Regenerative Therapy 22 (2023) 203–209

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

Dried human-cultured epidermis accelerates wound healing in a porcine partial-thickness skin defect model

Takashi Nakano [a], Michiharu Sakamoto [a, *], Yasuhiro Katayama [a], Yoshihiro Shimizu [b], Masukazu Inoie [b], Yuanjiaozi Li [a], Hiroki Yamanaka [a], Itaru Tsuge [a], Susumu Saito [a], Naoki Morimoto [a]

[a] Department of Plastic and Reconstructive Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan
[b] Japan Tissue Engineering, Co., Ltd., Gamagori, Japan

ARTICLE INFO

Article history:
Received 30 December 2022
Received in revised form
6 February 2023
Accepted 16 February 2023

Keywords:
Dried cultured epidermis
Allogeneic cultured epidermis
Burn treatment
Regenerative medicine
Acute wounds

ABSTRACT

Introduction: Autologous cultured epidermis (CE) is an effective approach for overcoming the deficiency of donor sites to treat extensive burns. However, the production of autologous CE takes 3–4 weeks, which prevents its use during the life-threatening period of severe burns. In contrast, allogeneic CE can be prepared in advance and used as a wound dressing, releasing several growth factors stimulating the activity of recipient cells at the application site. Dried CE is prepared by drying CEs under controlled temperature and humidity conditions until all the water is completely removed and no viable cells are present. Dried CE accelerates wound healing in a murine skin defect model and is potentially a new therapeutic strategy. However, the dried CE safety and efficacy have not yet been studied in large animal models. Therefore, we studied the safety and efficacy of human-dried CE in wound healing using a miniature swine model.

Methods: Human CE was manufactured using Green's method from donor keratinocytes. Three types of CEs (Fresh, Cryopreserved, and Dried) were prepared, and the ability of each CE to promote keratinocyte proliferation was confirmed *in vitro*. Extracts of the three CEs were added to keratinocytes seeded in 12-well plates, and cell proliferation was evaluated using the WST-8 assay for 7 days. Next, we prepared a partial-thickness skin defect on the back of a miniature swine and applied three types of human CE to evaluate wound healing promotion. On days 4 and 7, the specimens were harvested for hematoxylin-eosin, AZAN, and anti-CD31 staining to assess epithelialization, granulation tissue, and capillary formation.

Results: The conditioned medium containing dried CE extract significantly enhanced keratinocyte proliferation compared to the control group ($P < 0.05$). *In vivo* experiments revealed that human-dried CE significantly accelerated epithelialization at day 7 to the same extent as fresh CE, compared to the control group ($P < 0.05$). The three CE groups similarly affected granulation formation and neovascularization.

Conclusions: Dried CE accelerated epithelialization in a porcine partial-thickness skin defect model, suggesting that it may be an effective burn treatment alternative. A clinical study with a long-term follow-up is needed to assess the applicability of CEs in clinics.

1. Introduction

Thermal injuries affect millions of adults and children worldwide and are associated with high morbidity and mortality [1]. Extensive burn injuries can be fatal because the effects of thermal injuries are not limited to localized wounds but also can affect the entire body by causing decreased circulating plasma volume and increased inflammatory cytokine levels. In addition to general

Abbreviations: CE, cultured epidermis; allo-CE, allogeneic CE; EGF, epidermal growth factor; NSS, normal saline solution; HKGS, human keratinocyte growth supplement; WST-8, water-soluble tetrazolium salt; PBS, phosphate-buffered saline; HE, hematoxylin-eosin; AZAN, azocarmine, and aniline blue.

* Corresponding author. Department of Plastic and Reconstructive Surgery, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawaharacho, Sakyo-ku, Kyoto 606-8507, Japan.
E-mail address: dojis@kuhp.kyoto-u.ac.jp (M. Sakamoto).

Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2023.02.003

systemic management, such as fluid resuscitation and nutrient support, rapid and consistent wound healing is a key determinant for survival from extensive burns. The current treatment standard for burn wounds requires the excision of necrotic tissue within 72 h post-burn injury [1]. This early excision reduces the source of inflammatory stimuli and attenuates detrimental systemic reactions, including hypermetabolic responses [2,3]. Autologous skin grafting is the gold standard [4] for reconstructing skin defects. However, skin grafting is invasive and causes morbidity in the uninjured skin area. In addition, a larger burn leads to smaller healthy skin areas available for autologous skin grafting [1].

Autologous cultured epidermis (CE) is an effective approach for addressing the deficiency of donor sites in treating extensive burns. A serial cultivation method for human keratinocytes established by Green and Rheinwald in 1975 made it possible to produce cultured keratinocyte sheets large enough to cover the entire body from a small skin biopsy [5]. Since then, many reports have demonstrated the efficacy of autologous CE in burn wound treatment. However, the production of autologous CE takes 3–4 weeks, which prevents its use during the life-threatening period of severe burns. In addition, manufacturing autologous CE from each patient as a customized treatment is expensive, which presents a major socioeconomic problem [6].

Allogeneic CE (allo-CE) compensates for the shortcomings of autologous CE [7] because it can be prepared in advance and used as a wound dressing. Allo-CE releases several growth factors that stimulate the activity of the recipient's cells at the application site [8,9]. Also, allo-CE is usually cryopreserved and stored in hospitals to keep it available for patients. For instance, a cryopreserved allogeneic CE, Kaloderm® (Tego Science, Seoul, Korea), is available in Korea for treating dermal burns and diabetic foot ulcers [10]. However, the need for long-term preservation without changing the product quality and freezers during the storage and transportation of cryopreserved products represents a problem in clinics.

In a previous study, we examined the relationship between cell viability and the effectiveness of CEs in promoting wound healing. We found that cell-depleted dried CEs could promote wound epithelialization in a diabetic mouse model [11,12]. Dried allo-CE was prepared by drying CEs under controlled temperature and humidity conditions until all the water was completely removed and no viable cells were present. The absence of viable cells in dried CE allows sterilization at the end of the manufacturing process, thereby reducing the risk of microorganism contamination. In addition, dried allo-CE can be stored at room temperature for a long period and be used immediately off the shelf, which is an advantage during the acute phase of burn treatment while waiting for autologous CE to be ready.

Dried allo-CE is a potentially new therapeutic strategy for the treatment of burns. However, dried allo-CE has not been studied in a large animal model before initiating a clinical trial. Therefore, this study evaluated the safety and efficacy of human-dried CE on wound healing in a xenograft partial-thickness skin defect model using miniature swine and revealed the wound-healing-promoting effect of dried CE.

2. Methods

2.1. Ethics statement

Human keratinocytes were obtained from the skin of a supernumerary finger resected from a patient with polydactyly at Kyoto University Hospital. The donor's parents provided written informed consent before specimens were obtained. This protocol was approved by the Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine (permit numbers R0467-1 and R0690-3) and was in accordance with the Declaration of Helsinki. Our experimental protocol for animal experimentation was approved by the Animal Research Committee of the Kyoto University Graduate School of Medicine (No. Med kyo 20,125). The number of animals used in this study was minimum, and all efforts were made to reduce animal suffering in compliance with the protocols established by the Animal Research Committee.

2.2. Preparation of cultured epidermis

Human CE was prepared using a modified Green's method as previously described with some modifications [5,13]. The skin without subcutaneous tissue was sliced into pieces. After trypsinization, keratinocytes were isolated from the supernatant, disseminated on an irradiated feeder layer of 3T3-J2 cells in a flask, and cultured in a 3:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium supplemented with fetal bovine serum, insulin, hydrocortisone, cholera toxin, triiodothyronine, epidermal growth factor (EGF), and antibiotics in an atmosphere of 10% CO_2 at 37 °C. For the CEs preparation, keratinocytes were cultivated for approximately one week until confluence. Fresh CE was obtained as keratinocyte sheets, detached from the flasks after treatment with dispase II and then backed with non-woven gauze as a carrier (10 × 8 cm) for easy handling.

The dried CE was prepared by drying fresh CEs. The fresh CE was transferred to a blank plate and dried under controlled temperature and humidity conditions until the water was completely removed. The dried CEs quickly recovered similar flexible physical properties to those before drying when they were rehydrated with normal saline solution (NSS) immediately before use. Cryopreserved CEs were prepared by cryopreservation at −80 °C for over one day. Precisely, cryopreserved CEs were backed with non-woven gauze and then put in a cryotube with a cryopreservation medium containing 10% dimethyl sulfoxide and cryopreserved at −80 °C for at least one day. The cryopreserved CEs were thawed immediately before use by incubation in a water bath at 37 °C for 8 min, followed by the removal of the cryoprotectant by gentle washing twice with NSS.

2.3. Keratinocyte proliferation assay

2.3.1. Preparation of a conditioned medium from CE

A sheet of CEs (dried, cryopreserved, and fresh) (10 × 8 cm) was incubated in a 50 mL tube containing 20 mL serum-free medium (EpiLife; Gibco, Waltham, MA, USA) at 4 °C for 24 h. The supernatant was used as the conditioned medium.

2.3.2. Preparation of keratinocyte

Human keratinocytes were used at 5–6 passages. In total, 2.0×10^4 keratinocytes per 1 mL of EpiLife supplemented with 1% human keratinocyte growth supplement (HKGS)(S0015; Thermo Fisher Scientific, MA, USA) were seeded in each well of a 12-well plate (Thermo Fisher Scientific, Waltham, MA, USA) (n = 30) and incubated at 37 °C in a 5% CO_2 atmosphere for 24 h. After incubation, viable cells on day 0 were evaluated using a Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan). The water-soluble tetrazolium salt (WST-8; 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) was reduced by an intracellular dehydrogenase to produce water-soluble formazan. Upon directly measuring the absorbance of formazan at 450 nm, the amount of formazan dye was proportional to the number of living cells. The remaining 24 samples were divided into four groups (control, dried, cryopreserved, and fresh) and further cultured.

2.3.3. Evaluation of keratinocyte proliferation

The medium was changed to the medium required for each group (mentioned below) after washing twice with phosphate-buffered saline (PBS) and incubating at 37 °C in 5% CO_2. (Control group: 1 mL EpiLife, dried group: 900 μL EpiLife and 100 μL conditioned medium from dried CE, cryopreserved group: 900 μL EpiLife and 100 μL conditioned medium from cryopreserved CE, and fresh group: 900 μL EpiLife and 100 μL conditioned medium from fresh CE.)

On days 2, 4, and 7, keratinocytes were observed under a phase-contrast microscope (ECLIPSE TS100; Nikon, Tokyo, Japan). Cell proliferation was evaluated on days 4 and 7 using the WST-8 assay (n = 3 at each time point). In 12-well plates, 100 μL WST-8 solution was added to each well and incubated for 2 h, and absorbance at 450 nm was measured using a microplate reader (Versa Max, Molecular Devices, Sunnyvale, CA, USA).

2.4. Evaluation of the wound healing promotion of CEs in a porcine partial-thickness skin defect model

2.4.1. Experimental design and operative procedure

The 13-month-old male minipig, CLAWN miniature swine (Shimizu Experimental Materials, Kyoto, Japan), weighing approximately 27.5 kg, was used for this study. The animal was cared for as outlined in the Public Health Services Policy on Human Care and Use of Laboratory Animals. General symptoms, such as feeding status and limping, were assessed daily. The minipig fasted from the evening before the operation and was sedated via subcutaneous injection of 30 mg/kg ketamine (Ketalar, DAIICHI SANKYO, Tokyo, Japan) and 5 mg/kg xylazine hydrochloride (Celactal, Bayer, Tokyo, Japan). Then the minipig was anesthetized by inhaling a mixture of air and oxygen containing 2.0–2.5% isoflurane and placed under positive pressure control. Both sides of the back were shaved with a hair clipper, and four partial-thickness skin defects (8 × 10 cm, 1.5 mm with depth) were prepared on both backs using an electric dermatome (Keisei Co., Tokyo, Japan). Four wounds were allocated to each group (dried, cryopreserved, fresh, and control), and the wounds in the three CE groups (dried, cryopreserved, and fresh) were covered with a respective CE sheet. All wounds, including the control group, were covered with a silicone-faced wound dressing (SI-Mesh, ALCARE, Tokyo, Japan) as a contact layer and sterile gauze and fixed using a stapler and an elastic adhesive bandage (Elatex 10; ALCARE, Tokyo, Japan).

On postoperative day 4, all dressings were removed and the wounds were evaluated macroscopically under general anesthesia. After taking photographs, skin biopsies (n = 6 in each group) were harvested for histological evaluation, using 5 mm-diameter punch biopsy tools (Kai Industries, Gifu, Japan) from the inside of the wounds in each group. The wounds were then covered and fixed with dressings, as previously described. On postoperative day 7, the animals were sacrificed by intravenous potassium chloride injection, the wounds were photographed, and skin biopsies (n = 6) were harvested in the same way as on day 4.

The skin specimens were fixed in a 10% neutral-buffered formalin solution. The specimens were embedded in paraffin to prepare 5-μm sections, which were then subjected to hematoxylin-eosin (HE) staining, azocarmine and aniline blue (AZAN) staining, and immunohistochemical staining for CD31.

2.4.2. Assessment of epithelialization

The epithelialization ratio on days 4 and 7 was measured on the HE-stained sections of skin biopsies using an optical microscope (KEYENCE BZ-810, KEYENCE Japan, Osaka, Japan). Epithelialization ratio (%) was determined using the following equation:

$$\textit{Epithelialization ratio}\ (\%) = \frac{L_E}{L_o} \times 100 \quad (1)$$

where L_E is the total epithelial length of the section, and Lo is the overall length of the section.

2.4.3. Assessment of regenerated tissue

The newly formed granulation and epithelium areas on days 4 and 7 were measured using AZAN-stained sections. The overall area, including the epithelium above the remaining dermis, was measured using an optical microscope.

2.4.4. Assessment of newly formed capillaries

Immunohistochemical staining for CD31 was performed to detect newly formed capillaries. Anti-CD31 rabbit polyclonal antibodies (dilution 1:10,000, code ab182981, Abcam) and Simple Stain Rabbit MAX-PO (Nichirei Biosciences Inc., Tokyo, Japan) were used as the primary and secondary antibodies, respectively. Staining was visualized using 3-3′-diaminobenzidine-4HCl (DAB), counter-stained with hematoxylin, and micrographs were taken under an optical microscope (KEYENCE BZ-810). We measured the number and area of the newly formed capillaries in the granulation area of each section. A threshold was set for the brown tint stained with DAB, and regions with a color density higher than this threshold were counted using the BZ-X800 Analyzer software (Keyence Corp., Osaka, Japan). For the capillary area, the area in which the tubular structure of the blood vessels was visible was measured, and the sum of the areas was calculated. Both the capillary number and area were measured in the entire area of the newly formed granulation tissue, as determined by AZAN staining [14,15].

2.5. Statistical analysis

Statistical significance was determined using the Tukey-Kramer test. All data are expressed as the mean ± standard deviation. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Enhancement of keratinocyte proliferation by CE extracts

Fig. 1 shows the ability of CE extracts (dried, cryopreserved, and fresh) to promote keratinocyte growth. The absorbance at 450 nm in each CE group (dried, cryopreserved, and fresh) was significantly higher than that in the control group on days 4 and 7, indicating that the conditioned medium containing CE extract significantly enhanced keratinocyte proliferation.

3.2. Acceleration of epithelialization by CE in a porcine partial-thickness skin defect model

3.2.1. Macroscopic evaluation

The wounds on days 0, 4, and 7 are shown in Fig. 2. On day 4, the applied CE was visible as a translucent membrane on the wound surfaces in each CE group (dried, cryopreserved, and fresh), and no differences in wound appearance were observed among the four groups. On day 7, the wounds were covered with a crust. No obvious signs of infection were observed at any time.

3.2.2. Histological evaluation

3.2.2.1. Assessment of epithelialization. The HE-stained sections on days 4 and 7 are shown in Fig. 3. On day 4, epithelialization from the hair follicles and granulation tissue formation began slightly. On day 7, epithelialization and granulation tissue formation progressed

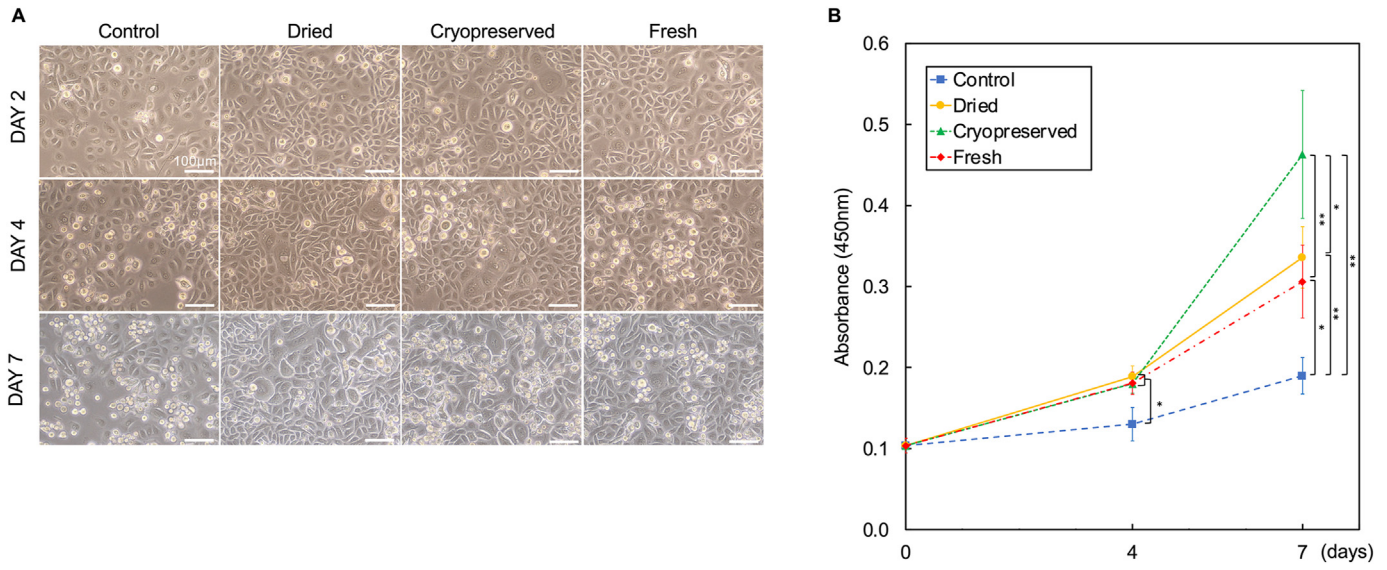

Fig. 1. Enhancement of keratinocyte proliferation by CE extracts. (A) On days 2, 4, and 7, keratinocytes were observed under an inverted microscope. (B) On days 0, 4, and 7, keratinocyte proliferation was evaluated with the WST-8 assay (n = 3, at each time point). Error bars show standard deviation. Absorbance (450 nm) in the CE groups (dried, cryopreserved, and fresh) were significantly larger than that in the control group at day 4 and 7. (*$P < 0.05$, **$P < 0.01$). In addition, absorbance (450 nm) in the cryopreserved CE group was significantly larger than that in the dried and fresh CE groups at day 7 (*$P < 0.05$, **$P < 0.01$).

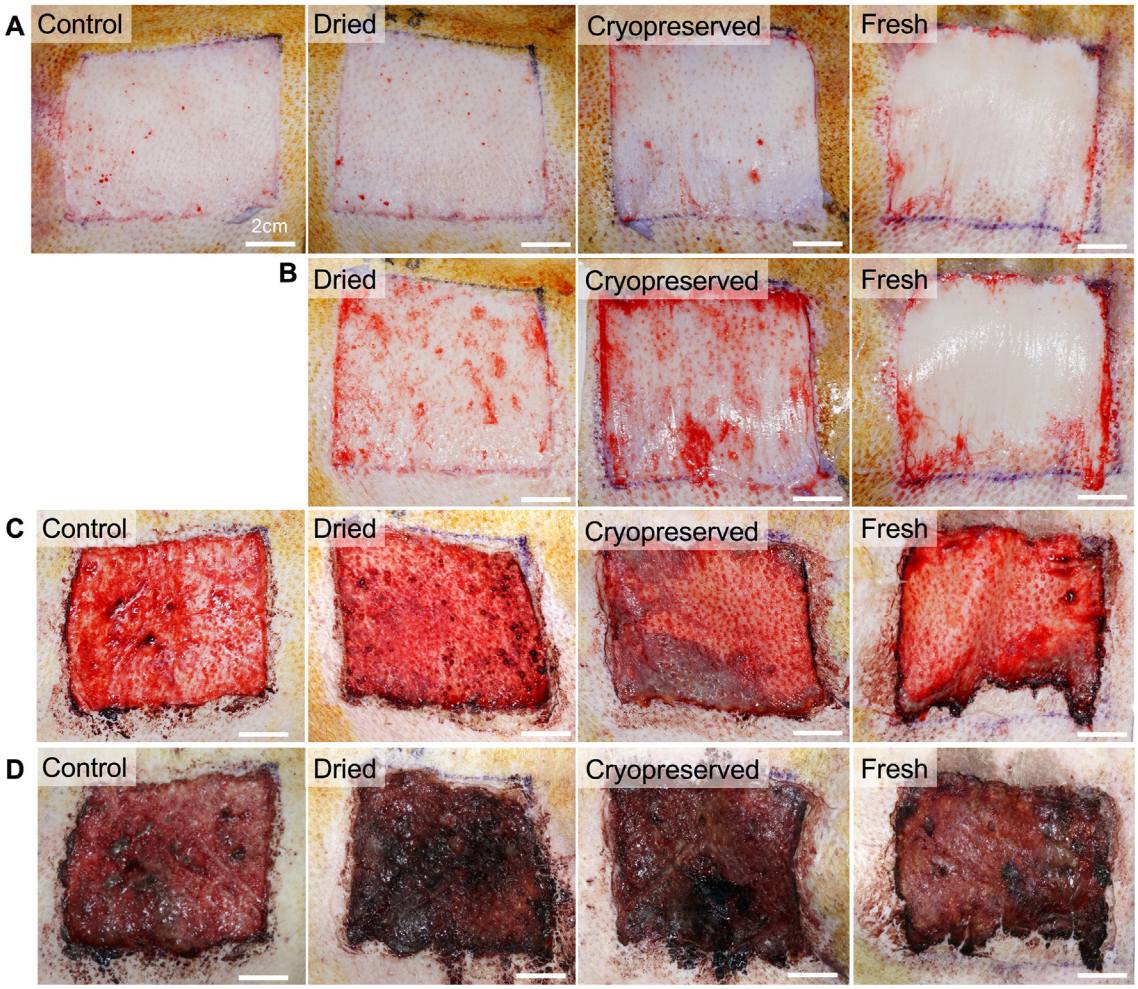

Fig. 2. The wounds in the control, dried, cryopreserved, and fresh CE groups. (A) Partial-thickness skin wounds (8 × 10 cm in size, 1.5 mm with depth) were created at four locations on the back of minipig with an electric dermatome. (B) Three types of CEs (dried, cryopreserved, and fresh) were applied to cover the entire wound surface. (C) The wounds on postoperative day 4. (D) The wounds on postoperative day 7.

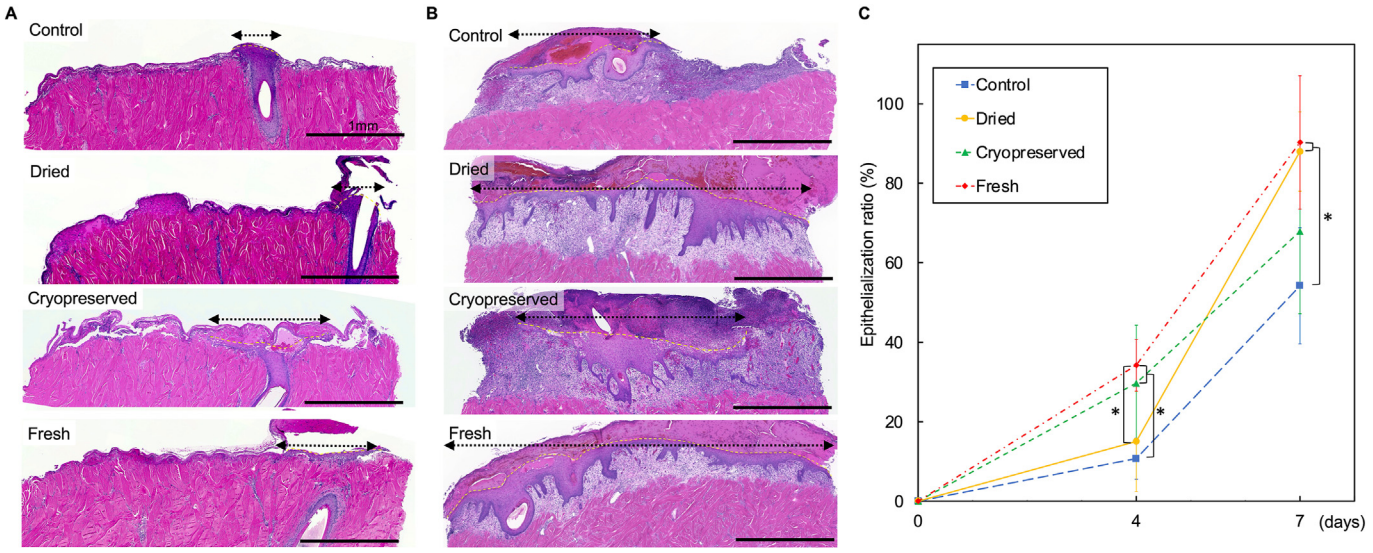

Fig. 3. The HE-stained sections in a porcine partial-thickness skin defect model. The arrows indicate the newly formed epithelium. (A) On postoperative day 4, epithelialization (yellow dashed line) was observed from the hair follicles. (B) On postoperative day 7, epithelialization (yellow dashed line) was more advanced in dried and fresh CE groups, compared to control group. (C) The epithelialization ratios of four groups. The epithelialization ratios of dried group and fresh group were significantly larger than that of the control group on day 7 (*$P < 0.05$, n = 6). Error bars show standard deviation.

further, and the epidermis was stratified. On day 7, the epithelialization ratios in the dried and fresh CE groups were significantly higher than those in the control group (Fig. 3C).

3.2.2.2. Assessment of regenerated tissue. AZAN-stained sections on day 7 are shown in Fig. 4A. In the AZAN-stained sections, regenerated granulation tissue was observed in light blue over the remaining dermis. The area of regenerated tissue in each group was not significantly different (n = 6) (Fig. 4B).

3.2.2.3. Assessment of newly formed capillaries. Immunohistochemical staining of CD31 sections on day 7 is shown in Fig. 5A. The number of capillaries in the cryopreserved CE group was significantly larger than that in the control group, and the capillary areas in each group were not significantly different (n = 6) (Fig. 5B and C).

4. Discussion

The clinical utility of allo-CE was reported in the 1980s. Allo-CE is a cultured keratinocyte sheet manufactured from donor cells that promotes wound healing when used in deep dermal burns [16], donor sites [17], and chronic ulcers [18]. Keratinocytes in allo-CE transplanted to the recipient site do not permanently survive and

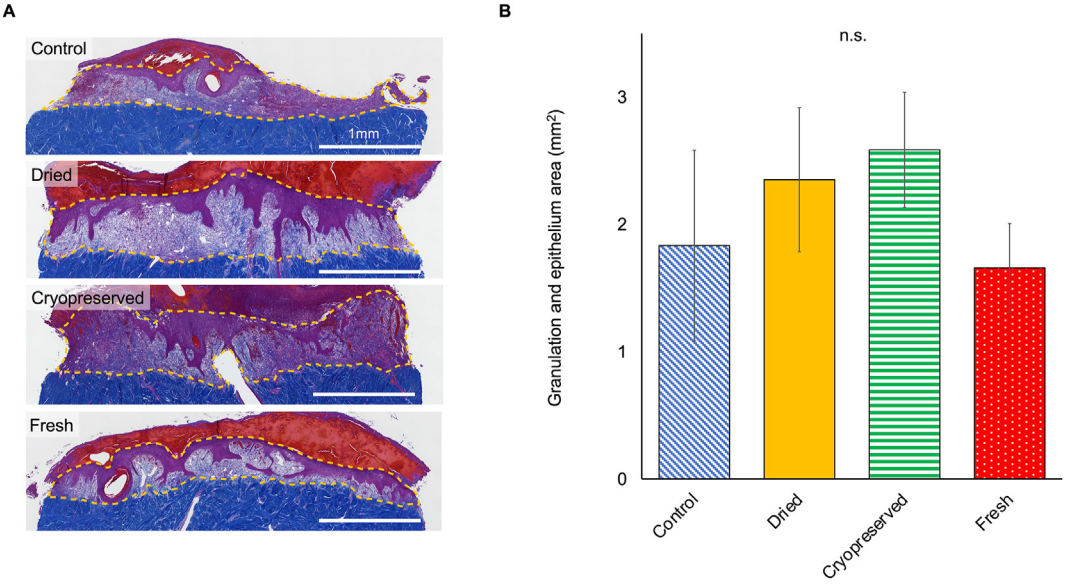

Fig. 4. The Azan-stained sections in a porcine partial-thickness skin defect model. (A) On postoperative day 7, the area of newly formed granulation tissue and epithelium was indicated by the yellow dashed line enclosure. (B) The area of newly formed granulation tissue and epithelium. The granulation area in the dried group was significantly larger than that in the control group (*$P < 0.05$, n = 6). Error bars show standard deviation.

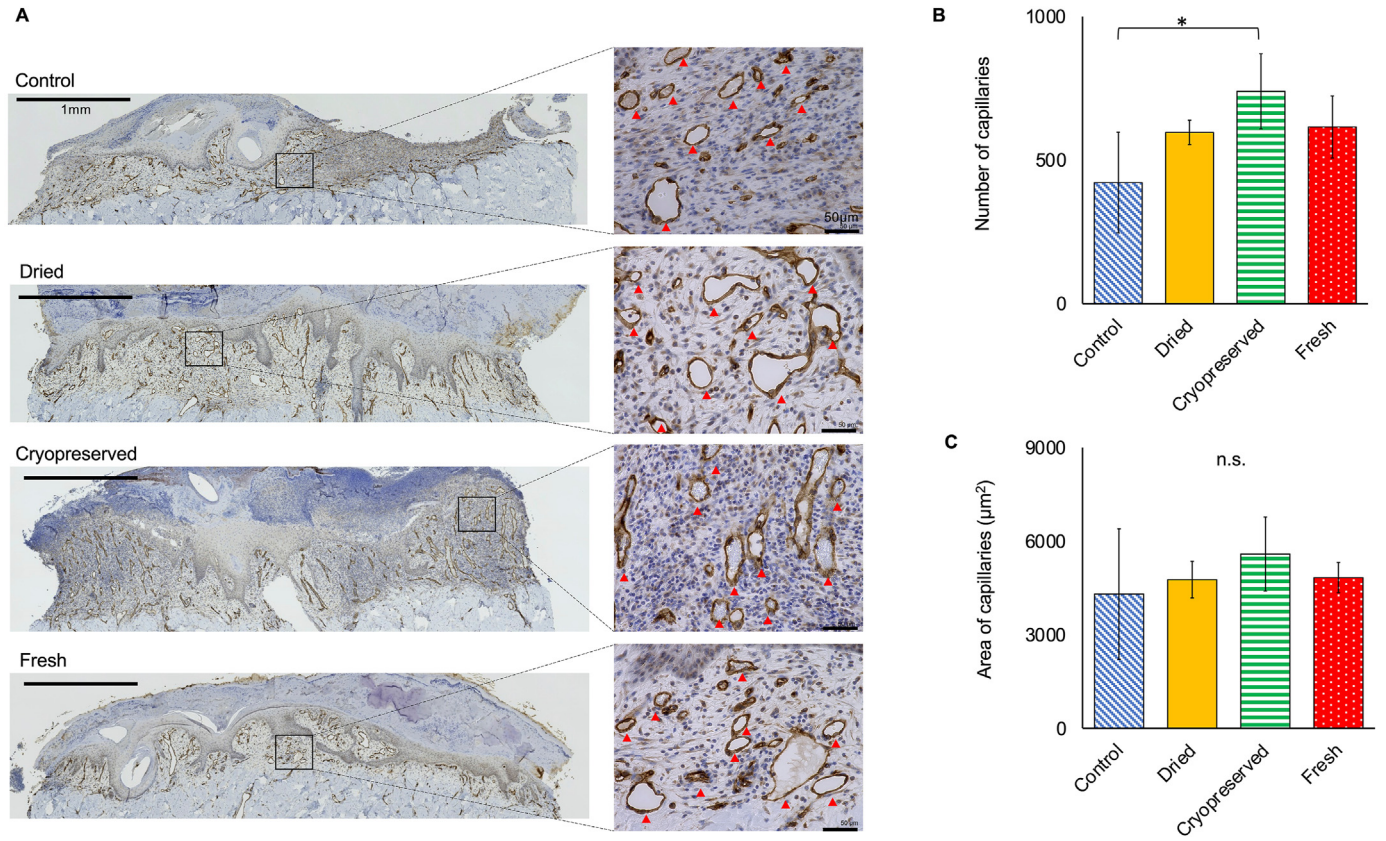

Fig. 5. The immunohistochemical-stained for CD31 sections in a porcine partial-thickness skin defect model. (A) On postoperative day 7, newly formed capillaries was indicated by red arrowheads (▲). (B) The number of newly formed capillaries in each group. The number in the dried group was significantly larger than that in the control group (*$P < 0.05$, n = 6). (C) The area of newly formed capillaries in each group. The area in the dried group was significantly larger than that in the control group (*$P < 0.05$, n = 6).

are eventually replaced by recipient cells, that is, they serve as physiological wound dressings [19,20]. In our previous reports, human CE containing no viable cells after repeated freeze-thaw cycles promoted epithelialization and accelerated wound area reduction as effectively as fresh CE in murine skin defect wounds [11]. This suggests that human CE promotes wound healing by covering the wound surface with multiple layers of keratinocytes and releasing various bioactive substances to create an appropriate environment for recipient cells to proliferate and migrate even in the absence of proliferative cells. Owing to a lack of clinically available allo-CE products in Japan, establishing a quality-controlled cell bank is mandatory. We therefore established a keratinocyte and fibroblast bank of clinically accessible human cultured cells in Japan [21].

Several reports have been published on the effectiveness of dried CE. Dried CE had similar morphological and physical properties compared with those of cryopreserved CE, and promoted epithelialization and accelerated the reduction of the wound area as well as cryopreserved CE in murine skin defect models [12,22]. Thus, dried CEs are a promising modality for burn wound treatment. However, the safety and efficacy of dried CEs have not been investigated in non-clinical studies using large animal models. Therefore, we evaluated the safety and efficacy of human-dried CE in miniature swine.

We prepared a partial-thickness skin defect on the back of a miniature swine and used three types of human CE (fresh, cryopreserved, and dried) to evaluate wound healing. The back of a minipig has many hairs, resulting in epithelialization primarily from the hair follicles and other skin appendages inside the partial-thickness wound. Human-dried CE significantly accelerated epithelialization arising from this skin appendage at day 7 to the same extent as fresh CE, compared to the control group. In terms of granulation tissue formation and neovascularization, the three CE groups were comparable, and those in the dried CE group were larger than those in the control group, although the differences were not statistically significant. These CEs can be considered to have promoted wound healing by acting as versatile wound dressings that secrete bioactive substances, as previous reports have shown [11].

In this study, we confirmed that the bioactive substances contained in CE indeed have physiological bioactivity in a keratinocyte proliferation assay. Previous reports have shown using cytokine arrays that the dehydration process to produce dried CE does not reduce the amount of cytokines and chemokines [12]; however, being detected on a cytokine array does not necessarily guarantee that the detected proteins have biological activity. Therefore, we confirmed that conditioned medium from each CE promoted keratinocyte proliferation. This indicates that dried CE contains factors that promote keratinocyte proliferation as much as fresh CE, which is consistent with previous reports [12].

Thus, dried CE can be used as a physiological and versatile wound dressing, which can be stored for a long time at room temperature and used off-the-shelf when needed. The best use of dried CE is in cases where hair follicles or other skin appendages remain in the wound, such as in second-degree burn wounds or donor sites. In addition, dried CE can be used in combination with autologous meshed skin grafts to treat extensive and third-degree burns [23,24]. Dried CE can be a useful modality in the treatment of severe burns,

especially during the first three weeks of preparing autologous CE, to promote wound reduction and improve survival and functional prognosis in patients with severe burns. Furthermore, dried CE has advantages in terms of socio-medical economics because it can be produced at a much lower cost than autologous CE.

Certain limitations of the current study are as follows: first, the study was performed using a xenograft model because of which the observed safety and efficacy of dried CE is not always certified as allografts in clinical practice; second, this experiment was conducted on a partial-thickness skin defect model and therefore, cannot be directly applied to second-degree burn wounds. In clinical practice for burn wounds, allo-CE should be applied only to viable wound surfaces after adequate debridement of all necrotic tissue. Third, the follow-up period was relatively short. In the future, we intend to perform a clinical study with long-term follow-up to assess the applicability of CEs in clinical situations.

5. Conclusions

Dried CE accelerated epithelialization in a porcine partial-thickness skin defect model, suggesting that dried CE could be an effective option for burn treatment. A clinical study with a long-term follow-up is needed to assess the applicability of CEs in clinical situations.

Funding

This research was funded by the Translational Research Network Program (21he0122014j0001) of the Japan Agency for Medical Research and Development (AMED). The funder had no role in the study design, data collection, analysis, decision to publish, or manuscript preparation.

Declaration of competing interest

Japan Tissue Engineering, Co., Ltd. (J-TEC) supported the manufacturing of the cultured epidermis used in this study. Y.S. and M.I. were employed by J-TEC, and the funder provided support in the form of salaries for Y.S. and M.I. but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The other authors declare no competing interests.

Acknowledgments

The authors thank Rie Tatsumi and Yasuko Minaki for their generous assistance, and Editage (www.editage.com) for English language editing.

References

[1] Amini-Nik S, Dolp R, Eylert G, Datu AK, Parousis A, Blakeley C, et al. Stem cells derived from burned skin - the future of burn care. EBioMedicine 2018;37:509–20. https://doi.org/10.1016/j.ebiom.2018.10.014.
[2] Jeschke MG, Patsouris D, Stanojcic M, Abdullahi A, Rehou S, Pinto R, et al. Pathophysiologic response to burns in the elderly. EBioMedicine 2015;2:1536–48. https://doi.org/10.1016/j.ebiom.2015.07.040.
[3] Stanojcic M, Vinaik R, Jeschke MG. Status and challenges of predicting and diagnosing sepsis in burn patients. Surg Infect 2018;19:168–75. https://doi.org/10.1089/sur.2017.288.
[4] Kearney JN. Clinical evaluation of skin substitutes. Burns 2001;27:545–51. https://doi.org/10.1016/S0305-4179(01)00020-1.
[5] Rhelnwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell 1975;6:331–44.
[6] Domaszewska-Szostek AP, Krzyżanowska MO, Czarnecka AM, Siemionow M. Local treatment of burns with cell-based therapies tested in clinical studies. J Clin Med 2021;10. https://doi.org/10.3390/jcm10030396.
[7] Brychta P, Adler J, Řihová H, Suchánek I, Kaloudová Y, Koupil J. Cultured epidermal allografts: quantitative evaluation of their healing effect in deep dermal burns. Cell Tissue Bank 2002;3:15–23. https://doi.org/10.1023/A:1021898704210.
[8] Hefton JM, Finkelstein JL, Madden MR, Thomas Shires G. Grafting of burn patients with allografts of cultured epidermal cells. Lancet 1983;322:428–30. https://doi.org/10.1016/S0140-6736(83)90392-6.
[9] Brain A, Purkis P, Coates P, Hackett M, Navsaria H, Leigh I. Survival of cultured allogenic keratinocytes transplanted to deep dermal bed assessed with probe specific for Y chromosome. Br Med J 1989;298:917–9. https://doi.org/10.1136/bmj.298.6678.917.
[10] Kim EH, Lee SH. Efficacy of cultured allogenic keratinocytes in treatment of deep second-degree burn. J Burn Care Res 2021;42:533–7. https://doi.org/10.1093/jbcr/iraa191.
[11] Sakamoto M, Ogino S, Shimizu Y, Inoie M, Lee S, Yamanaka H, et al. Human cultured epidermis accelerates wound healing regardless of its viability in a diabetic mouse model. PLoS One 2020;15:1–14. https://doi.org/10.1371/journal.pone.0237985.
[12] Sakamoto M, Nakano T, Tsuge I, Yamanaka H, Katayama Y, Shimizu Y, et al. Dried human cultured epidermis accelerates wound healing in diabetic mouse skin defect wounds. Sci Rep 2022;12:1–11. https://doi.org/10.1038/s41598-022-07156-w.
[13] Green H, Kehinde O, Thomas J. Growth of cultured human epidermal cells into multiple epithelia suitable for grafting. Proc Natl Acad Sci U S A 1979;76:5665–8. https://doi.org/10.1073/pnas.76.11.5665.
[14] Kanda N, Morimoto N, Takemoto S, Ayvazyan AA, Kawai K, Sakamoto Y, et al. Efficacy of novel collagen/gelatin scaffold with sustained release of basic fibroblast growth factor for dermis-like tissue regeneration. Ann Plast Surg 2012;69:569–74. https://doi.org/10.1097/SAP.0b013e318222832f.
[15] Sakamoto M, Morimoto N, Ogino S, Jinno C, Taira T, Suzuki S. Efficacy of gelatin gel sheets in sustaining the release of basic fibroblast growth factor for murine skin defects. J Surg Res 2016;201:378–87. https://doi.org/10.1016/j.jss.2015.11.045.
[16] De Luca M, Bondanza S, Cancedda R, Tamisani AM, Di Noto C, Muller L, et al. Permanent coverage of full skin thickness burns with autologous cultured epidermis and reepithelialization of partial skin thickness lesions induced by allogeneic cultured epidermis: a multicentre study in the treatment of children. Burns 1992;18(Suppl 1):S16–9. https://doi.org/10.1016/0305-4179(92)90105-4.
[17] Duinslaeger LA, Verbeken G, Vanhalle S, Vanderkelen A. Cultured allogeneic keratinocyte sheets accelerate healing compared to Op-site treatment of donor sites in burns. J Burn Care Rehabil 1997;18:545–51. https://doi.org/10.1097/00004630-199711000-00013.
[18] Lindgren C, Marcusson JA, Toftgård R. Treatment of venous leg ulcers with cryopreserved cultured allogeneic keratinocytes: a prospective open controlled study. Br J Dermatol 1998;139:271–5. https://doi.org/10.1046/j.1365-2133.1998.02364.x.
[19] Gielen V, Faure M, Mauduit G, Thivolet J. Progressive replacement of human cultured epithelial allografts by recipient cells as evidenced by HLA class I antigens expression. Dermatologica 1987;175:166–70. https://doi.org/10.1159/000248820.
[20] van der Merwe AE, Mattheyse FJ, Bedford M, van Helden PD, Rossouw DJ. Allografted keratinocytes used to accelerate the treatment of burn wounds are replaced by recipient cells. Burns 1990;16:193–7. https://doi.org/10.1016/0305-4179(90)90038-x.
[21] Nakano T, Katayama Y, Sakamoto M, Shimizu Y, Inoie M, Shimizu N, et al. Establishment of a keratinocyte and fibroblast bank for clinical applications in Japan. J Artif Organs Off J Jpn Soc Artif Organs 2022. https://doi.org/10.1007/s10047-022-01331-6.
[22] Jang H, Kim YH, Kim MK, Lee KH, Jeon S. Wound-healing potential of Cultured Epidermal Sheets is unaltered after lyophilization: a preclinical study in comparison to cryopreserved CES. BioMed Res Int 2013;2013:907209. https://doi.org/10.1155/2013/907209.
[23] Akita S, Hayashida K, Yoshimoto H, Fujioka M, Senju C, Morooka S, et al. Novel application of cultured epithelial autografts (CEA) with expanded mesh skin grafting over an artificial dermis or dermal wound bed preparation. Int J Mol Sci 2017;19. https://doi.org/10.3390/ijms19010057.
[24] Sakamoto M, Morimoto N, Inoie M, Takahagi M, Ogino S, Jinno C, et al. Cultured human epidermis combined with meshed skin autografts accelerates epithelialization and granulation tissue formation in a rat model. Ann Plast Surg 2017;78:651–8. https://doi.org/10.1097/SAP.0000000000001058.