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Original Article

Effect of the orientation of microskin on the survival rate of transplantation and improving the method

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Abstract: The direction of microskin transplantation is difficult to control, and the survival rate is critically affected. In this study, we show for the first time that survival rate of transplantation was improved by changing the direction of microskin. A human split-thickness skin graft was prepared as microskin (size of 1 mm × 1 mm), and was transplanted onto a wound in nude mice. The effect of the orientation of microskin on the survival rate of transplants was observed. The collagen membrane was first attached to the epidermal surface of pig skin, which was then cut into microskin and then they were floated on physiological saline. The effect of the collagen membrane on the orientation of microskin was observed. Then the microskin of pig with an epidermal surface attached to the collagen membrane was transplanted to the wound of the pig, and the survival rate of transplants was observed. In the 2nd, 3rd and 4th week after transplantation of nude mice, the wound healing rate in group A (all of the microskin's epidermal surface was upward) was significantly higher than in other groups ($P < 0.01$). The floating rate and the forward floating rate in the experimental group were significantly higher than those in the control group ($P < 0.01$). Four weeks after microskin transplantation of pigs, the wound contraction rate in group A, compared with group B, was significantly lower, and the wound healing rate was significantly higher ($P < 0.01$). In microskin grafting, the direction of microskin significantly affects the survival rate of transplantation. The method of adhering the collagen membrane to the epidermal surface of microskin may ensure complete floating of microskin on the physiological saline with the epidermal surface facing up. This is a new method to improve the survival rate of microskin grafting.

Keywords: Wound repair, microskin graft, direction, collagen membrane, scar

Introduction

Repairing of an extensive deep wound often faces the problem of a shortage of autologous skin [1]. Current methods for repairing a large area of deep wound include cultured epithelial autografts (CEA) [2], Meek technique [3], and microskin grafting [4]. CEA requires less skin. Theoretically, it can be amplified to 1000~10,000 times. To a certain extent, this solves the problem of lack of autologous skin. But after many years of clinical practice, it becomes apparent that due to the lack of dermal tissue, the renascent skin can easily form ulcers repeatedly, and scar hyperplasia and contracture are obvious, which affect the appearance and function. In addition, the culturing time in vitro is rather long, thus it is difficult to repair the wound timely [5]. Meek technique is simple, time-saving, and has a good appearance after wound healing [6-8]. The maximum extensive ratio, however, is only 1:9 and the top dressing may provide an environment for growth of microorganisms [9].

Microskin grafting can be extended to 10~20 times, which makes full use of the limited source of autologous skin. This has become the main method to repair extensive deep wound since birth [10]. However, it is difficult to control the direction and uniformity of microskin, resulting in an unstable survival rate [10, 11]. Therefore, in this study, the effect of the direction of microskin on the survival rate of trans-

plantation was observed, and the survival rate was significantly improved by changing the orientation of microskin.

Methods and materials

Ethics statement

The animal studies were approved by the Animal Care and Use Committee of Naval Medical University, and all of the protocols were approved by the Ethics Committee of Changhai Hospital, Naval Medical University, Shanghai, China.

The effect of the orientation of microskin on the survival rate of transplantation

Preparation of microskin: Ten groups of circular blades were arranged in parallel with the blade pitch of 1 mm, thus a roller cutter was made. The skin was prepared into microskin (size of 1 mm × 1 mm) for one cross-cutting.

Microskin grafts in wounds of nude mice: Eighty nude mice (provided by the animal experimental center of Naval Medical University) were used, 8 weeks old of age, male or female, body weight 24~26 g. The full-thickness skin with a diameter of 1.5 cm of the nude mice was excised from the middle of the back after routine disinfection. The mice were randomly divided into group A (all of the microskin's epidermal surface was upward), group B (half of the microskin's epidermal surface was upward and the rest was downward), group C (the microskin's epidermal surface was downward) and group D (no microskin were transplanted). They were all transplanted with microskin (fresh human foreskin, thickness of 0.2~0.3 mm, size of 1 mm × 1 mm, expansion ratio of 1:10), covered with transparent silicone membranes, then sutured and fixed [11]. In the 2^{nd}, 3^{rd} and 4^{th} weeks after transplantation, the wound healing condition was observed respectively and the wound healing rates were calculated with transparent paper tracings combined with computer-aided imaging system: the wound healing rate (%) = (total wound area - unhealed wound area) (cm^2)/total wound area (cm^2) × 100%. Four weeks after transplantation, tissue specimens were cut for immunohistochemical staining of HLA-class I to identify the remainder of cells of human source, as well as the Hematoxylin-Eosin (H&E) and Masson staining, to observe the cellular infiltration and collagen arrangement.

Preparation and toxicity test of the collagen membrane

Preparation of the collagen membrane: Flatfish skin was dissolved in the acetic acid (0.5 mol/L), salted out and centrifuged. The supernatant was extracted collagen (the concentration was 90%). Then the supernatant was poured into a container, and vacuum freeze-dried to a collagen membrane [12]. H&E staining and scanning electron microscopy were used to observe the structure of the collagen membrane. The density and floating properties were measured.

Toxicity test of the collagen membrane: A human fibroblast suspension was prepared into a concentration of 2×10^4 L^{-1}, and 200 μl of the suspension was cultured per well in a 96 well plate, and incubated for 24 h. The original culture medium was discarded when the cells attached well. In the experimental group, the collagen membrane extraction was added, and the control group received added DMEM medium containing 10% fetal bovine serum. The CCK8 (Cell Counting Kit 8) was used for detection and the absorbance (OD value) at 450 nm was measured. The relative proliferation rate of cells was calculated as follows: relative proliferation rate of cells (%) = OD value of day n/OD value of day 1 × 100%.

Floating test of microskin

Two pieces of pig skin (thickness of 0.2~0.3 mm) and 1 piece of collagen membrane (size of 2.5 cm × 4.0 cm) were taken out. Meek gel was sprayed on the epidermal surface of one of the pig skin to attach it to the collagen membrane. This was dried for 5 min and cut into microskin (size of 1 mm × 1 mm). According to the conventional floating method of microskin grafting [13], microskin in both the experimental group (microskin of pig with the epidermal surface adhered to the collagen membrane) and the control group (microskin of pig with the epidermal surface adhered to nothing) were respectively and evenly dispersed in physiologic saline with an area of 10 cm × 10 cm. The number of microskins floating and positively floating (float with the epidermal surface facing up) on the

physiologic saline was counted respectively. The floating rate and the positive floating rate were calculated as follows: the floating rate (%) = the number of floating microskins/the number of total microskin × 100%. The positive floating rate (%) = the number of positive floating microskins/the number of total microskins × 100%. The gauze was lifted, with microskin left on it, then flipped with both hands. One side of the gauze containing microskin was covered to the dermal surface of sheet alloskins (size of 5 cm × 5 cm). We uncovered the gauze, counted the number of microskins with the dermal surface facing up, and calculated the same direction transfer rate: the same direction transfer rate (%) = the number of microskins with the dermal surface facing up/the number of total microskins × 100%. The experiment was repeated 20 times.

Microskin graft in wounds of pigs

Five Bama minipigs (provided by the experimental animal center of Naval Medical University) were used, weighing about 15 kg, male or female. Their hair was cut after routine disinfection. Split thickness skins were cut in the center of the back and were respectively prepared into microskin adhered to the collagen membrane, pure pig microskin, and pure collagen membrane microskin. After floating on physiologic saline, they were all transferred to sheet alloskins (size of 5 cm × 5 cm). Four pieces of the full-thickness skin (size of 5 cm × 5 cm) were cut on both sides of the back. Thus 4 wounds were formed. After adequate hemostasis the wounds were randomly divided into group A, group B, group C, and group D. Then they were covered with alloskins respectively containing microskin adhered with the collagen membrane, pure pig microskin, pure collagen membrane microskin, and no microskin (the expansion ratio is 1:10). The wound healing condition was observed respectively in the 2^{nd}, 3^{rd} and 4^{th} week after transplantation, and in the 4^{th} week the rates of wound healing and wound contraction were respectively calculated with transparent paper tracings combined with computer-aided imaging system: the wound healing rate (%) = (total wound area - unhealed wound area) (cm^2)/total wound area (cm^2) × 100%, wound contraction rate (%) = (total wound area - present wound area) (cm^2)/total wound area (cm^2) × 100%. A part of the tissue specimens was cut for H&E staining and Masson staining to observe cell infiltration and collagen arrangement.

H&E staining: After cutting into 10 μm sections, deparaffinization and rehydration, the sections were stained with hematoxylin, rinsed and cleared in hydrochloric acid and alcohol. Then the sections were rinsed with running water, and the cytoplasm was dyed with eosin dye solution. After dehydration, the sections were sealed with neutral gum.

Masson staining: After soaking the paraffin sections (10 μm) in Bouin solution overnight, they were rinsed with running water until they were colorless. The sections were stained with Celestine Blue and Mayer's hematoxylin for 5 minutes, cleared with 1% hydrochloric acid and alcohol, and rinsed with running water. Then they were stained with Ponceau acid fuchsin solution for 5-10 minutes and rinsed with distilled water. After 5 minutes of treatment with 1% phosphomolybdic acid aqueous solution, they were stained directly with aniline blue for 5 minutes, 1% glacial acetic acid for 5 minutes, and 95% alcohol multiple times. They were placed in glue that was anhydrous alcohol based, to xylene transparency, and sealed with-neutral gum.

Immunohistochemical staining of HLA-class I: After routine deparaffinization and hydration, the sections were washed with PBS, blocked with 3% methanol-H_2O_2 solution, washed with PBS, and then subjected to autoclave antigen heat retrieval, washed with PBS again, and blocked with normal goat serum at room temperature. Primary antibody, 4°C was added overnight. They were rewarmed at 37°C for 45 minutes, washed with PBS; secondary antibody was added. They were washed at 37°C for 1 hour, washed with PBS, and color was developed with DAB. Slides were mounted and checked on the microscope.

Statistical analysis

All data were statistically analyzed using SPSS 22.0 (SPSS Inc., Chicago, USA), and the normality and homogeneity of variance were both tested. The wound healing rate of nude mice and the relative proliferation rate of cells were analyzed with Repeated Measurement Analysis of Variance (RMANOVA), and at the same time

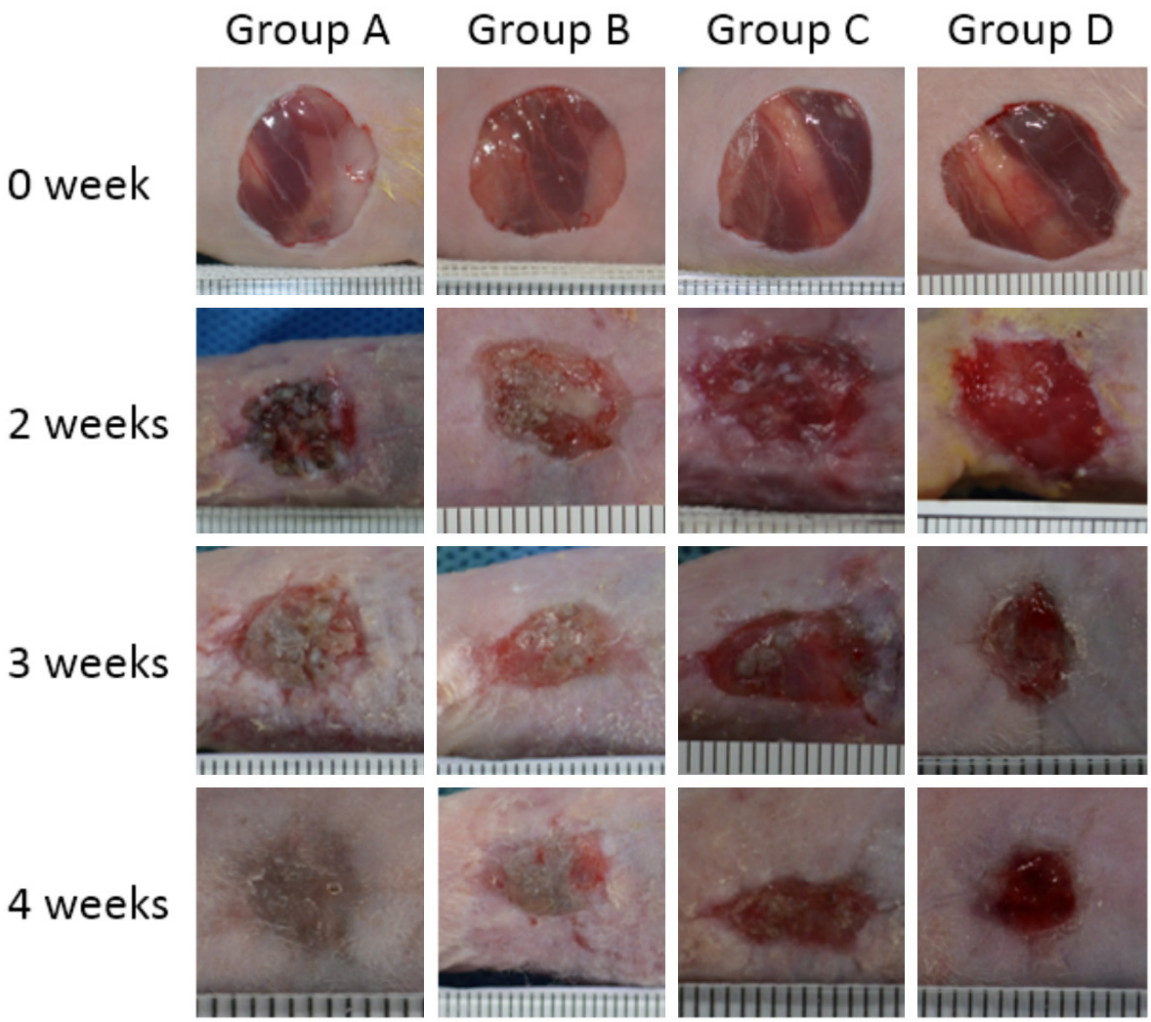

Figure 1. Gross observations at 2, 3, and 4 weeks after transplantation of human microskin to full thickness skin excision in nude mice. Group A: forward transplantation group, Group B: forward and reverse transplantation group, Group C: reverse transplantation group, Group D: control group. 2 weeks after transplantation, the transplanted skin was almost completely alive, with a clear outline of the fine microskin. 4 weeks after transplantation, the wound completely transformed to epithelium.

Bonferroni test was used for pairwise comparison. The floating rate, the positive floating rate and the same direction transfer rate were all analyzed with t-test. The rates of wound healing and wound contraction of pigs were analyzed with One-Way Analysis of Variance (One-Way ANOVA), and then the Bonferroni method was used for pairwise comparison. Differences were statistically significant when $P < 0.05$.

Results

Wound healing of nude mice

Nearly all of the microskins in group A survived 2 weeks after transplantation, and a visible clear microskin contour could be observed in this group. The survival numbers of microskins were relatively small in other groups. Four weeks after transplantation, most of the wounds in group A presented good epithelization, while the healing area of other groups was much smaller (**Figure 1**). In the 2nd, 3rd and 4th weeks after transplantation, the wound healing rates of group A was (54.75 ± 3.05)%, (84.80 ± 3.17)% and (94.80 ± 2.78)%, respectively, which were significantly higher than those of group B ((44.85 ± 2.76)%, (74.85 ± 2.80)%, (84.95 ± 2.96)%), group C ((35.85 ± 2.87)%, (45.25 ± 2.29)%, (55.90 ± 2.00)%) and group D ((4.95 ± 1.96)%, (14.85 ± 2.06)%, (24.50 ± 2.35)%) ($P < 0.01$) (**Figure 2**). Positive HLA-

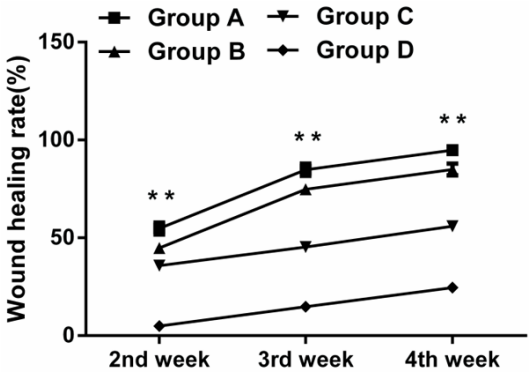

Figure 2. Comparison of wound healing rate of 2, 3, and 4 weeks after transplantation of human microskin to full thickness skin excision in nude mice. Group A: forward transplantation group, Group B: forward and reverse transplantation group, Group C: reverse transplantation group, Group D: control group. The wound healing rates at each time point of forward transplantation group were significantly higher than those of other groups (**$P < 0.01$).

class I staining was observed in the basal layer of the renascent skin, which proved that human cells had taken part in the process of wound healing (**Figure 3**). H&E staining showed that the epidermal layer of the renascent skin in group A was continuous, with a few inflammatory cells, fibroblast infiltration and frequent neovascularization. The epidermal layers of other groups were discontinuous. Masson staining showed that there were massive collagen fibers in all groups, which were arranged in order, with no significant difference (**Figure 4**).

Character of the collagen membrane

The collagen membrane was smooth, flexible, and appropriate, with good shape, and the thickness was about 0.3 mm. When placed in physiological saline, it could completely float, so the density ((42.76 ± 0.13) kg/m^3) was less than physiological saline. H&E staining showed that the collagen fibers were connected into a porous structure, and the mesh size was relatively consistent. Scanning electron microscope (SEM) showed that the pore size was 100 μm~200 μm (**Figure 5**).

Toxicity test of the collagen membrane

The relative proliferation rates of cells in both the experimental group and the control group increased with extension of the culture time, and there was no significant difference between the two groups ($P > 0.05$) (**Figure 6**).

Improvement of floating method of microskin

Microskin in the experiment group could completely float on the surface of saline with a small amount resting underwater. The majority of the epidermis was upward and the distribution was relatively uniform. There was less microskin floating on the surface of saline in the control group. When microskin had been transferred to sheet alloskins, the majority of the dermis was upward in the experimental group, while it was downward in the control group. Compared with the control group, the floating rate ((95.05 ± 2.09)%), the forward floating rate ((91.25 ± 2.88)%), and the same direction transfer rate ((89.75 ± 2.07)%) of the experimental group were significantly higher than those of the control group, all ($P < 0.01$) (**Table 1**).

Wound healing condition of pigs

Four weeks after transplantation, no obvious residual tissue of the collagen membrane was found in all groups. The wound healing rate of group A ((95.15 ± 2.28)%) was significantly higher than that of group B ((65.05 ± 2.33)%), group C ((30.00 ± 2.53)%) and group D ((30.00 ± 2.90)%) ($P < 0.01$). The wound contraction rate in group A ((12.45 ± 2.01)%) was significantly lower than that in group B ((20.05 ± 2.11)%), group C ((29.50 ± 3.19)%), and group D ((2.98 ± 2.88)%) ($P < 0.01$). There was no significant difference in the above indexes between group C and group D ($P > 0.05$) (**Figures 6**, **7**). H&E staining showed that the epidermal layer in group A was continuous, and there was no formation of epidermal cysts in the dermis and subcutaneous tissue. In group B, epidermal islands were observed in the dermis and subcutaneous tissue, and were connected with the epidermis, forming an obvious epidermal cyst structure. Masson staining showed that the collagen fibers in each group were arranged in an orderly manner, with no significant difference (**Figure 8**).

Discussion

Microskin grafting was first successfully applied clinically by Zhang *et al.* in 1986. Due to its expansion ratio, microskin grafting became the

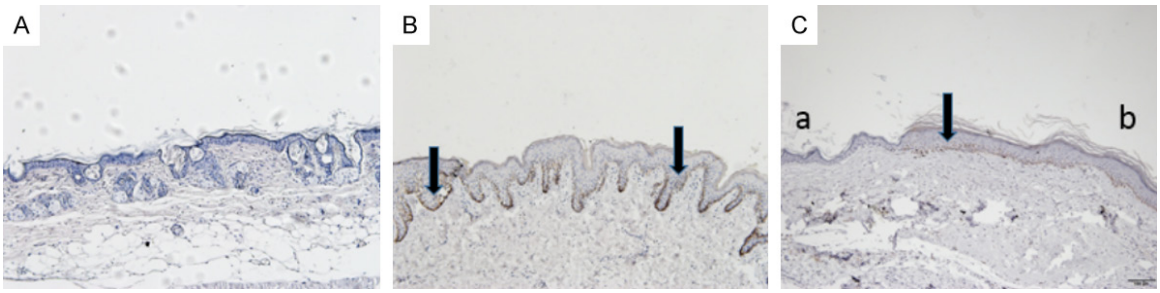

Figure 3. HLA-class I (10 ×) stained by immunohistochemistry. (A) normal nude mice skin, no positive staining, (B) normal human skin, positive staining shown in basal cell layer (arrow), (C) nude mice full-thickness skin excision wound transplantation of human microskin. 4 weeks after transplantation, new skin formed. Area "a" showed normal nude mice skin, area "b" was new skin. There was positive staining in the basal cell layer (arrow).

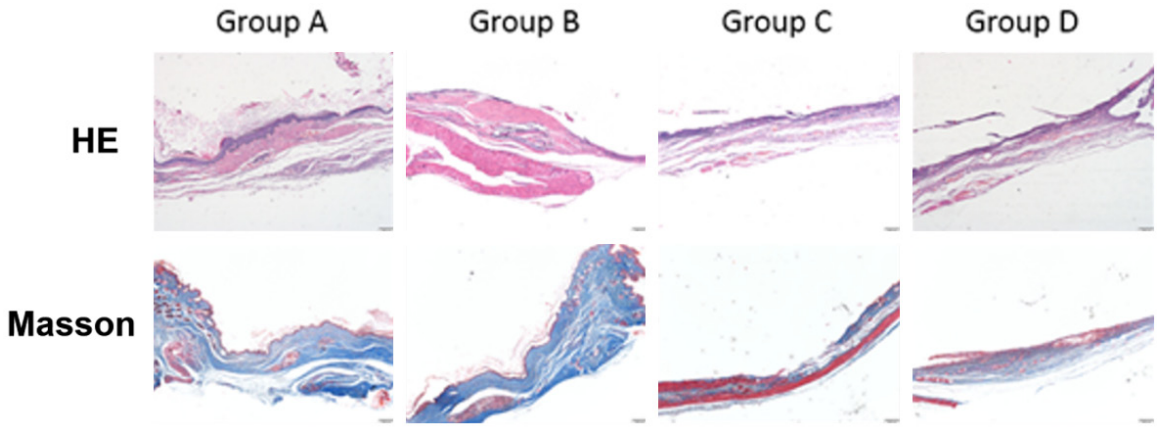

Figure 4. H&E and Masson staining of the wound 4 weeks after transplantation of human microskin to full thickness skin excision in nude mice. Group A: forward transplantation group, Group B: forward and reverse transplantation group, Group C: reverse transplantation group, Group D: control group. H&E staining showed that the epidermis in Group A was more continuous than other groups, with many inflammatory cells and fibroblast infiltration, and formation of new blood vessels. Masson staining showed that the collagen fibers were arranged regularly. There were no significant differences between different groups.

main method of treatment for extensive burn patients. Currently, it is still widely used in China, which is one of the reasons for the high success rate of the treatment of severe burns in China [14-17]. In addition, microskin grafting is also used for repairing wounds after resection of giant nevus, wounds after trauma and extensive skin avulsion, and wounds of a chronic skin ulcer.

In conventional microskin grafting, microskin is floated in the physiological saline, transferred to sheet alloskin, and then transplanted to the wound. Orientation is one of the key points to affect the survival rate of transplantation. Microskin is a hexahedron. When transplanted, it may have three orientations: positive transplantation (with epidermal surface facing up), lateral transplantation (with epidermal surface facing to one side), reverse transplantation (with epidermal surface facing down). In theory, microskins can survive only when they are transplanted with the epidermal surface facing up. Although Hackl *et al*. [18] and Singh [19] have proven through animal experiments that when the size of microskin is 0.8 mm × 0.8 mm and 0.3 mm × 0.3 mm and , the direction had no significant effect on the survival rate, this remains controversial [17, 20]. However, the smaller the microskins are, the lower the orientation requirement. Part of the microskin may be buried in the wound, and can obtain nutrition from the wound in any direction. But the survival rate is very low. Epidermal cysts may form in the subcutaneous tissue or dermis, and the renascent skin is also irregular, which seri-

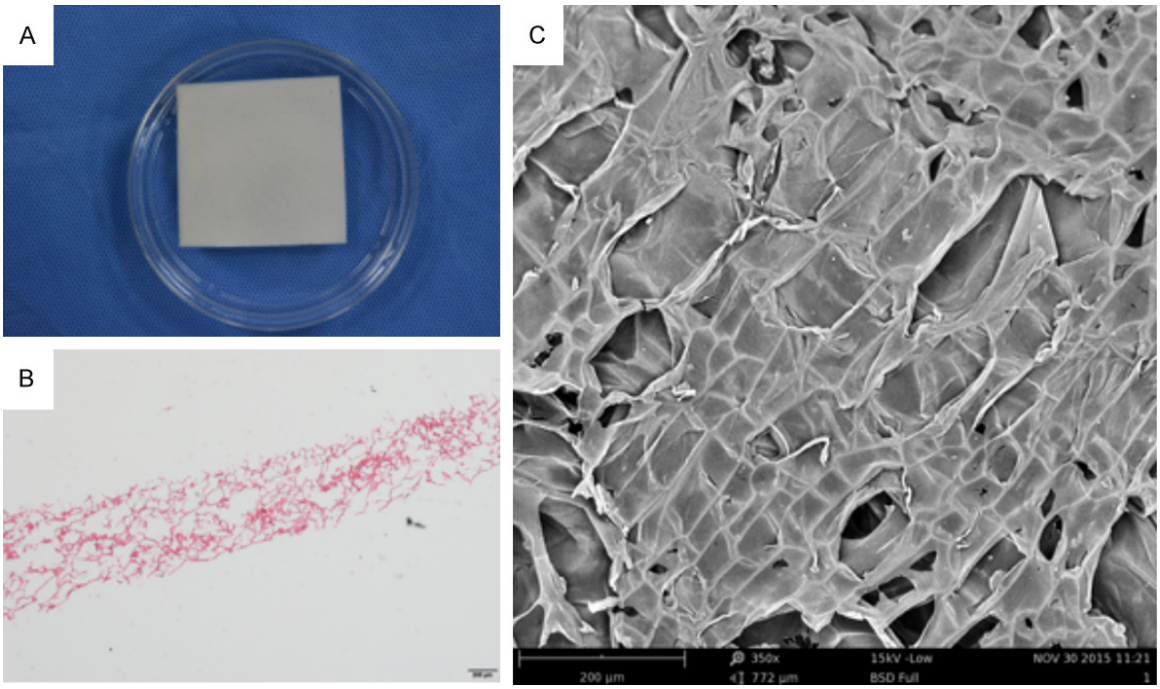

Figure 5. A: A collagen membrane is shown. The surface is smooth, the structure is compact, and it can float in the physiological saline completely. B: Shows the collagen membrane with H&E staining (5 ×): collagen fibers connect with each other to form a porous structure, with basic uniform mesh size, arranged in an orderly manner. C: Scanning electron microscope showed that the collagen fibers were irregular in size, and the pore size was 100 μm~200 μm.

ously affects healing quality. We also confirmed that in nude mice when the size of microskin was 1 mm × 1 mm, with an expansion ratio 1:10, the wound healing rate of positive transplantation was significantly higher than that of the lateral and reverse transplantation. Orientation actually affects the survival rate of microskin grafts.

A recent study showed that the epidermis of split-thickness skin contains much total lipid, and the hydrophobicity is high. Therefore, microskin can float in the physiological saline with the epidermal surface facing up. The above principle ensured positive transplantation of microskin. However, a large number of clinical applications showed that this method would be affected by the skin thickness, size and shape of microskin, and could not guarantee that all microskin would float or that the epidermal surface would completely face up. In this study, we also observed that the floating rate of pure pig microskin was only 50%, and the forward floating rate was less than 70%.

Collagen is the main protein of the extracellular matrix [21]. In recent years, fish scale-derived collagen has been more widely applied in clinical practice due to its abundant source, low cost, and low risk of infectious disease [22]. The density of the collagen membrane we made was (42.76 ± 0.13) kg/m^3, which was much less than that of water. Thus it could float completely on water and could be adhered to the epidermal surface of microskin to increase the floating performance. The forward floating rate of collagen membrane + pig microskin was above 90%, which could guarantee the forward transplantation of microskin. Animal experiments on pigs also confirmed that using collagen membrane to change the direction of microskin could improve the wound healing rate. The method of using the collagen membrane to change the direction of microskin could significantly improve the survival rate of microskin grafting, and the surface of the renascent skin was smooth. Conversely, there was a portion of microskin in the dermis and subcutaneous tissue using the conventional microskin grafting, growing laterally and rin reverse. Cells in the basal layer of microskin formed epithelial

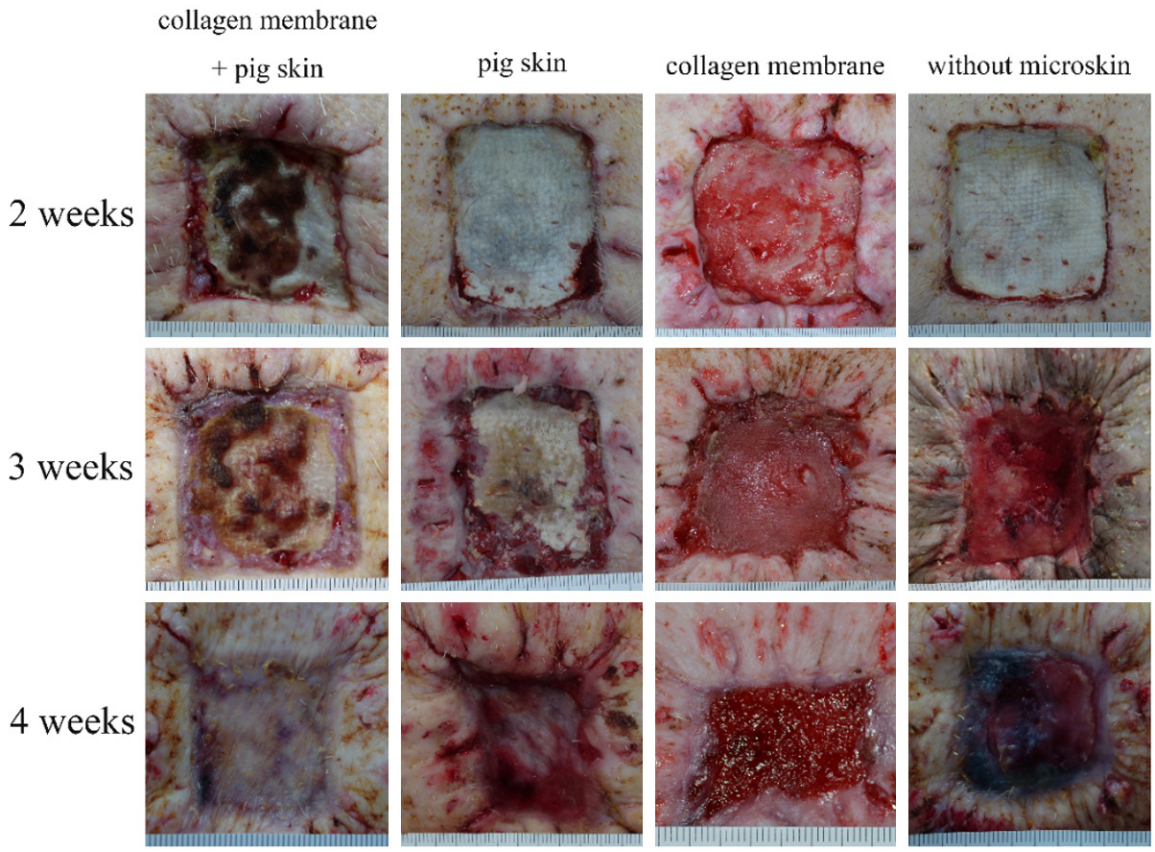

Figure 6. 2, 3 and 4 weeks of the wound surface after transplantation of autologous microskin to full thickness skin excision in pig. At 4 weeks, no collagen membrane was found in the wound, and the wound was completely epithelialized inthe collagen membrane and microskin group.

Table 1. Rate of floating, epidermal surface facing up and dermal surface facing up

	Floating rate (%)	Forward floating rate (%)	Same direction floating rate (%)
Experimental group	95.05 ± 2.09	91.25 ± 2.88	89.75 ± 2.07
Control group	52.20 ± 5.93	68.05 ± 4.65	66.40 ± 4.62
t	30.457	18.964	20.635
P	0.000	0.000	0.000

t-test was used respectively.

islands which expended outwardly, connected to the epidermis of the wound surface and forming an epidermal cyst structure. In addition, the collagen membrane we made had no cytotoxicity, and its degradation time was appropriate. When the dressing was opened, there was no residual collagen membrane, and the extension and fusion of the skin were not affected in all groups. In addition, the protein in tilapia's skin has been reported to promote quick and effective wound healing [23].

Uniformity is another important factor affecting the survival rate of microskin grafts. The wound healing with very fine microskin distribution was better, and the wound healing with less distribution or no distribution of fine microskin was poor. There was at least one microskin per square centimeter to ensure complete repair of the wound in theory. It is still difficult to guarantee the uniform distribution of microskin by using the method of floating on physiological saline, and this needs further study.

Conclusion

In microskin grafting, when the size of microskin is 1 mm × 1 mm, expanding to 10 times, the direction of microskin can significantly affect the survival rate of transplantation. Adhesion of a collagen membrane

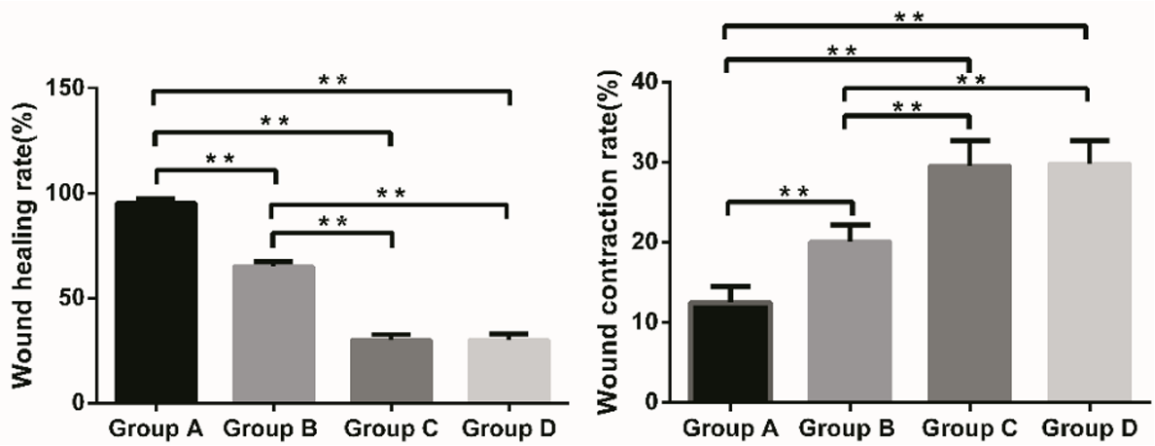

Figure 7. Comparison of wound healing rate and shrinkage rate 4 weeks after transplantation of autologous microskin to a full thickness skin excision in pig. Group A: collagen membrane + micro-skin group, Group B: conventional micro-skin group, Group C: collagen membrane group, Group D: control group. The wound healing rate of Group A was significantly higher than those of other groups, while the wound contraction rate was much less than in the other groups, $**P < 0.01$. The wound healing rate and contraction rate of the collagen membrane group had no significant difference with the control group.

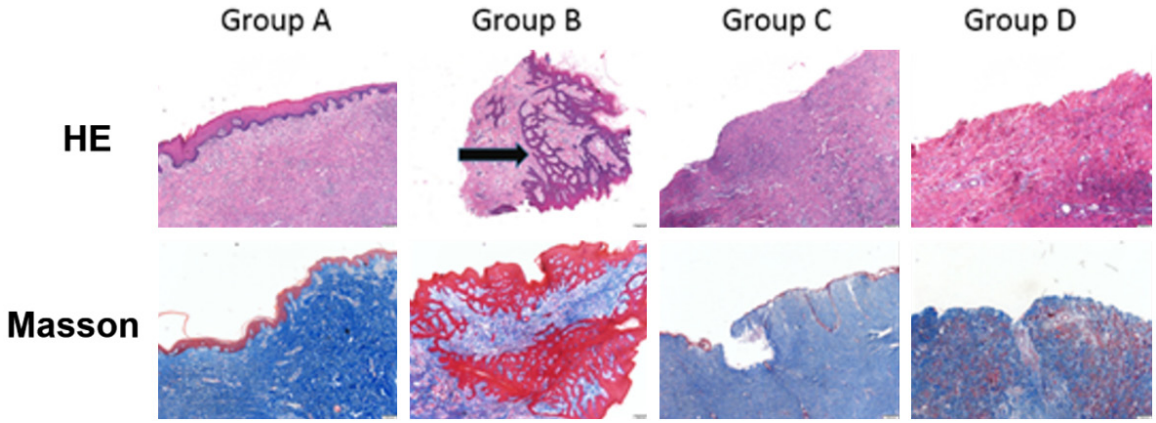

Figure 8. H&E (5 ×) and Masson (5 ×) staining 4 weeks after transplantation of autologous micro-skin to full thickness skin excision in pig. Group A: collagen membrane + micro-skin group, Group B: conventional micro-skin group, Group C: collagen membrane group, Group D: control group. H&E staining showed that the epidermal layer of Group A was continuous, and the subcutaneous tissue and dermis had no epidermal cyst formation. Obvious epidermal cyst-like structures (arrow) formed in Group B. Masson staining showed that coarse collagen fibers were arranged regularly in all groups, and no significant difference was found.

to the epidermal surface of microskin can ensure that the microskin floats completely on the physiological saline with the epidermal surface facing up. Thus this provides a new method to improve the survival rate of microskin grafting.

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Disclosure of conflict of interest

None.

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References

[1] Yuru S, Dawei L, Chuanan S, Kai Y, Li M, Longzhu L, Dongxu Z and Wenfeng C. The ben-

efit of microskin in combination with autologous keratinocyte suspension to treat full skin loss in vivo. J Burn Care Res 2017; 38: 348-353.
[2] Chua AWC, Khoo YC, Truong TTH, Woo E, Tan BK and Chong SJ. From skin allograft coverage to allograft-micrograft sandwich method: a retrospective review of severe burn patients who received conjunctive application of cultured epithelial autografts. Burns 2018; 44: 1302-1307.
[3] Houschyar KS, Tapking C, Nietzschmann I, Rein S, Weissenberg K, Chelliah MP, Duscher D, Maan ZN, Philipps HM, Sheckter CC, Reichelt B, Branski LK and Siemers F. Five years experience with meek grafting in the management of extensive burns in an adult burn center. Plast Surg (Oakv) 2019; 27: 44-48.
[4] Zheng X, Ding Z, Cheng W, Lu Q, Kong X, Zhou X, Lu G and Kaplan DL. Microskin-inspired injectable MSC-laden hydrogels for scarless wound healing with hair follicles. Adv Healthc Mater 2020; 9: e2000041.
[5] Lo CH, Chong E, Akbarzadeh S, Brown WA and Cleland H. A systematic review: current trends and take rates of cultured epithelial autografts in the treatment of patients with burn injuries. Wound Repair Regen 2019; 27: 693-701.
[6] Lee SZ, Halim AS, Wan Sulaiman WA and Mat Saad AZ. Outcome of the modified meek technique in the management of major pediatric burns. Ann Plast Surg 2018; 81: 295-301.
[7] Almodumeegh A, Heidekrueger PI, Ninkovic M, Rubenbauer J, Hadjipanayi E and Broer PN. The MEEK technique: 10-year experience at a tertiary burn centre. Int Wound J 2017; 14: 601-605.
[8] Quintero EC, Machado JFE and Robles RAD. Meek micrografting history, indications, technique, physiology and experience: a review article. J Wound Care 2018; 27: S12-S18.
[9] Alawi SA, Limbourg A, Strauss S and Vogt PM. Imaging of bacteria in burn wounds treated with split-thicknessgrafts in MEEK/MESH technique: a pilot study with first experiences in clinical wound evaluation with autofluorescence. Handchir Mikrochir Plast Chir 2019; 51: 130-138.
[10] Zhang ML. Retrospection and future of microskin grafting. Zhonghua Shao Shang Za Zhi 2008; 24: 343-345.
[11] Rissin Y, Fodor L, Talmon G, Fishelson O and Ullmann Y. Investigating human microskin grafting technique in a new experimental model. Burns 2009; 35: 681-686.
[12] Chandika P, Ko SC, Oh GW, Heo SY, Nguyen VT, Jeon YJ, Lee B, Jang CH, Kim G, Park WS, Chang W, Choi IW and Jung WK. Fish collagen/alginate/chitooligosaccharides integrated scaffold for skin tissue regeneration application. Int J Biol Macromol 2015; 81: 504-513.
[13] Zhang ML, Wang CY and Chang ZD. Microskin grafting in animal experiments and clinical practice. Zhonghua Wai Ke Za Zhi 1986; 24: 219-221, 253-214.
[14] Zhang ML, Wang CY, Chang ZD, Cao DX and Han X. Microskin grafting. II. Clinical report. Burns Incl Therm Inj 1986; 12: 544-548.
[15] Zhang ML, Chang ZD, Han X and Zhu M. Microskin grafting. I. Animal experiments. Burns Incl Therm Inj 1986; 12: 540-543.
[16] Zhang ML, Chang ZD, Wang CY and Fang CH. Microskin grafting in the treatment of extensive burns: a preliminary report. J Trauma 1988; 28: 804-807.
[17] Zhang M, Zhou G and Zhang P. Microskin grafting in recent 15 years. Zhonghua Wai Ke Za Zhi 2001; 39: 708-710.
[18] Hackl F, Bergmann J, Granter SR, Koyama T, Kiwanuka E, Zuhaili B, Pomahac B, Caterson EJ, Junker JP and Eriksson E. Epidermal regeneration by micrograft transplantation with immediate 100-fold expansion. Plast Reconstr Surg 2012; 129: 443e-452e.
[19] Singh M, Nuutila K, Kruse C, Robson MC, Caterson E and Eriksson E. Challenging the conventional therapy: emerging skin graft techniques for wound healing. Plast Reconstr Surg 2015; 136: 524e-530e.
[20] Chen X, Qin FJ, Yu DN and Yonghua S. History, present and future of microskin grafting. Chin J Injury Repair and Wound Healing (Electronic Edition) 2019; 14: 9-12.
[21] Song WK, Liu D, Sun LL, Li BF and Hou H. Physicochemical and biocompatibility properties of type I collagen from the skin of nile tilapia (oreochromis niloticus) for biomedical applications. Mar Drugs 2019; 17: 137.
[22] Shi Y, Zhang H, Zhang X, Chen Z, Zhao D and Ma J. A comparative study of two porous sponge scaffolds prepared by collagen derived from porcine skin and fish scales as burn wound dressings in a rabbit model. Regen Biomater 2020; 7: 63-70.
[23] Chen J, Gao K, Liu S, Wang S, Elango J, Bao B, Dong J, Liu N and Wu W. Fish collagen surgical compress repairing characteristics on wound healing process in vivo. Mar Drugs 2019; 17: 33.