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Original Article

A high-hydrostatic pressure device for nevus tissue inactivation and dermal regeneration for reconstructing skin defects after giant congenital melanocytic nevus excision: a clinical trial

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ABSTRACT

Background: A novel treatment has been developed to reconstruct large skin defects caused by the excision of giant congenital melanocytic nevi. It involves the reimplantation of high-hydrostatic pressurized nevus tissue as a cell-inactivated autologous scaffold for dermal regeneration, followed by the implantation of cultured epithelial autografts on the regenerated dermis. Because this treatment has shown promise in a first-in-human clinical trial which used a prototype pressure machine, a novel pressure device was specifically designed for clinical use.

Methods: In a prospective investigator-initiated clinical trial involving three patients, we evaluated the safety and efficacy of the skin regeneration treatment using a pressure device. All three patients underwent surgical excision of the nevus tissue, primary reimplantation of the inactivated nevus tissue, and secondary implantation of cultured epithelial autografts.

Results: Engraftment of inactivated nevus tissue and cultured epithelial autografts was successful in all three cases, with over 90% epithelialization at 8 weeks post-surgery. No serious adverse events or device malfunction were observed during the trial.

Conclusion: The novel pressure device safely and effectively enabled dermal regeneration using the nevus tissue as an autologous scaffold. This innovative approach offers several advantages, including reduced invasiveness due to minimal sacrifice of normal skin for skin grafting and high curative potential resulting from full-thickness removal of the nevus tissue.

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1. Introduction

A giant congenital melanocytic nevus is a type of large melanocytic nevus present at birth and measures ≥6 cm on the body or ≥9 cm on the head of neonates, and >20 cm in diameter in adults [1]. It carries a risk of malignant melanoma [2,3], and early prophylactic surgical excision is recommended to reduce this risk [4]. However, complete removal of these skin lesions results in large full-thickness skin defects because nevus cells are histologically present throughout the dermal layer which usually requires harvesting of large amounts of normal skin for reconstruction.

To address this issue, a novel treatment has recently been developed to reconstruct full-thickness skin defects after excision of giant congenital melanocytic nevi [5,6]. This involves the primary reimplantation of high-hydrostatic pressurized nevus tissue as a cell-inactivated autologous scaffold for the dermis regeneration, followed by secondary implantation of cultured epithelial autografts on the regenerated dermis. Nevus tissue is inactivated by applying high-hydrostatic pressurization at 200 MPa for 10 min, which effectively kills all cells in human skin and nevus tissue [7–11].

In a first-in-human clinical trial conducted from 2016 to 2018 [6], full-thickness nevi were inactivated using a portable high-hydrostatic pressure machine (Kitaoka Iron Works Co. Ltd., Osaka, Japan) at 200 MPa for 10 min [7–11], and subsequently reimplanted onto the original site. Four weeks post-surgery, the cultured epithelial autografts were implanted onto the regenerated dermis. The reconstructed skin was epithelized by more than 96% in each graft at 12 weeks, and no recurrence was observed at 52 weeks. This suggests that inactivated nevus tissue can provide a suitable graft bed for cultured epidermal autografts. However, the pressure machine used in the trial was a prototype, and the required manual pressure adjustment posed the risk of inaccuracy and lack of reproducibility. Therefore, to improve the safety and reproducibility of the treatment and facilitate insurance approval, we developed a new medical device, a high-hydrostatic pressure device, and a skin container specifically to inactivate nevus tissue for clinical use. A clinical assessment of the treatment outcomes is necessary to evaluate the performance of these devices. Hence, we conducted this prospective investigator-initiated clinical trial to evaluate the safety and efficacy of skin regeneration treatments using inactivated nevus tissues created using this new device and cultured epithelial autografts.

2. Patients and methods

2.1. Study objectives, design, and participants

This was a prospective, open-label, nonrandomized, noncomparative, single-arm clinical trial to confirm the safety and efficacy of the following treatment methods: surgical excision of nevus tissue, primary reimplantation of inactivated nevus tissue created with the investigational devices, and secondary implantation of cultured epithelial autografts. The eligibility criteria included patients with giant congenital melanocytic nevi deemed unsuitable for resection using standard treatments, including primary closure, tissue expansion, and skin grafting. The exclusion criteria included extensive scarring from previous therapies, where the successful engraftment of the inactivated nevus was deemed unlikely (Table 1). We selected the target nevus to be within 10% or less of the body surface area of each trial patient who met the eligibility and exclusion criteria. A target sample size of three cases was set, considering the rarity of the disease and the feasibility of accumulating cases in this study. The study was registered in the Japan Registry of Clinical Trials (ID: jRCT2052210044) and was conducted between July 2021 and March 2022 at Kyoto University Hospital, Japan.

This study was conducted in accordance with the Declaration of Helsinki and the Pharmaceutical Affairs Law. Following the review process of the Pharmaceuticals and Medical Devices Agency, the study design and amendments received Institutional Review Board approval from Kyoto University Hospital (approval no. K077). Written informed consent was obtained from all the patients. The study was conducted in accordance with the principles of good clinical practice.

2.2. Investigational devices

2.2.1. High-hydrostatic pressure device

The pressure device (Fig. 1A) was manufactured by Sugino Machine Co., Ltd. (Toyama, Japan) according to the Japanese safety requirements for electrical equipment (JIS C1010-1). It was delivered to Kyoto University Hospital by Japan Tissue Engineering Co., Ltd. (J-TEC, Aichi, Japan). The main components of the device include a pressure container, pressure sensor, servomotor, and water supply tank with dimensions of 950 × 850 × 1440 mm (width × depth × height). It was designed to apply hydrostatic pressures of up to 300 MPa on the samples inside a pressure container.

2.2.2. Skin container

The skin container (Fig. 1B) was specifically designed to hold the excised nevus tissue during pressurization. It was manufactured by Hanshin Kasei Kogyo Co., Ltd. (Toyama, Japan) using polyethylene and was 45.0 mm in diameter and 89.9 mm in height. The container was sterilized by Radia Industry Co., Ltd. (Gunma, Japan) and delivered to Kyoto University Hospital by J-TEC.

2.3. Intervention

2.3.1. Preparation of cultured epithelial autografts

Two weeks prior to the initial surgical treatment of the target nevus, a normal skin sample of approximately 1 × 2 cm was harvested under either general or local anesthesia and transported to J-TEC. It took three weeks on average to prepare cultured epithelial autografts (JACE®) by J-TEC.

2.3.2. Staged surgical interventions

During the initial surgery, the target nevus was excised to full thickness. A punch biopsy specimen was obtained to evaluate the deep margins. The excised nevus tissue was adjusted to a thickness of 0.5 mm using a Padgett dermatome, and in some cases, it was also meshed. The nevus tissue was placed in a skin container filled with saline solution and sealed. The skin container was set into the pressure container of the device, and pressurized at 210 MPa for 10 min. The inactivated nevus tissue thus obtained was sutured to the original site, fixed, and stabilized using a tie-over dressing and/or splint. The second surgery was performed at 2 (±1) weeks after the initial surgery, during which cultured epithelial autografts were grafted onto the reimplanted nevus tissue. If an area was observed where the inactivated nevus did not survive and the subcutaneous tissue was exposed, it was covered with split-thickness skin grafts and cultured epithelial autografts. Moreover, if needed, additional application of split-thickness skin grafts and/or cultured epithelial autografts was allowed once during 2–6 weeks after the second surgery.

Digital photographs of the target nevus and the reconstructed skin were taken before excision, after reimplantation of the inactivated nevus, 8 weeks after the second surgery, and 24 weeks after the initial surgery. Digital photographs of the donors were obtained

Table 1
Study eligibility and exclusion criteria.

Eligibility Criteria:
1 Giant congenital melanocytic nevi that could not be resected with standard treatments, including primary closure, tissue expansion, and skin grafting
2 Pathological confirmation of the presence of nevus cells in the deep dermal layer
3 Patients ≥6 months of age who could undergo surgery under general or local anesthesia
4 Provision of written informed consent
Exclusion Criteria:
1. Patients with extensive scarring from previous therapies, where engraftment of the inactivated nevus was not expected
2. Patients receiving systemic immunosuppressants or steroids
3. Patients with allergies to penicillin, kanamycin, streptomycin, and amphotericin B or a history of allergy to penicillin and aminoglycoside antibiotics
4. Patients allergic to cows, mice, or pigs
5. Patients with a history of malignant skin tumors or with suspected malignant skin tumors
6. Patients who had participated in other clinical trials within the last 3 months
7. Patients who previously participated in this clinical trial and underwent transplantation for an inactivated autologous nevus
8. Patients judged by the investigators as being inappropriate for participation in the clinical trial

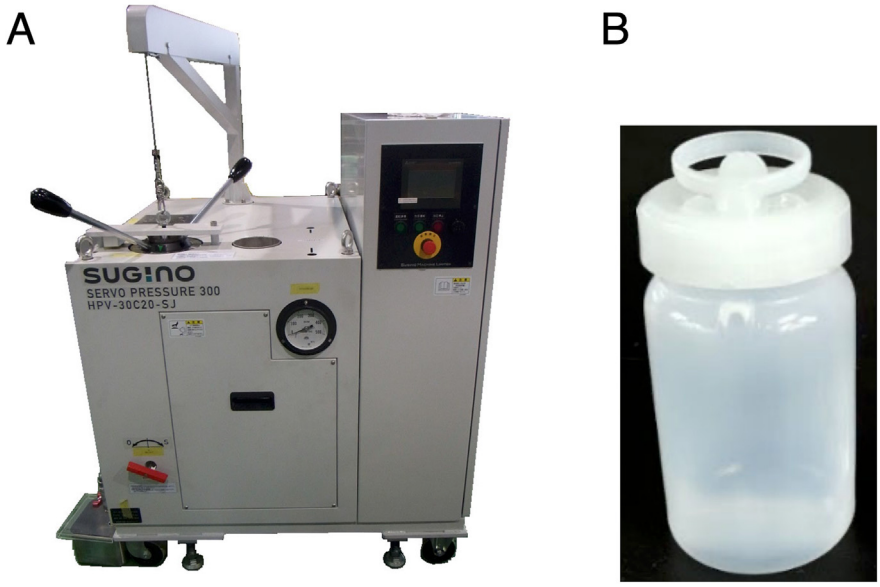

Fig. 1. (A) High-hydrostatic pressure device and (B) skin container.

when additional skin grafting was required. Blood examinations were performed before the initial surgery and 2 (±1) and 24 (±4) weeks after the initial surgery. After 24 (±4) weeks, a punch biopsy specimen of the reconstructed skin was obtained to diagnose nevus recurrence.

2.4. Outcomes and statistical analysis

The primary outcome was successful engraftment of both the inactivated nevus tissue and cultured epithelial autografts, both of which were evaluated 8 weeks after the second surgery. This evaluation timeframe was set to align with the assessment conducted in a previous clinical trial of JACE® for expanding insurance coverage to giant congenital melanocytic nevi. Successful engraftment was defined as an epithelialization rate (ratio of the epithelialized area to the surface area of the target nevus) exceeding 90%. An independent committee comprising three plastic surgeons assessed the epithelial area of the reconstructed skin at 8 weeks. The point estimate of the proportion of cases judged to have engraftment, along with the two-sided 95% confidence interval (CI) was calculated using the Clopper-Pearson method. The secondary endpoints involved safety assessment, including but not limited to any instance of device malfunction, and adverse events such as nevus recurrence, within 24 weeks after the initial surgery. Efficacy was also assessed by calculating the skin grafting ratio (the ratio of the donor area of the skin grafts required to reconstruct the area where the inactivated autologous nevus did not survive to the area of the reimplanted inactivated autologous nevus). Additionally, the color change between the target nevus and the reconstructed skin was evaluated using Commission Internationale de l'Eclairage (CIE) L × a*b* [12]. Continuous variables were expressed as means (± standard deviations).

3. Results

3.1. Patient demographics and summary

A participant flowchart is shown in Fig. 2. Three patients (one man and two women) with giant congenital melanocytic nevi covering 21.7% (±15.3%) of their body surface area and no history of other disorders were enrolled in the study. All were younger than 1 year, with a mean of 8.7 (±2.1) months. Their height was 67.8 (±2.2) cm and their weight was 7.4 (±0.4) kg. The target nevi were located on the upper back (case 1), lower back (case 2), and left lower leg (case 3), respectively, and their sizes were 4.7 (±2.5) % of the body surface area. A patient summary is shown in Table 2, and the gross appearance of the target nevus at each evaluation point in each patient is shown in Fig. 3.

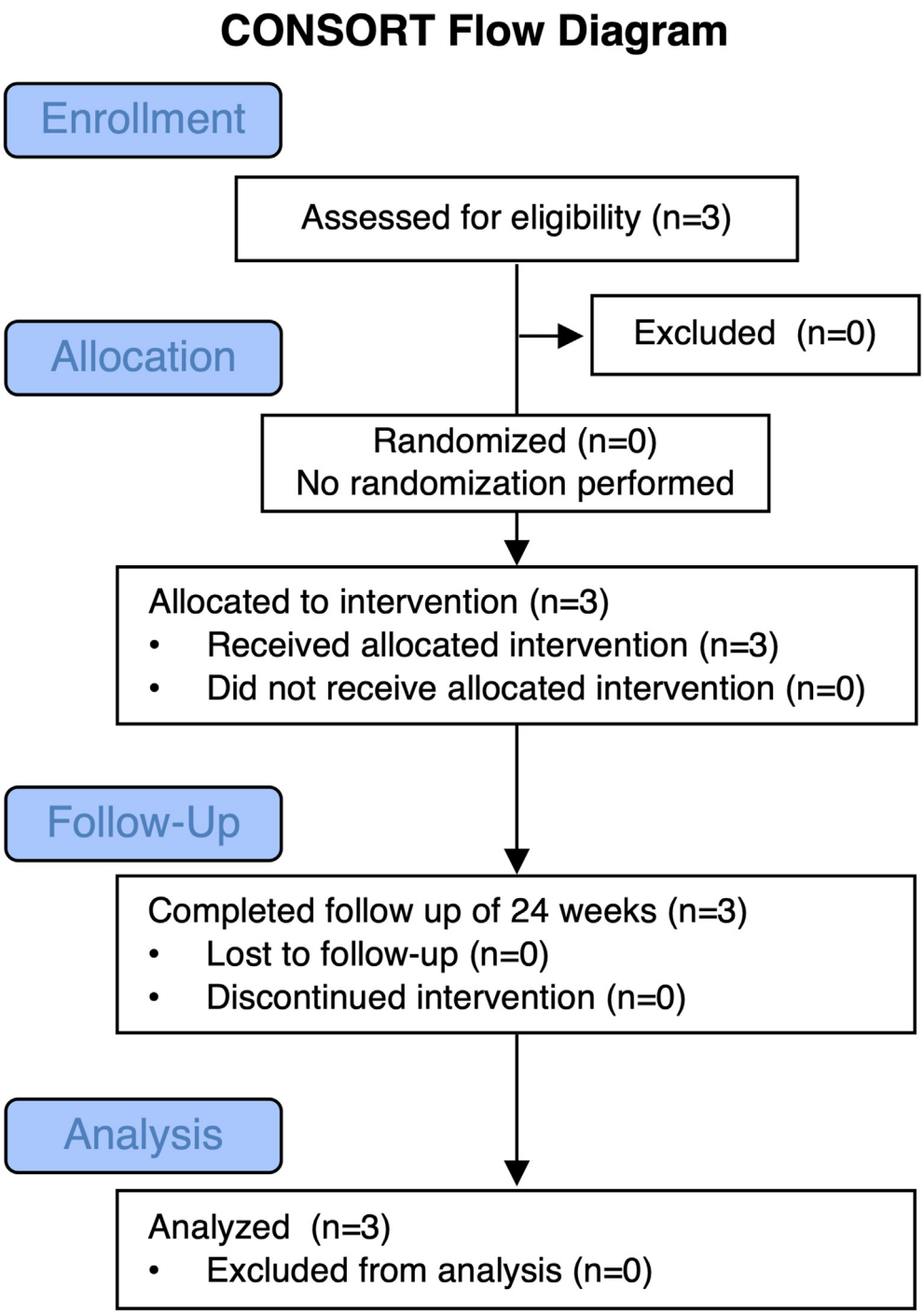

Fig. 2. Participant flow chart.

3.2. Efficacy

3.2.1. Engraftment of inactivated nevus tissue and cultured epithelial autografts

In all three cases, the engraftment of inactivated nevus tissue and cultured epithelial autografts was successful. Eight weeks after the second surgery, in all patients, epithelialization was achieved in over 90% of the target area in all patients (97.9%, 98.7%, and 99.5%, respectively) (Fig. 3). The 95% CI for the successful engraftment rate, calculated using the Clopper-Pearson method, was 29.2–100%.

3.2.2. Skin grafting ratio

In case 1, epithelialization was achieved with only cultured epithelial autografts on the inactivated nevus tissue without the need for additional skin grafting after inactivated nevus reimplantation (skin grafting ratio:0%). In case 2, an additional skin grafting of 1.33 cm^2 (skin grafting ratio:1.37%), obtained from normal skin excised simultaneously as small nevi outside the target area was applied four weeks after the second surgery. In case 3, an additional skin grafting of 13.23 cm^2 (skin grafting ratio:13.37%) obtained from the scalp was applied during the second surgery.

Table 2
Patient summary.

Case	Age (months)	Sex	Target site	Target area (cm^2)	Additional SG during the second surgery	Additional SG or CEA after the second surgery	Engraftment of inactivated nevus and CEA at 8 weeks after the second surgery (epithelialization rate, %)	Skin grafting ratio (%)	L* value		Recurrence
									before	24 weeks	
1	8	M	Upper back	109.4	No	No	Successful (97.86)	0	92.19	100.68	No
2	7	F	Lower back	99.57	No	SG + CEA	Successful (98.71)	1.37	93.47	145.21	Yes
3	11	F	Lower leg	69.93	Yes	CEA	Successful (99.50)	13.37	70.93	122.02	No

M, male; F, female; SG, skin graft; CEA, cultured epithelial autograft.

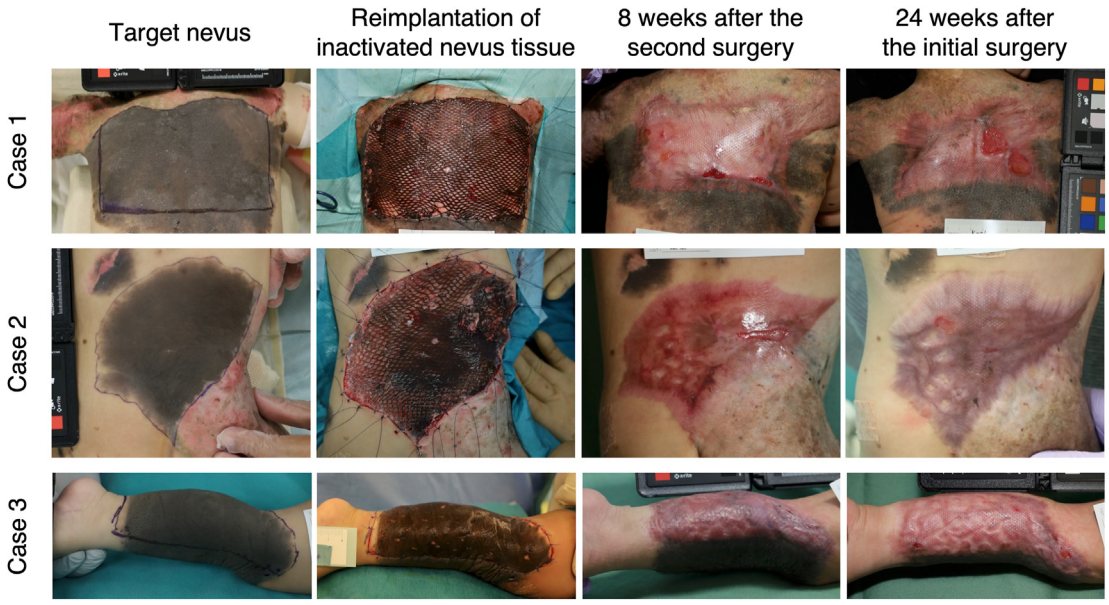

Fig. 3. Gross appearances of target lesions at each timepoint.

3.2.3. Color of the reconstructed skin

The color of the inactivated and reimplanted nevus tissue initially remained black, but improved over time. Moreover, the L* values of the reconstructed skin were significantly higher than those of the target nevi.

3.3. Safety

3.3.1. Recurrence

The biopsy results of the excised nevi were negative for deep margins in cases 1 and 3 while they were positive in case 2. Biopsy of the reconstructed skin 24 weeks after the initial surgery showed no presence of nevus cells in cases 1 and 3, but the presence of nevus cells only in the epidermal layer in case 2, indicating recurrence (Fig. 4).

3.3.2. Adverse events and device malfunction

No serious adverse events were observed in the study population (n = 3). The adverse events that could not be ruled out as having a causal relationship with the investigational devices and treatments included anemia (n = 1), fever (n = 2), pain during postoperative dressing changes (n = 3), wound infection (n = 1), elevation of inflammatory markers (n = 1), blister formation (n = 1), erosion formation (n = 3), hypertrophic scarring (n = 3), and pruritus (n = 1). No device malfunctions were observed during the trials.

4. Discussion

Cultured epithelial autografts prepared using Green's technique [13] have played a major role in advancing the development of regenerative medicine. In Japan, JACE® cultured epithelial autograft has been used for the treatment of severe burns since 2007 and of giant congenital melanocytic nevi since 2016. However, the take rate of cultured epithelial autografts on full-thickness skin defects is low at less than 20%, whereas the rate for partial-thickness skin defects is high at 80% [14]. A 6-year multicenter surveillance in Japan also revealed that the take rate of JACE® on the artificial dermis was only 43% at four weeks and improved to 74% when combined with a wide-mesh autograft [15]. An effective method for regenerating the dermis to provide a suitable wound bed for cultured epithelial autografts has not been established.

This study enrolled three patients who underwent surgical excision of the nevus tissue, primary reimplantation of inactivated nevus tissue created with the investigational devices, and secondary implantation of cultured epithelial autografts. Our previous study showed that full-thickness, inactivated nevus tissue did not survive successfully, while reimplantation of split-thickness tissue resulted in good epithelialization [6]. This suggests a limitation to the thickness at which inactivated nevus tissue can be recellularized and revascularized. Therefore, in this study, the excised nevus tissue was adjusted to a thickness of 0.5 mm. Engraftment of inactivated nevus tissue and cultured epithelial autografts was

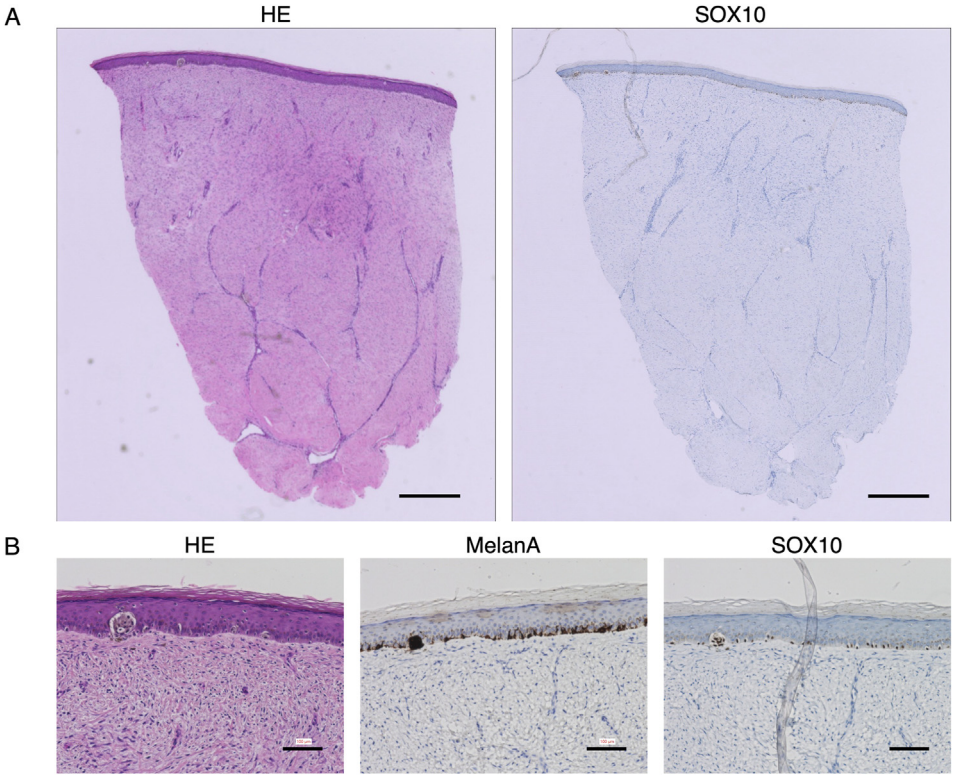

Fig. 4. Histological sections of the reconstructed skin at 24 weeks in case 2 revealed the presence of nevus cells forming nests only in epidermal layer. (A) Hematoxylin-eosin and SOX10-stained sections. Scale bar: 500 μm. (B) Magnified views of sections stained using hematoxylin-eosin, MelanA, and SO×10. Scale bar: 100 μm.

successful in all three cases, with over 90% epithelialization achieved 8 weeks post-surgery. These results are consistent with those of a first-in-human clinical trial, which also supports the idea that inactivated nevi can provide a suitable graft bed for cultured epidermal autografts [6]. In general, skin grafting for a large full-thickness skin defect requires harvesting of normal skin, which is 17% (1:6 Meek graft) to 60% (1:3 mesh graft) of the defect [16–19]. However, in this clinical trial, the maximum skin grafting ratio was 13.4%, suggesting that our treatment method reduces the sacrifice of normal skin for skin grafting.

No serious adverse events were reported, and no device malfunction was observed during the trial. In case 2, nevus cells were confirmed to be present in the epidermal layer of the reconstructed skin, indicating nevus recurrence. However, the Data and Safety Monitoring Committee determined that this recurrence was not due to device performance issues. Rather, nevus cells likely migrated from the remaining nevus tissue in the surrounding area or deep tissues, as no nevus cells were found in the dermis of the reconstructed skin, and non-clinical studies have shown that nevus cells are completely killed by high-pressure treatment [7–11].

Consequently, the present clinical trial suggests that the investigational devices safely and effectively enable dermal regeneration using the nevus tissue. This method offers several advantages, including reduced invasiveness due to minimal sacrifice of normal skin for reconstruction and high curative potential resulting from full-thickness removal of the nevus tissue. One disadvantage is the requirement for ex vivo removal of the nevus tissue and subsequent inactivation. However, treatments such as laser and cryotherapy without nevus removal are insufficient to kill nevus cells completely and uniformly [20,21], and no alternative treatments have been developed. This innovative approach has the potential to yield favorable outcomes in the treatment of giant congenital melanocytic nevi.

5. Conclusions

A high-hydrostatic pressure device was developed for the reconstruction of full-thickness skin defects resulting from the excision of giant congenital melanocytic nevi. The pressure device safely and effectively enabled dermal regeneration using the excised nevus tissue itself.

Declaration of competing interest

The authors (MS and NM) were financially supported by Japan Tissue Engineering Co., Ltd. (J-TEC) for their collaboration. J-TEC supplied cultured epithelial autografts for this study; however, the company did not play any additional role in the study design, data collection and analysis, decision to publish, or manuscript preparation.

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