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Conceptual Paper

Recombinant Epidermal Growth Factor, from Bench to its Front Setting Line

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Epidermal growth factor (EGF) was firstly isolated by Cohen S [1] from a mouse submaxillary gland, and further purified mouse EGF. Its amino acid sequences was found to the relationship to urogastrone [2,3]. EGF consisted of 53 amino acids and contain three intramolecular disulfide bridges bonds that are required for biological activity. In recent, it has been suggested that EGF could be beneficial in burn [4], wound healing [5-7], diabetic foot ulcers [8,9] and digestive ulcer, and provide an attractive perspective. In addition, cosmetic containing EGF was found to be effective to improve the plasticity, to remove wrinkle, to show whitening and anti-aging, and control of erythema amount and sebum amount on the human skin care. Zhu G in this field has successfully prepared a series of 68 bottles of Shampoo liquid (New Wash) into market, Moreover, production of EGF from animal's source is not suitable for human application [10,11]. Based on these data, we introduce recombinant epidermal growth factor(rhEG) to investigate the effects of various doses of locally applied EGF on animal wounds and its preliminary trials.

As shown in figure 1, synthetic hEGF gene was cloned into a T7/lac Z pET-28a expression vector. After identified by screening and sequencing, recombinant pET-28a-hEGF plasmid containing hEGF gene was transformed into *E. coli* strain BL21（DE3) competent cells using $CaCl_2$ methods, plated on LB agar containing Kanamycin and incubated at 37°C overnight. An individual positive colony was selected from LB agar plate and incubated into LB liquid medium containing Kanamycin. The *E. coli* culture was incubated in shaking incubator 12 - 16 hours at 37°C, then was continuously incubated to expanding at 1:100 ratio. After reaching to OD600 = 0.4, protein expression was induced by the addition of IPTG with final concentration of 0.5 mmol/l. After 5 hours of growth, cells was harvested partially in form of inclusion body and resuspend in phosphate buffer solution (PBS). After smashing by ultrasonic disintegrator, the supernatant of cell lysate was collected by centrifugation and the residue was added with 8 mol/l urea lysis solution and recentrifuged. The supernatant fluids were collectively combined and then purified by Ni^{2+}-NTA affinity chromatography. The purified rhEGF was obtained and its biological activity was detected by MTT methods. When obtaining rhEGF crude donated from Prof. Zhi QW [11], the drug formulation was further prepared by manufacturer Dr. Zhu G [12] at doses of 2, 5, 10 ug/ml of rhEGF spray and 10 and 40 ug rhEGF/g of ointment. *In vitro*, the prepared purified rhEGF spray was shown a potent stimulation of cell growth and proliferation when added to the cultured 3T3 cells at 3.13 ~ 6.25 ng/ml of rhEGF concentration (Table 1). *In vivo* animal model, after daily application of various doses of rhEGF spray or its ointment, a significant decreased wound healing time was observed [12].

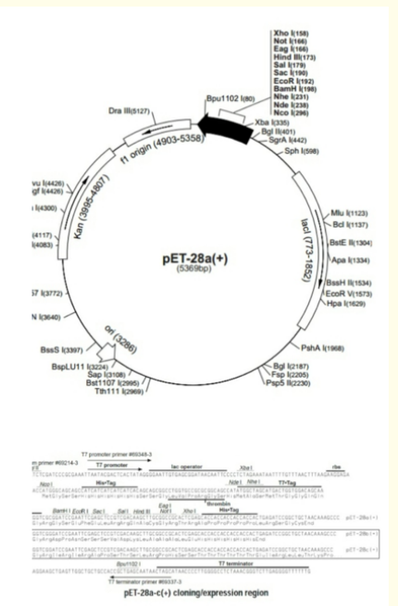

Figure 1: *The structure of pET-28a plasmid.*

EGF ng/mL	0	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100
OD	1.1415	1.1344	1.1766	1.1943	1.1977	1.2064	1,2595	1.3044	1.3166	1.4123	1.4512	1.3938
	1.1058	1.1228	1.1829	1.154 I	1.1871	1.2294	1.3060	1.3122	1.3260	1.4293	1.4131	1.4079
	1.1625	1.1884	1.1564	1.1586	1.1921	1.2170	1.2465	1.2555	1.3097	1.3428	1.3569	1.3715
Mean	1.1366	1.1485	1.1720	1.1690	1.1923	1.2176	1.2707	1.2907	1.3174	1.3948	1.4071	1.3911
In-crease rate %	100.00 ± 2.87	101.05 ± 3.50	103.11 ± 1.38	102.85 ± 2.20	104,90 ± 0.53	107,13 ± 1.16	111.79 ± 3.13	113.56 ± 3.07	115.91 ± 0.82	122.72 ± 4.58	123.80 ± 4.74*	122.39 ±1.84
Mean ± SD												

Table 1: *The proliferative activity of 3T3 culture cells following various of rhEGF concentration (MTT assay).*

In preliminary clinical trials, Dr. Zhu has experienced a 2.5 x 2.5 cm brush burn, the wound healed without scar at 5 ug of rhEGF spray for 1 week. The soles of his little finger with chilblain (0.6 x 0.8 cm) was also healed with 5 ug/ml of rhEGF spray within 5 days. In recent, his colleague Dr. Tang caught a knife wound (2 x 2.5 cm) by a knife accident. The entire epidermis was stripped off his forefinger. Blood flowed profusely from his right index finger. The wound healed without scar by the use of the combination of 5 ug of rhEGF spray every other day and a traditional medicine Yunan Baiyao spread within 20 days. Moreover, in Zhu's observation, a bone fracture attack by accident caused severe swollen on the right leg of an animal chicken. The obvious disappearance of serious swollen was found at 3 days after local use of 5 ug/ml of rhEGF spray and 1 week later, the chicken could go on foot with a broken leg. The wound was gradually healing through the continuous combination of local rhEGF spray and topical EGF-Silvadene ointment (10 ug/g) for next 6 days. The results suggested that rhEGF might play its antibiotic role and its some benefits in bone fracture. More data, a 73-year-old female with her right lumbar burn, the superficial burn area reached 12 x 8 cm, the burn wound was recently healed following cefuroxime sodium 2 g/day for 8 days and local 5 ug and 10 ug/ml of rhEGF spray over 20 days. All these data indicate clearly that rhEGF prepared by our group has the ability to become an efficient therapeutic drug for superficial or deep partial-thickness wound in skin. This is testable.

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