# Bio-inductive effects of inorganic elements on skin wound healing

( Published on Chinese Journal of Burns )

ZHOU Lai-sheng, LIAO Zhen-jiang, ZHANG Qin, LUO Min, LU Gang, ZHONG Wei.

**[Abstract] Objective** To explore the bio-inductive effects of inorganic elements ( Dermlin$^{TM}$ ) on the human epithelial proliferation and differentiation and their promoting effects on skin wound healing. **Methods** (1) Cellular test: Normal human skin epithelial cells were cultured with 20 g/L Dermlin supplemented culture medium ( E group ) and regular culture medium ( C group ), respectively. The cell proliferation rate and the expressions of type IV collagen and epidermal growth factor ( EGF) in the supernatant were determined in 12 and 20 post cultre days ( PCD ). (2) Animal test: Self-consubstantiality control was employed in the study. Sixty Sprague – Dawley rats were inflicted with two symmetric 10% TBSA of superficial or deep partial thickness scald on the back of each rat, and were divided into control [ C, with topical application of silver sulfadiazine ( SD – Ag ) cream to the wounds ] and treatment ( T, with 1 g/100 cm$^2$ Dermlin topical application to the wounds ) groups. The pathological changes in wound skin were observed and the wound healing rate was calculated on 3, 5, 7, 10, 14 and 18 post treatment day ( PTD ). (3) Randomized, double-blinded and consubstantiality control method was employed in the clinical trial. Ninety patients were enrolled in the clinical study, among them 30 cases with 60 donor site wounds, 30 with 60 superficial and 30 with 60 deep partial thickness burn wounds were included. Dermlin in dose of 1 g/100 cm$^2$ was applied to the wounds in T group and SD – Ag cream in C group for up to 18 days. Furthermore, sixty patients with diabetic foot ulcers were included for 1 g/100 cm$^2$ Dermlin treatment. The wound healing rate was observed. And the blood and urine test and the indices of hepatic and renal function were determined. **Results** (1) Cellular test: The cell

proliferation rate and the expression of type IV collagen and EGF in the culture supernatant were obviously higher than those in control group at the same time points ( $P < 0.01$ ). (2) Animal test: Hyperplastic granulation tissue occurred in the rat wound in the T group since 5 PTD, while that occurred in the C group since 7 PTD. The healing rate of superficial partial thickness wound in T group on 7, 10, 14 PTD, and that of deep partial thickness wound in T group on 5, 10, 18 PTD were obviously higher than that in the C group ( $P < 0.05$ ). (3) Clinical study indicated that the wound healing rate of the patients with superficial or deep partial thickness scald in the T group was evidently higher than that in the C group on 5 and 10 PTD ( $P < 0.05$ ), but the wound healing time of the superficial, deep partial thickness wound and donor site wound in the T group was significantly shorter than that in the C group ( $P < 0.05$ ). Before treatment, the square of the ulcers on the foot of the patients with diabetic was ( $39 \pm 28$ ) $cm^2$, and it was reduced to ( $19 \pm 23$ ) $cm^2$ 2 weeks later, with the therapeutic efficacy reaching 62.5%. For all patients, no obvious change was found in the blood test and hepatic and renal function indices. **Conclusion** The inorganic element ( Dermlin ) is beneficial to wound healing and to the proliferation and differentiation of epithelial cells.

**[Key words]** Burns; Biocompatible material; Diabetic foot ulcer; Wound healing; Inorganic elements

The basic condition for wound healing is the proliferation, differentiation and migration of epithelial cells. The direction of research in wound field is to explore a kind of preparation, which can stimulate the proliferation and differentiation of epithelial cells, and also being of biological stability and safety.

## Material and methods

1. The source and features of inorganci elements

The inorganical elements (Dermlin$^{TM}$), with the batch number of 20040321, were provided by Jiangsu Yenssen Biotech Co., Ltd.. The active ingredients are calcium

and silicon. These material are white powder, with the granule diameter of 20 ~ 350 μ m. This kind of granule contains micropores with nm level, its specific surface area being 165 $m^2$ / g.

2. Cellular test
    a) Culture of epithelial cells: Select residual normal skin tissue with the square of 1 cm x 2 cm from abdominal surgeries of 7 male patients ( from 18 to 25 years old). The epithelial cells were screened repeatedly and cultured by combined collagenase.
    b) Test groups: Use the third generation epithelial cells into the test and divede the cells into E group and C group. Take the epithelial cells from one patient as one sample. Each data of each group need to be tested 7 samples. The cells of E group were cultured with 20 g / L Dermlin supplemented DMEM culture medium, while that of C group were cultured with regular DMEM culture medium.
    c) Test indices: ( 1 ) The cell proliferation rate: Adhering cells were determined in 16 post culture hour and in 12, 20 post culture day ( PCD ) by the staining of crystal violet. The cell proliferation rate = ( number of adhering cells on PCD – number of adhering cells in 16 h ) / number of adhering cells in 16 h. ( 2 ) The expressions of type IV collagen and epidermal growth factor ( EGF ) were determined by enzyme-linked immunosorbent assay ( ELISA ). Collect the supernatant on 12 and 20 PCD, being marked by antibody of rat anti-human collagen type IV and rabbit anti-human EGF, respectively. And then add related AP goat anti-rat or anti-rabbit IgG. Taking Nitrophenyl phosphate as substrate, the expressions of type IV collagen and EGF were determined by enzyme-labeled instrument under the wave length of 405 nm.
3. Animal test
    a) Animal models and groups: Sixty Sprague-Dawley rats, with the weight of 220 g to 240 g, were inflicted with two symmetric 10% TBSA of superficial ( 30 rats with 60 wound surface ) or deep partial ( 30 rats with 60 wound

surface ) thickness scald on the back of each rat. Self-consubstantiality control was employed in the study. Devide the wounds on the same rat into treatment ( T, with 1 g / 100 $cm^2$ Dermlin topical application to the wound, covered by a vaseline pad ) and control [ C, with topical application of silver sulfadiazine ( SD – Ag ) cream to the wound, covered by a vaseline pad ] group.

b) Observation indices: Cut the full layers of wound skin on 3, 5, 7, 10, 14, 18 post treatment day ( PTD ) to observe the pathological changes and calculate the healing rate. For superficial partial thickness wounds, observe the indices on the first five PTD, while for deep partial wounds, observe the indices on the last five PTD. Select 6 rats for observation on each PTD.

4. Clinical trial

a) Application to burn treatment: ( 1 ) Approved by Jiangsu FDA, the clinical trials of Dermlin were conducted in Burn Department of Ruijin Hospital affiliated to Shanghai 2nd Medical University, Burn Institute of 304 Hospital of PLA, and Burn Derparment of the Military General Hospital of Beijing PLA. Rondomized, double-blinded and consubstantiality control method was employed in the clinical trial. Ninety patients were enrolled in the clinical study, among them 30 cases with 60 donor site wounds, whose deepness from 0.15 mm to 0.25 mm, 30 with 60 superficial and 30 with 60 deep partial thickness burn wounds, whose area from 5% TBSA to 10% TBSA. ( 2 ) Grouping: T group ( 30 wounds of superficial partial thickness, 30 wounds of deep partial thickness, 30 wounds of donor site wounds) were treated with Dermlin in dose of 1 g / 100 $cm^2$, and covered by vaselin pads. Change Dermlin once three days. C group ( 30 wounds of superficial partial thickness, 30 wounds of deep partial thickness, 30 wounds of donor site wounds) were treated with 5 g / L Iodophor or 10 g / L SD-Ag cream, and also coverd by vaseline pad. ( 3 ) Observation indices: Calculate the healing rate and record the healing time of superficial and deep partial thickness wounds on 5, 10

PTD. And the blood and urine test and the indices of hepatic and renal function were determined.

b) Application to the treatment of diabetic foot ulcer: ( 1 ) Approved by Jiangsu FDA, the clinical trials of Dermlin applied to 60 patients with diabetic foot ulcer were conducted in the Endocrinology Department of Ruijin Hospital affiliated to Shanghai 2nd Medical University. The standard for the selected patients: Theses patients didn't gain effective curative effect through current treatment for diabetic foot ulcer. ( 2 ) Treatment: Wash the wound surface on diabetic foot ulcer, evenly apply a thin layer of Dermlin in dose of 1 g / 100 $cm^2$ to the affected area, being covered by a vaseline pad. Change Dermlin once daily. The diabetic foot ulcers were treated for 2 weeks. ( 3 ) Observation indices: Measure the square of diabetic foot ulcer before and after treatment and observe the blood test and the indices of hepatic and renal function.

5. Statistical processing

The datum were expressed by $x \pm s$. And the datum of cellular test were analized by ANOVA and Tukey HSD. The results of aninal test and clinical trial were analized concerning intentionality and effective datum. And then do two-side statistical test. The statistical analysis were finished by software of NIH and SPSS 11.0.

## Results

1. Cellular test

The cell proliferation rate and the expression of type IV collagen and EGF in the culture supernatant were obviously higher than those in control group at the same points ( $P < 0.01$ ). See Tab 1.

Tab 1 The proliferation rate of the epithelial cells and the content of type IV

collagen and EGF in the culture supernatant in the two groups ( $\bar{x} \pm s$ )

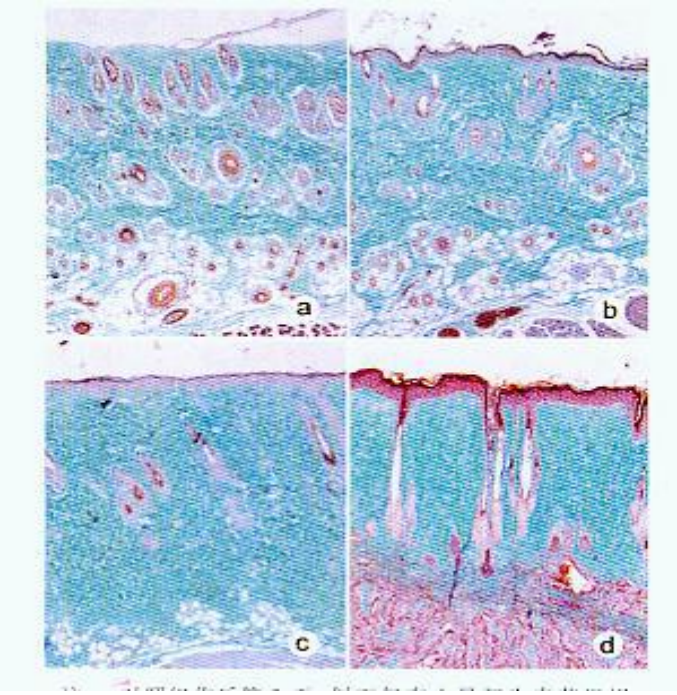
| Group | Number of samples | Culture time ( d ) | |
|---|---|---|---|
| | | 12 | 20 |
| C group | | | |
| Proliferation rate | 14 | 0.51±0.13 | 0.81±0.06 |
| Type IV collagen ( ng / 1 x $10^5$ cells ) | 14 | 1.89±0.21 | 1.22±0.19 |
| EGF ( ng / 1 x $10^5$ cells ) | 14 | 0.019±0.003 | 0.089±0.008 |
| E group | | | |
| Proliferation rate | 14 | 2.75±0.48* | 6.21±0.89* |
| Type IV collagen ( ng / 1 x $10^5$ cells ) | 14 | 5.82±0.88* | 7.10±0.92* |
| EGF ( ng / 1 x $10^5$ cells ) | 14 | 0.144±0.018* | 0.371±0.021* |

Note: Compare with C group, * $P < 0.01$

2. Animal test

a) As to observation results of pathology for SD rats with superficial partial thickness scald on the back, please see Fig 1. The inflammatory cells of wounds in T group and C group were obviously infiltrated since the 5th day after the deep partial thickness scald on SD rats. Hyperplasticc granulation tissue occured in the rat wound i the T group since 5 PTD, while that occured in the C group since 7 PTD.

Fig 1 Histological staining of the collagen fibers in the superficial partial thickness burn wound in the two groups Masson x 100




Note: a. Only a little hyperplastic granulation tissue occured in the wound in C group since 3 PTD; b. Hyerplastic granulation tissue obviously occured in the wound in T group since 3 PTD; c. A little epithelium occured in the wound in C group since 10 PTD; d. The whole epithelium occured in the wound in T group since 10 PTD.

b) Healing rate of wounds on SD rats: See Tab 2 and Tab 3.

Tab 2 Comparison of the healing rate of the superficial partial thickness burn wound in the two groups ( %, $\bar{x} \pm s$)

| Group | No. of wounds | PTD | | | | |
|---|---|---|---|---|---|---|
| | | 3 | 5 | 7 | 10 | 14 |
| C group | 30 | 1.0±2.6 | 17.5±12.4 | 49.2±11.4 | 64.4±15.4 | 80.0±7.1 |
| T group | 30 | 1.6±4.7* | 24.7±15.0* | 60.8±13.8* | 83.1±14.4* | 92.5±5.0* |

Note: Compare with C group, * $P < 0.05$

Tab3 Comparison of the healing rate of the deep partial thickness burn wound in the two groups ( %, $\bar{x} \pm s$ )

| Group | No. of wounds | PTD | | | | |
|---|---|---|---|---|---|---|
| | | 5 | 7 | 10 | 14 | 18 |
| C group | 30 | 0.5±1.5 | 5.9±5.5 | 22.9±5.8 | 70.0±6.0 | 83.8±4.8 |
| T group | 30 | 2.5±3.4* | 9.1±5.8* | 33.8±9.1 | 76.2±4.4* | 96.2±4.8* |

Note: Compare with C group, * $P < 0.05$

3. Clinical trial

a) See Tab 4 for the healing rate and healing time of the patients with superficial or deep partial thickness burn on 5, 10 PTD. For the treatment of donor site wound, the healing time in T group was ( 7.4±1.9 ) d, while that in C group was ( 11.4±2.4 ) d ( $P < 0.05$ ). For all patients, no obvious change was found in the blood test and hepatic and renal function indices.

Tab 4 Comparison of the healing time and healing rate of the superficial or deep partial thickness burn wound in the two groups ( $\bar{x} \pm s$ )

| Group | No. of wounds | Healing rate on 5 PTD (%) | Healing rate on 10 PTD (%) | Healing time ( d ) |
|---|---|---|---|---|
| C group | | | | |
| Superficial | 30 | 70±8 | 92±4 | 11.1±3.1 |
| Deep | 30 | 16±5 | 63±14 | 17.4±2.9 |
| T group | | | | |
| Superficial | 30 | 87±5* | 100±0* | 8.1±1.1* |
| Deep | 30 | 38±5* | 88±8* | 13.5±2.2* |

Note: Compare with C group, * $P < 0.05$

b) Before treatment, the square of the ulcers on the foot of the patients with diabetic was ( 39±28 ) $cm^2$, and its was reduced to ( 19±23) $cm^2$ 2 weeks later, with the therapeutic efficacy reaching 62.5% ( $P < 0.01$ ). For all patients, no obvious change was found in the blood test and hepatic and renal function indices.

## Discussion

The repair of wound surface begins with epithelial proliferation, differentiation and migration. According to traditional theory, biological material is considered as inert material. However, since this author first put forward the theory of "Molecular Bio-compatibility" in 1995, people have new understanding to the bio-inductive effect of inorganic elements.

The results of this study demonstrate that the inorganic element ( Dermlin ) can effectively promote the wound healing. It can stimulate the proliferation of epithelial cells and the formation of type IV collagen and EGF. During the study,

the wound surface in T group did not occur any infection, which showed that inorganic element ( Dermlin ) has the obvious function of bacteriostasis. Meanwhile, since the chemical composition of these inorganic elements used in the study is similar to that of inherent elements in human body, these inorganic elements are safe and stable. Therefore, the inorganic bio-inductive material is likely to be a new research direction in wound treatment.