards, a paper mask soaked in the same solution was applied on the face for 20 min to allow restoration. The same protocol was employed if HA enhanced with abFGF was used.

Results and Discussion

Rationale for the study

Our previous findings showed that when treatments of diabetic or scald wounds [16,17,26] were administered under the supervision/care of a physician/nurse, even with the use of a simple protocol of topical application of aEGF to the sites of injury, the outcomes were
Authentic Human Epidermal Growth Factor: A Panacea for Wound Healing

highly effective [16,17,26]. Despite the high efficacies of αEGF shown in wound treatments, due to various difficulties, which include: (1) successful attainment of the national patent concerned; (2) accomplishment of αEGF production under current Good Manufacturing Practice Regulations; (3) fulfilment of FDA clinical trial requirements; (4) the presence of numerous biologically inactive and/or unstable EGF variants possessing unauthentic structures which have negative impacts on the identity and functional performance of αEGF; (5) long durations and expensive investments to overcome the above mentioned hurdles, αEGF has not been registered as a drug in first world countries [10,27]. Thus, a large population of potential users may not be aware of the availability of αEGF for applications. Fortunately, αEGF may still be employed for use as an active ingredient in healthcare products. The results revealed below involved patients whose skin injuries/disorders were successfully treated with αEGF, either alone or in combination with abFGF, even when the operations were undertaken by laypersons at home.

Treatments of diabetic wounds

Patients suffering from Diabetes mellitus may experience many health-threatening complications, among which the development of Diabetic Foot Ulcers (DFU) is a common example. Globally, about 6.3% of diabetic patients will develop DFU [28], whereas in China, the rate of occurrence of DFU is 4.1% [28]. The annual amputation rate for those suffering from DFU in China is 5.1% [29]. Thus, considering the large population of China, 56 million people are afflicted with DFU, and 2.8 million individuals will receive amputations. All these numbers are shockingly high, and the incidents impose difficult challenges on not only the patients and their families, but also on the health and social welfare system. However, the figures shown above might not represent the entire picture as individuals with mobility difficulties might not be officially registered, and thus they might not receive the expected care services.

Previously, through a collaboration with United Christian Hospital in Hong Kong, we reported that αEGF was employed successfully in treating DFU [17,26]. In the current study, we were interested in knowing whether αEGF was as effective in promoting the healing of DFU when the application was administered at home in the absence of professional assistance. Two elderly women living in China were referred to us for assistance. Both patients were diabetic and shown to be afflicted with DFU, which were badly deteriorated and untreatable using conventional methods. Despite the pessimistic diagnosis of both DFU, they were successfully treated using an active cream supplemented with 0.04% (w/w) αEGF [17,18,26] and a facile topical application protocol [26] to achieve, delightfully and amazingly, complete healing (Figure 1 and 2). Once again, our results provide strong evidence for our previous findings [17,18,26] that αEGF is very effective in treating DFU.

---

**Figure 1: Treatments of diabetic wounds: case 1.**

An 85-year-old lady residing in Shenzhen, China, was afflicted with DFU. The affected areas essentially concentrated on the toes. After 15 days of topical application of Platinum cream to the ulcers, healing signs including wound healing and restoration of blood circulation displayed on the four small digits. Significant recovery was observed after 28 days of treatment and after 7 months, the last picture received from the patient, showed complete healing of the wounds.
Figure 2: Treatments of diabetic wounds: case 2.
A 96-year-old lady residing in Beijing, China, was afflicted with DFU on her left lower limb. In addition to edema, a deep ulcerated wound measuring approximately 1.4 cm in diameter was shown on the affected limb. After applying Platinum cream to the wound for 12 days or so, incredibly, remarkable healing of the wound was observed. The wound continued to heal and after 25 days of treatment, the wound healed completely.

Treatment of Steven-Johnson Syndrome
A teenage girl was suffering from acute allergy to some kind of traditional Chinese medicine which was used to ease premenstrual syndrome. A few days prior to admission to a local hospital, Kwong Wah Hospital, she was afflicted with sore throat, itchy eyes and skin allergies. Despite treatments with eye drops and antibiotics, no noticeable improvement was observed. Worse still, small blisters and rashes began to appear on her body and face. She was then admitted to the Children’s Intensive Care Unit (CICU) at Kwong Wah hospital. At CICU, however, her allergic symptoms deteriorated rapidly in the next two days. Worst of all, she experienced pain in her oral cavity and desquamation of her lips (Figure 3a); moreover, she was unable to eat solid food. She was then transferred to CICU of another local hospital, Queen Mary Hospital (QMH), where she was diagnosed to suffer from an autoimmune disease, Steven-Johnson syndrome (SJS). However, there was nothing that the doctors at QMH could do to help the victim recover from the serious illness. After reading our article previously published on treating SJS [19], we were approached by the victim’s sister for assistance. It was decided that the top priority was to treat her oral cavity and lips, thus enabling her to eat solid food. Her oral cavity and lips were rinsed and applied topically, respectively, with a saline preparation containing 0.01% (w/w) aEGF and 0.1% glycerol, two times daily. Incredibly, after 2 days, the lips started to heal and the swelling subsided (Figure 3b). On the other hand, after her face was treated with aqueous cream supplemented with 0.005% (w/w) aEGF for 7 days, the rashes and blisters faded significantly (Figure 3c).
Figure 3: Treatment of Steven-Johnson Syndrome.
A teenage girl suffering from Steven-Johnson Syndrome (SJS) was treated with aEGF. a) Before treatment stands for before treating with aEGF solution, the girl experienced severe pain in her oral cavity and desquamation of her lips. b) The wounded lips started to heal after 2 days of aEGF treatment. c) After 7 days of treatment with aEGF products, the conditions shown on her face and lips showed remarkable improvements.

The results support the notion that aEGF functions not only effectively on open wounds, but also well to heal ulcerations occurring in our oral cavity.

Treatment of bedsores

Due to lack of mobility or mobility dysfunctions, many elderly people residing in nursing homes suffer from bedsores or pressure sores, which are localized damages to the skin [30]. An old lady residing in an elderly home was found to suffer from an onset of bedsores in which inflamed patches began to appear around her hip (Figure 4). To prevent the bedsores to deteriorate, the ulcerated regions were topically treated [26] twice daily with Platinum cream (Materials and methods). Encouragingly, the ulcerated areas healed readily and complete healing was achieved within 10 - 12 days (Figure 4). The results support the view that aEGF might not only be effective in treating bedsores, but it might also be capable of preventing the development of these skin problems.

Figure 4: Treatment of bedsores.
An old lady suffered from an early stage of bedsores with inflamed patches detected around her hip region. The affected areas were topically treated with aEGF supplemented cream (Platinum cream). The duration (in days) of the treatment is shown on top of the picture. Day 0 stands for prior to the treatment.
Treatments of wounds resulting from micro-needling

Micro-needling, also known as collagen induction therapy, is a process that involves the use of needles to puncture hundreds of tiny holes into the skin. During the process, many invisible puncture wounds are generated on the skin. During the rejuvenation process, the skin is stimulated to produce more collagen and thus improve the texture of the skin. A common approach to treating fresh wounds generated by micro-needling is to avoid subsequent skin inflammation. In a case study, it was reported that a female client who had just been treated with micro-needling was shocked to see numerous tiny wounds accompanied by swelling and redness displayed on her face (Figure 5a). The results showed that the well-known hyaluronic acid (HA) is not effective enough to provide wound healing restoration, although all the unpleasant marks on her face faded away in a couple of weeks.

After three months, the same lady received micro-needling treatment again. This time, a major difference was that HA worked in conjunction with both aEGF and abFGF in both the treatment and the succeeding restoration steps (Materials and methods). Miraculously, subsequent to restoration, there was no trace of undesirable side effects, which swiftly emerged after the last micro-needling treatment (Figure 5a), appearing on the client’s face this time (Figure 5b). The lady remarked that she was extremely pleased with the outcome.

**Figure 5:** Treatments of wounds resulting from micro-needling: case 1.

*The micro-needling treatment and subsequent restoration of the skin (wound healing) were performed using: a) HA alone; b) HA together with aEGF and abFGF.*
In addition, it was well demonstrated in the current study that an assortment of aEGF and abFGF performed more efficiently than aEGF did alone, despite the high potency of aEGF in wound healing reported above and previously [16,17,19,26]. A male client was shown to undergo an apparently thorough restoration, using aEGF, abFGF in conjunction with HA, which prevented effectively the formation of wounds and other undesirable side effects on the face subsequent to micro-needling treatment (Figure 6b). However, if abFGF was excluded, given the same treatment process, aEGF was unable to attain the same flawless restoration (Figure 6a).

The results support the view that aEGF, being able to promote growth of epidermal cells, when functioning together with abFGF, being a potent angiogenic and mitogenic agent [2,31,32], may exhibit a synergistic effect on wound healing (Figure 6b). Additional research studies would help to elucidate whether the two skin growth factors might collaborate equally effective in treating other skin disorders including deep wounds and hard-to-heal chronic wounds.

Conclusions

Authentic EGF (aEGF), at a concentration of 0.04% (w/w), was shown to be effective in treating various skin disorders/problems including diabetic foot ulcers and bedsores, even when the treatments were administered by laymen. The factor was not only shown to

perform efficiently on open wounds, but also shown to work well to expedite the healing of oral ulcerations that occurred in a patient suffering from Steven-Johnson Syndrome. Moreover, as revealed in micro-needling treatments, the bioactivity formed between αEGF and abFGF was more potent than that of αEGF acting alone, suggesting the notion that the two factors might be employed co-operatively to treat more serious and a wider spectrum of skin disorders/problems.

**Acknowledgements**

This study was supported by Research Contract No.1617219-0 managed by the R&D Branch, HKUST.

**Conflict of Interest**

None.

**Bibliography**


4. PeproTech. Recombinant Human FGF-basic (146 a.a.).


27. Administration TG. Scheduling delegate’s final decisions. 3.7 Epidermal growth factor (2018).


Volume 3 Issue 4 September 2018
©All rights reserved by WKR Wong, et al.