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Research Article

The Importance of Authentic Human Epidermal Growth Factor in Offering Effective Treatments for Hard-To-Heal Wounds

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Abstract

Skin regeneration and repair of wounds in a timely and effective manner could highly reduce the risk of infection. Authentic human epidermal growth factor (aEGF) consisting of 53 amino acids is a functionally versatile protein, which is able to stimulate the proliferation of epidermal cells. However, due to the availability of unauthentic EGF derivatives (EGF isoforms) in the market, the identity of aEGF is mystified by the presence of these isoforms. Although aEGF has been shown to be safe and effective in promoting wound healing, it is suspicious that EGF isoforms could offer the same level of performance. In this communication, we present results to support that aEGF, which was derived from the extracellular production of a recombinant *Escherichia coli* excretion system, is highly effective in treating different kinds of wounds, including chronic disorders such as diabetic foot ulcers, bedsores, and various hard-to-heal wounds such as scalds and surgical injuries. In many cases, difficulties were encountered in initial treatments using other methods before aEGF was employed to result in successful healing.

Keywords: *Authentic EGF; Bioactivity; Bedsores; Diabetic Foot Ulcers; EGF Isoforms; Scalds; Surgical Wounds*

Abbreviations

aa: Amino Acid(s); aEGF: Authentic EGF; abFGF: Authentic bFGF; CABG: Coronary Artery Bypass Grafting; CAD: Coronary Artery Disease; DFU: Diabetic Foot Ulcers; bFGF: Human Basic Fibroblast Growth Factor; EGF: Human Epidermal Growth Factor; PC: Platinum Cream

Introduction

As a result of his discovery of human epidermal growth factor (EGF) in the early 1960s and subsequent research achievements on this 53 amino acid (aa) polypeptide, Stanley Cohen won the Nobel Prize in Physiology and Medicine in 1986 [1,2]. Subsequently, numerous studies have shown that EGF performs a wide range of physiological and wound healing functions in our bodies [3-12]. Due to its ability to stimulate proliferation and regeneration of epidermal cells, EGF has been applied as an active ingredient in cosmetic products and formulations prepared for the treatment of various skin ailments.

Diabetic foot ulcer (DFU) is a chronic disease which has been a growing concern worldwide. Globally, an estimated population of 400 million patients is currently afflicted with diabetic mellitus (DM) [13]. Of all DM victims, 15% of them (about 58 million) in their lifetime, will develop a foot ulcer, and among 15% of the DFU patients (about 1 million) will require amputations [14,15]. Several new treatment modalities including the applications of an oxygen chamber, platelet derived growth factor (PDGF), and various local dressings have been

reported with various levels of success and a long healing time of at least 6 months [7,16,17]. In the early 2000s, a research collaboration between our team and Dr. M. W. Tsang's team at United Christian Hospital, Hong Kong, successfully applied EGF, in conjunction with wound cleansing and debridement, to achieving a phenomenally high healing rate (95%) in treating DFU, which was 57% higher than the DFU wounds treated with cleansing and debridement alone [4]. Despite the high healing rate, the high market price of EGF (HK$2 million g^{-1}) [18,19] and the prevalent availability of counterfeit (unauthentic) EGF molecules (derivatives or isoforms) prevent the widespread application of the 53 aa long authentic EGF (aEGF) polypeptide [11].

To help much reduce the market price of aEGF, our group has developed a cost-effective recombinant protocol for the production of it [20,21]. More importantly, the resulting aEGF has been employed as an active ingredient for effective treatments of various hard-to-heal wounds and other skin disorders [4-12]. In the studies, aEGF has been shown to be highly effective, stable and safe as an active ingredient for applications [4-12].

In this communication, the results of our application of EGF to the treatments of various types of problematic wounds including DFU, bedsores, scalded skin and large surgical wounds are reported. Many of the treatments were accomplished at home; the results reinforce that the treatment protocol is convenient, effective and safe for implementation.

Materials and Methods

Source of EGF

Expression of authentic EGF (aEGF) in *E. coli* JM101 [pWKW2] transformants was achieved as described previously [21]. Details of purification and characterization of aEGF were reported previously [20].

Assessment of bioactivity, toxicity and hypersensitivity of aEGF

The bioactivity of aEGF was determined according to published protocols [20]. To ensure that aEGF would not impose health harmful effects or elicit hypersensitivity, the polypeptide was certified to be safe for applications by Guangdong Medical Laboratory Animal Centre, which is affiliated with the Department of Health of Guangdong Province in China.

Source of aEGF cream employed for the treatments

The Actovegin cream plus 0.04% (w/w) or 0.02% (w/w) aEGF employed for treating patients suffering from DFU at United Christian Hospital was developed as described previously [4]. The "Platinum Cream" (PC) containing 0.04% (w/w) aEGF for treating various wounds at home was available from a Hong Kong company: Gene-vinate Limited (www.gene-vinate.com).

Treatments of different types of wounds

For treating diabetic foot ulcers, bedsores and other wounds at home, an affected area was first cleansed thoroughly with Dettol [9,10] diluted as recommended by the manufacturer. The disinfected area was then topically applied with a slight film of PC according to the protocol described previously [9] and summarized as follows: 1) cleanse with diluted Dettol (or other disinfectants such as hydrogen peroxide); 2) air dry the disinfected wound; 3) apply a slight film of PC topically onto it; 4) cover the treated wound with a gauze pad. Monitor the progress of healing; repeat (1) to (4) once or twice daily if necessary.

Results

Treatments of Diabetic Foot Ulcers

An adverse effect commonly found in DM patients is insufficient blood supply in their extremities, thus leading to the development of a health-threatening complication, DFU [22]. In view that the effectiveness of applying EGF to the treatments of DFU [4,11,23] was controversial and that EGF might be carcinogenic upon applications [24] in the early days, we started off to express recombinant authentic EGF

(aEGF) which shares the same primary structure, comprising 53 aa, with native EGF identified in our body [20,21,25,26]. It was hopeful that aEGF would be as safe and effective as native EGF when it was employed for use in wound healing.

In a randomized, controlled, double-blind study conducted by a collaborative effort between our group and United Christian Hospital in the early 2000s, aEGF produced in our laboratory was shown to be highly effective in treating DFU [4]. In the investigation, 95% of DFU successfully healed with a topical treatment regimen employing 0.04% (w/w) aEGF (Figure 1 and 2), which was 50% higher than the wounds treated with a lower, 0.02% (w/w), aEGF or the placebo Actovegin Cream that did not contain aEGF [4].

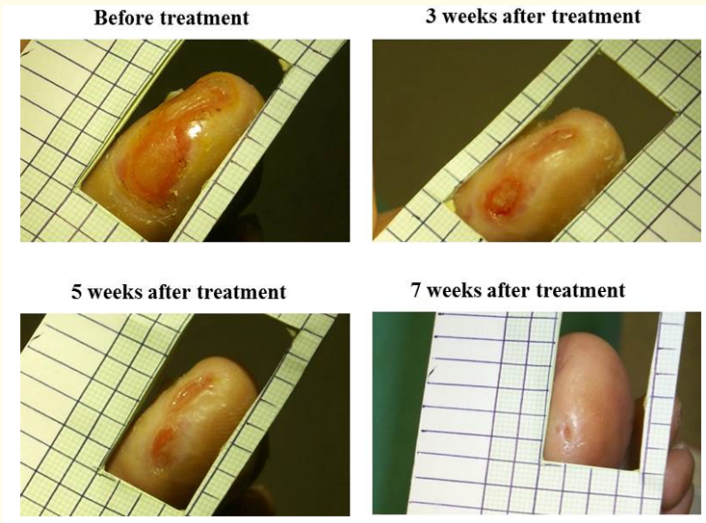

Figure 1: *Treatment of DFU located on a big toe.*
The ulcerated toe on the left foot of a diabetic patient was treated with Actovegin cream plus 0.04% (w/w). The healing progressed satisfactorily and reached a complete stage in about 7 weeks.

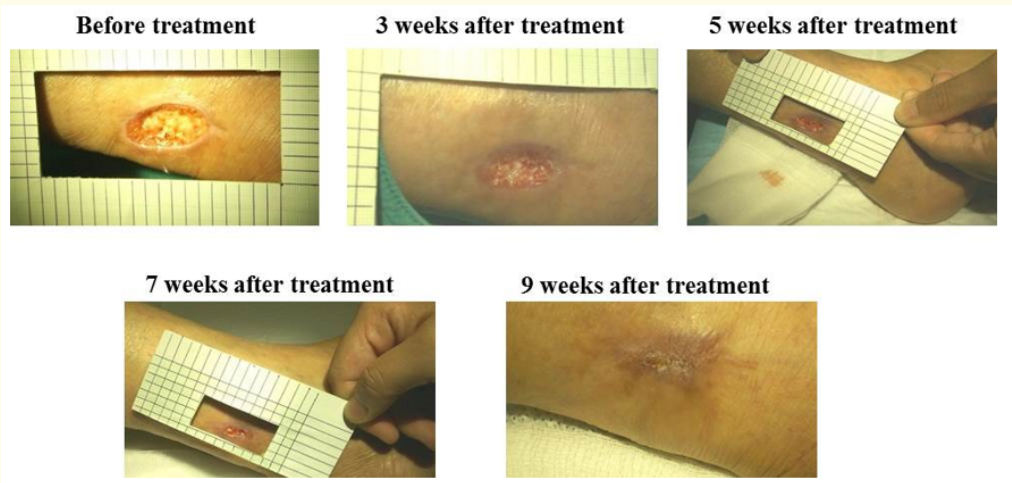

Figure 2: *Treatment of DFU located at the lower back region of a leg.*
The affected area at the lower back region of the left leg of a diabetic patient was treated with Actovegin cream plus 0.04% (w/w). The wound healed effectively and attained complete healing in about 9 weeks.

More importantly, to enable a larger population of patients to benefit from the healing effect of aEGF, this 53 aa polypeptide has been applied as an active ingredient to the development of commercial skincare products. One of them, the Platinum Cream (PC), which is simply made of Aqueous Cream comprising 0.04% (w/w) aEGF (www.gene-vinate.com), has been employed domestically to result in successful healing of serious DFU. An example was the successful treatment of the badly ulcerated toes of an 85-year-old woman with PC at home [12]. Despite over a rather long period of 7 months, the wounds healed nicely and completely (Figure 3).

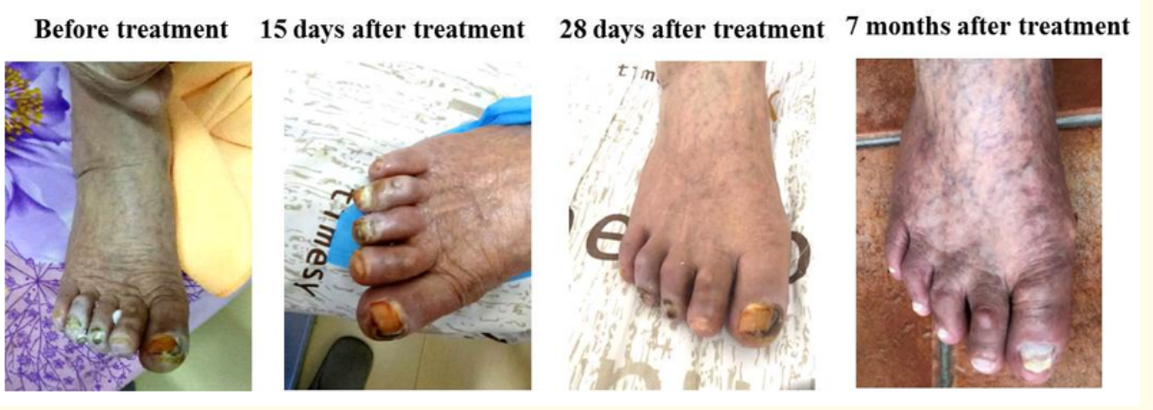

Figure 3: *Treatment of ulcerated toes.*

The ulcerated toes on the right foot of an 85-year-old lady afflicted with DFU were treated with Platinum Cream (PC; Materials and methods). After 15 days of treatment, signs of improvement including wound healing and restoration of blood circulation were noted on the digits. The improvements continued and after 7 months of treatment, complete healing of the wounds was achieved.

Treatments of bedsores

PC has been shown to be effective in treating other hard-to-heal wounds, e.g., bedsores, scalds and incisions. Due to lack of mobility or mobility dysfunctions, a large population of elderly people, e.g., those residing in nursing homes, suffer from bedsores or pressure sores, which are localized damages to the skin.

Two 80-year-old ladies accommodated in two separate elderly homes were found to suffer from different extents of bedsore [7,12]. One of them suffered from an early onset of bedsore where inflamed patches began to appear on the surface of her hip region (Figure 4) [12]. As aEGF treatment (once daily) was started quite early at the developmental stage of the bedsore, the result was highly impressive and the wound healed in about 12 days (Figure 4).

Figure 4: *Treatment of bedsores located on an 80-year-old lady.*

The affected areas located around the hip region of an 80-year-old lady suffered from an early stage of bedsores were treated with PC. After 12 days of treatment (Day 12), complete healing of the wound was noted. Before treatment stands for prior to PC treatment.

On the other hand, the other lady was found to develop a severe bedsore (Figure 5) [7]. Moreover, the affliction resulted in the formation of pemphigoid and blisters; the lady was treated with prednisone for more than 8 months. In view of the ineffectiveness of the steroid hormone treatment, she was then referred to medical services for assistance and was taken care by Dr. M.W. Tsang of United Christian Hospital. Due to infection, the ulcerated region was first treated with hibitane dressing for two weeks, followed by aEGF treatment (twice daily) with PC for a prolonged period of 3 months. Despite the seriousness of the bedsore, the skin disorder received complete healing in about 10 weeks (Figure 5).

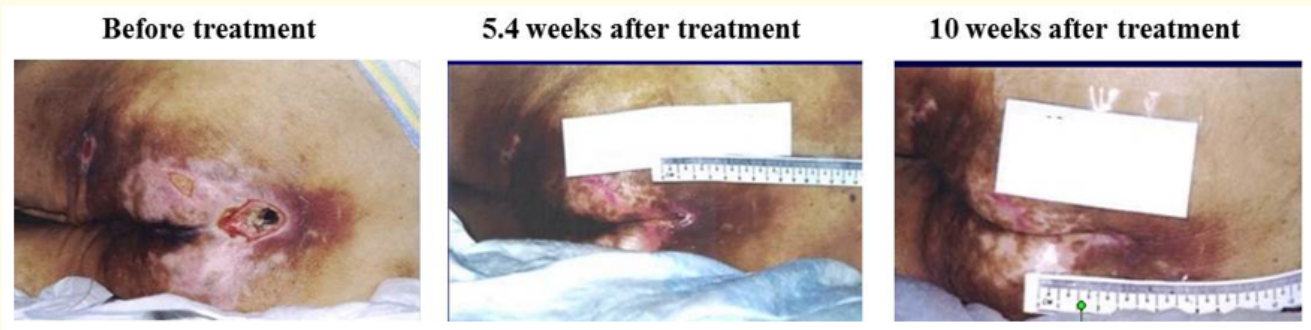

Figure 5: *Treatment of bedsores located on a bedridden old lady.*
The affected areas located around the hip region of a bedridden old lady suffered from severe bedsores were treated with PC. The ulcerated area received complete healing after around 10 weeks of treatment. Before treatment stands for prior to PC treatment.

Treatments of scalds

PC has also been employed to treat various scalded injuries of differing levels of severity. In a recent incident, a 52-year-old woman was scalded at a region right between the arm and the forearm of her left upper limb (Figure 6). Although the wound was treated initially with a commercial ointment for burns, there was no sign of recovery and infections appeared on the third day of the accident (Figure 6). Subsequently, the scald was treated twice daily with PC for 6 days. Despite the discouraging observations to begin with, the wound completely healed on the sixth day of the PC treatment program (Figure 6).

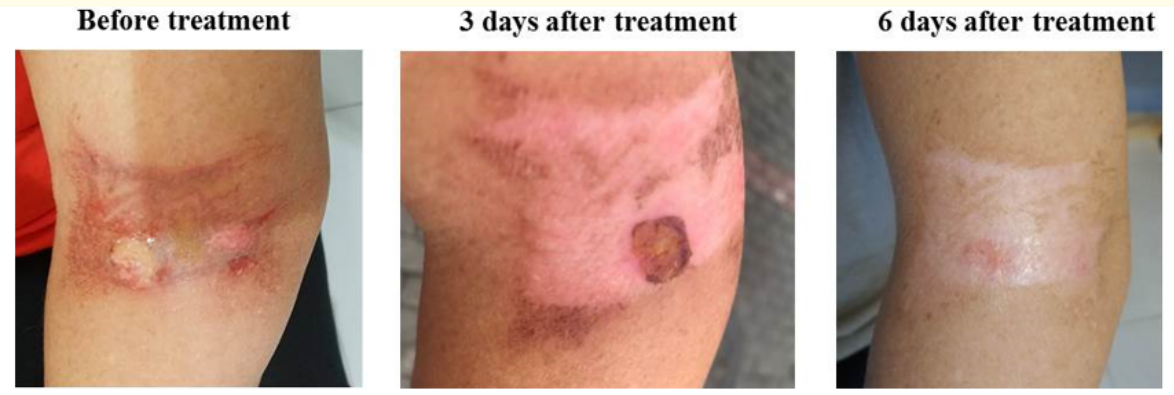

Figure 6: *Treatment of a scald located on an arm.*
A scald injury located on a region of the left hand between the arm and the forearm of a 52-year-old woman was treated with PC. Effectively, the wound healed after 6 days of treatment.

In another accident, a 30-year-old female was scalded while she was ironing clothes one night at home. The calf of her left leg was critically injured by steaming water spilt from the hot iron (Figure 7) [10]. During the night, the blisters popped and the injuries were highly painful and susceptible to infections. In the morning, despite consulting a doctor, not much he could help except prescribing some painkillers and antibiotics as well as a burn ointment for the lady to take home. In the afternoon, the lady sought assistance from us and her badly scalded wounds started to receive (twice daily) PC treatment. Miraculously, the wounds received an impressive healing effect for merely a treatment for 5 days (Figure 7) [10].

Figure 7: *Treatment of scalds located on a leg.*
Scald injuries located on the left calf region of a 30-year-old lady were treated with PC. The wound healed speedily after 5 days of treatment.

Treatments of surgical wounds

Even for poorly healing wounds such as surgical wounds, PC treatment has also been shown to work with high efficacies on them. In one case, a 64-year-old diabetic male, who was diagnosed with Coronary Artery Disease (CAD), received an operation of Coronary Artery Bypass Grafting surgery (CABG), in which a 48 cm great saphenous vein derived from his left thigh was used to attain three vessels for CABG [9]. Despite a successful bypass surgery, unfortunately, one of the four incisions, the 2nd wound, measuring approximately 4 - 6 cm × 0.8 cm, present on the left thigh did not heal well and sloughs began to emerge from it (Figure 8) [9]. Although the wound was treated with Cloxacillin, an antibiotic, there was no sign of improvement in healing for a long period of 2 months. We were then approached for assistance and the patient was recommended to try using the PC treatment protocol to manage the hard-to-heal incision. Despite dealing with a recalcitrant wound, the PC treatment regimen (twice daily) was shown to be highly effective in promoting the healing of it. On the 10th day, due to the observation of successful wound healing, the treatment was stopped (Figure 8). Subsequently, the self-healing effect continued to take place under normal conditions until the wound completely healed after a few more days (Figure 8).

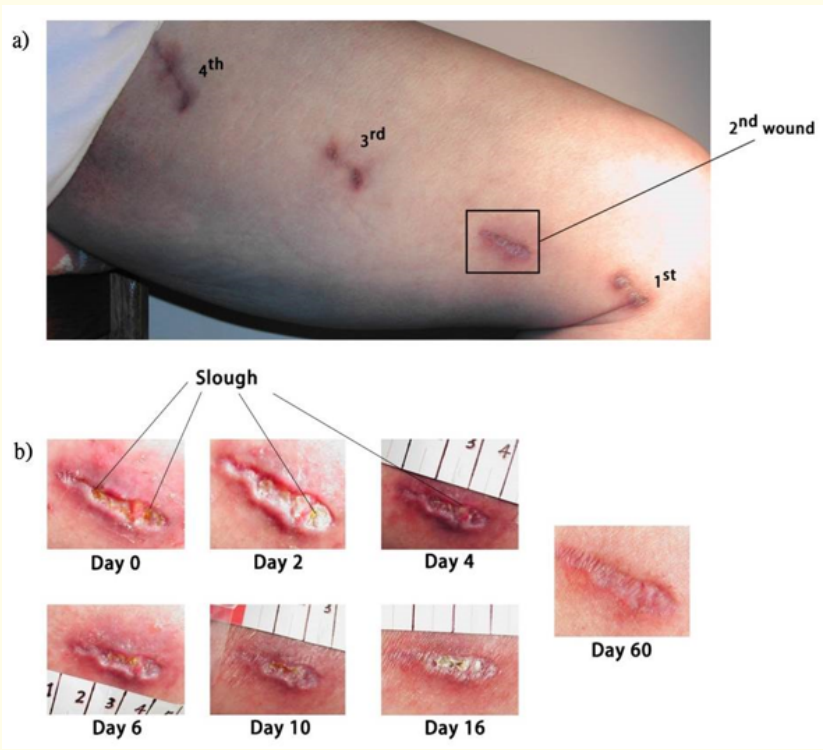

Figure 8: *Treatment of a recalcitrant surgical wound located on a thigh.*
a) Four incisions (labeled 1st to 4th) resulting from CABG left behind on a 64-year-old diabetic patient's left thigh. The 2nd wound (boxed) refused to heal; it was then subjected to PC treatment.
b) Subsequent to PC treatment, the healing progress of the unhealed wound (2nd wound) was highly enhanced. In view that the healing approached the stage of completion, PC treatment was stopped after Day 10. The wound was concluded to attain complete healing on Day 16 and its status on Day 60 was also monitored. The presence of slough in the wound at different times of treatment is indicated.

Discussion

Although EGF has been discovered for over half a century and it has been shown to play a pivotal role in the proliferation of epidermal cells, the application of EGF as an active ingredient to the development of medical or skin care products has been controversial [11,27]. Essentially, there have been two major points of dispute. First, is EGF safe for applications? Second, is EGF effective in treating wounds?

It is not difficult to provide an explicit answer, which is "yes", to the first question. Out of the hundreds/thousands of research articles concerning EGF published so far, it is a rare, or even an unlikely, event that we come across a document providing supporting evidence to substantiate that EGF is carcinogenic or unsafe for applications. Moreover, since the emergence of EGF for use in commercial applications in the 1990s [28], in these 3 decades, despite millions of people being end users of EGF products, adverse or harmful health effects resulting directly from EGF usage have been rarely reported.

In a review article published by our group recently, we have addressed the detrimental effects of counterfeit (unauthentic) EGF molecules (EGF derivatives or isoforms) on two important aspects: 1) the bioactivity, thus the efficacy, and 2) the exact identity, of EGF [11]. As majorities of the users are laypersons, it is impossible for them to address whether aEGF or an EGF isoform is used in a final product. Unfortunately, sometimes even skilled individuals may not be able to discern the identity of EGF involved since in many cases the primary structure of the polypeptide is not disclosed. In view that EGF isoforms cannot perform as efficient as the 53 aa native EGF circulating in our body, it was decided that aEGF, which shares the same identity with native EGF, was cost-effectively produced by a recombinant approach for applications.

We have engineered an *E. coli* excretion system which furnishes a productive microbial factory for efficient extracellular production of aEGF, amounting to a high yield of 325 mg L^{-1} [21]. The availability of this efficient expression platform has not only enabled us to market aEGF at a price much lower (a reduction of 80% or so) than those of its commercial counterparts, but also to attain quality aEGF, which possesses not only potent bioactivity but also exceptionally high stability.

Employing aEGF as the active ingredient, we presented supporting data in this communication (see Results) and previous studies [4,7,12] that aEGF is highly effective in promoting healing of many different types of wounds, including recalcitrant chronic wounds such as DFU (Figure 1-3) and bedsores (Figure 4 and 5), as well as other hard-to-heal wounds such as scalds (Figure 6 and 7) and surgical injuries (Figure 8). Working in conjunction with a facile cleansing protocol (Materials and methods), the superb quality and affordability of aEGF has enabled it to be acquired as a commercial health care product, which is available for widespread distribution and domestic application. Recently, our group has also developed an intein-mediated approach for the expression of another authentic skin growth factor, basic fibroblast growth factor (abFGF), which plays an important role in triggering the production of collagen and elastin in the deeper layer of our skin, the dermis [29,30]. With both aEGF and abFGF working collaboratively, it is expected that they will form a formidable pair in promoting wound healing, in particular where deep wounds are longing for effective repair services and aEGF is unlikely able to manage them efficiently alone.

Conclusions

In this communication, we report successful treatments of different types of hard-to-heal skin problems, including DFU, bedsores and other recalcitrant wounds, using a facile protocol comprising a simple cleansing procedure and topical application of aEGF. We emphasize that our active ingredient is "authentic" since it shares the same properties with native EGF. More importantly, aEGF has been shown to be safe for applications as well as effective in treating various kinds of wounds. On the other hand, the emphasis on authenticity might help end users to differentiate whether aEGF or an unauthentic isoform, which is likely less effective in performance than its authentic counterpart, is being employed as the active ingredient.

Acknowledgements

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Conflicts of Interest

The authors declare that they do not have conflicts of interest.

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