Retrospective Cohort Study

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Early identification of delayed wound healing in complex diabetic foot ulcers treated with a dermal regeneration template: a novel clinical target and its risk factors

Ting-Yu Tai, MD[a], Kuan-Jie Lin, MD[b,c], Hao-Yun Chang, MD[d], Yi-Chun Wu, MD[c,e,f], Ching-Uen Huang, HN[e], Xin-Yi Lin, RN[e], Feng-Chou Tsai, MD, PhD[c,e], Ching-Sung Tsai, MD[c,e], Yu-Han Chen, NP[e], Fu-Yu Wang, RN[g], Shun-Cheng Chang, MD[c,e,*]

Background: The dermal regeneration template (DRT), a tissue-engineered skin substitute composing a permanent dermal matrix and an upper temporary silicone layer that serves as the epidermis, has demonstrated efficacy in treating uncomplicated diabetic foot ulcers (DFUs). Our institution has obtained good outcomes with DRT in patients with more complicated DFUs. Because of its chronicity, the authors are working to identify a clinical target that anticipates delayed healing early in the treatment in addition to determining the risk factors linked to this endpoint to increase prevention.

Materials and methods: This retrospective single-center study analyzed patients with DFUs who underwent wound reconstruction using DRT between 2016 and 2021. The patients were categorized into poor or good graft-take groups based on their DRT status on the 21st day after the application. Their relationship with complete healing (CH) rate at day 180 was analyzed. Variables were collected for risk factors for poor graft take at day 21. Independent risk factors were identified after multivariable analysis. The causes of poor graft take were also reported.

Results: This study examined 80 patients (38 and 42 patients in the poor and good graft-take groups, respectively). On day 180, the CH rate was 86.3% overall, but the poor graft-take group had a significantly lower CH rate (76.3 vs. 95.2%, $P = 0.021$) than the good graft-take group. Our analysis identified four independent risk factors: transcutaneous oxygen pressure less than 30 mmHg (odds ratio, 154.14), off-loading device usage (0.03), diabetic neuropathy (6.51), and toe wound (0.20). The most frequent cause of poor graft take was infection (44.7%), followed by vascular compromise (21.1%) and hematoma (15.8%).

Conclusion: Our study introduces the novel concept of poor graft take at day 21 associated with delayed wound healing. Four independent risk factors were identified, which allows physicians to arrange interventions to mitigate their effects or select patients more precisely. DRT represents a viable alternative to address DFUs, even in complicated wounds. A subsequent split-thickness skin graft is not always necessary to achieve CH.

Keywords: dermal regeneration template, diabetic foot ulcer, graft take, risk factor, skin substitute, wound healing

[a]Division of Cardiovascular Surgery, Heart Center, Cheng Hsin General Hospital, [b]Division of Cardiovascular Surgery, Department of Surgery, Shuang-Ho Hospital, [c]Department of Surgery, School of Medicine, College of Medicine, Taipei Medical University, [d]Department of Medical Education, Division of General Medicine, Far Eastern Memorial Hospital, [e]Division of Plastic Surgery, Integrated Burn and Wound Care Center, Department of Surgery, Shuang-Ho Hospital, [f]Department of Biomedical Engineering, National Yang Ming Chiao Tung University, Taipei, Taiwan and [g]Cabrini Hospital, Melbourne, Australia

Ting-Yu Tai and Kuan-Jie Lin equally contributed to this study and share the first author position.

Shun-Cheng Chang is the corresponding author of this article.

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**Corresponding author. Address: Division of Plastic Surgery, Integrated Burn and Wound Care Center, Department of Surgery, Shuang-Ho Hospital, No. 291, Zhong-Zheng Rd., Zhong-He Dist., New Taipei City 235, Taiwan. Tel.: +886 2 2249 0088 ext. 2715, +886 935 585 014; fax: +886 2 2245 5110. E-mail: csc901515@gmail.com (S.-C. Chang).*

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Introduction

Diabetic foot ulcers (DFUs) are a significant complication of diabetes mellitus (DM), affecting 6.3% of patients with diabetes worldwide and up to 13.0% in North America[1]. In Taiwan, the prevalence of DFU is estimated to be between 0.5 and 0.8%[2]. DFUs are challenging to treat because of their susceptibility to infection, relative ischemia, and association with neuropathy and multisystemic diseases[3]. Despite advancements in modern medicine, only one-third of DFUs heal within 12 months and one-sixth of DFUs require amputation within the first year of presentation to the health-care system. Patients with diabetes have a 10 times higher rate of lower extremity amputation compared to patients without diabetes. Those who undergo major amputation have a 5-year survival rate of less than 50%, which is comparable to many malignancies[4,5]. Therefore, DFUs remain a crucial issue requiring a standardized strategy and multidisciplinary approach for evaluation and treatment[6].

Traditional methods for reconstructing skin defects, such as skin grafts, local flaps, and free flaps, are commonly used for DFUs, but they may cause additional wounds and donor-site morbidity. Skin substitutes (SS) have emerged as a viable alternative for DFU treatment. The use of SS dates back to 3500 years ago and the field has undergone significant evolution since the late 20th century due to advances in tissue engineering and biotechnology, leading to the development of numerous SS products[7–11]. Research has shown the effectiveness of SS in various types of wounds, making them a new option in the reconstruction ladder. Several classification systems have been proposed to categorize SS, including those proposed by Balasubramani, Kumar, and Ferreira[12–14]. In 2018, Davison–Kotler *et al*.[15] proposed a new classification system that incorporated key factors from previous classifications. This system includes five factors (i.e. cellularity, layering, replaced region, materials used, and permanence), which allow for a more methodical description of the different commercially available SS.

Although there have been doubts about the use of SS in ischemic and infected wounds, there is increasing evidence that dermal and multilayered substitutes can improve the process of complete wound healing and lower the amputation rate in DFUs when used in conjunction with standard care[16]. However, most of the literature requires the wound area to be between 1 and 25 cm^2 and the Meggitt–Wagner (M–W) ulcer classification grade to be 1 or 2[17–20]. Two different SS are available in our hospital: that is, Integra dermal regeneration template (DRT) (Integra LifeSciences Corporation) and Terudermis (Terumo Corporation). Both are acellular, bilayered DRTs (or called 'acellular dermal matrix' or 'artificial dermis' in other articles) with a permanent dermal matrix and an upper temporary silicone layer that serves as the epidermis. They have been shown to be effective in treating DFUs[21–25]. When compared to standard care alone, the use of DRT has been shown to increase the likelihood of wound healing, reduce wound healing time, decrease the risk of major amputation within 1 year, and does not elevate the likelihood of complications[16,22,23]. Furthermore, among patients undergoing split-thickness skin grafts (STSGs), those who received DRT exhibited lower Manchester Scar Scale scores, lower wound recurrence rates within 12 months, and increased transcutaneous oxygen pressure ($TcPO_2$) values at 3 and 6 months after the application[26,27]. We have used both DRTs in various types of wounds, including large and high-graded DFUs, following an approach that differs from those of other hospitals.

Early detection of treatment failure and timely adjustments in management are crucial for facilitating complete healing (CH) in chronic DFUs. In DFUs treated with DRT; however, there is a lack of clinical evidence for predicting potential delays in healing. Therefore, we aimed to identify specific clinical targets to improve this emerging treatment approach. The typical processes of our two different DRTs involve removing the upper silicone sheet 14 days after the application (D14), which makes the subsequent period an optimal time to evaluate the treatment's effectiveness. Hence, in patients with DFUs treated with DRT, our hypothesis posits that poor graft take (GT) at post-DRT day 21 (D21) may be correlated with lower CH rates at post-DRT day 180 (D180). We chose to evaluate at D21 instead of D14 because the attachment status at D21 would be a more stable and representative state for slow-healing wounds, such as DFUs, based on our experiences. Our study proposes the clinical use of evaluating DRT status at D21 for the first time and identifies the risk factors (RFs) associated with poor GT and its possible causes.

HIGHLIGHTS

- Using a dermal regeneration template is a promising alternative for treating complex diabetic foot ulcers.
- Poor graft take of the dermal regeneration template at day 21 after its application is significantly associated with a longer time to complete healing (CH) and a decreased CH rate at day 180.
- Four distinct, independent risk factors for the graft take at day 21 have been identified, that is, transcutaneous oxygen pressure less than 30 mmHg and diabetic neuropathy for poor graft take, and the use of off-loading device and wound located on the toe for good graft take.
- The three most frequent causes of poor graft take in our patients were infection, vascular compromise, and hematoma.
- Despite the anticipated extended healing time, dermal regeneration templates have shown the potential to achieve CH without the need for subsequent split-thickness skin grafts.
- Early recognition of risk factors and prompt identification of poor graft take at day 21 may aid in implementing therapeutic interventions to prevent further healing delay.

Materials and methods

Study design, definition, and grouping

This retrospective, single-center study collected patients who received DRT for wound reconstruction from a single senior plastic surgeon at Taipei Medical University – Shuang Ho Hospital, Ministry of Health and Welfare, Taiwan. We examined all operation schedules performed between 2016 and 2021 for wound reconstruction using DRT. Patients without DFU were excluded, along with those with insufficient records for assessment and those who underwent reconstruction using DRT more than once (with only the first time being counted). All patients had to receive our 'standard of care' for DFUs to be eligible for the study. Patients who were simultaneously receiving anticancer treatment or had an

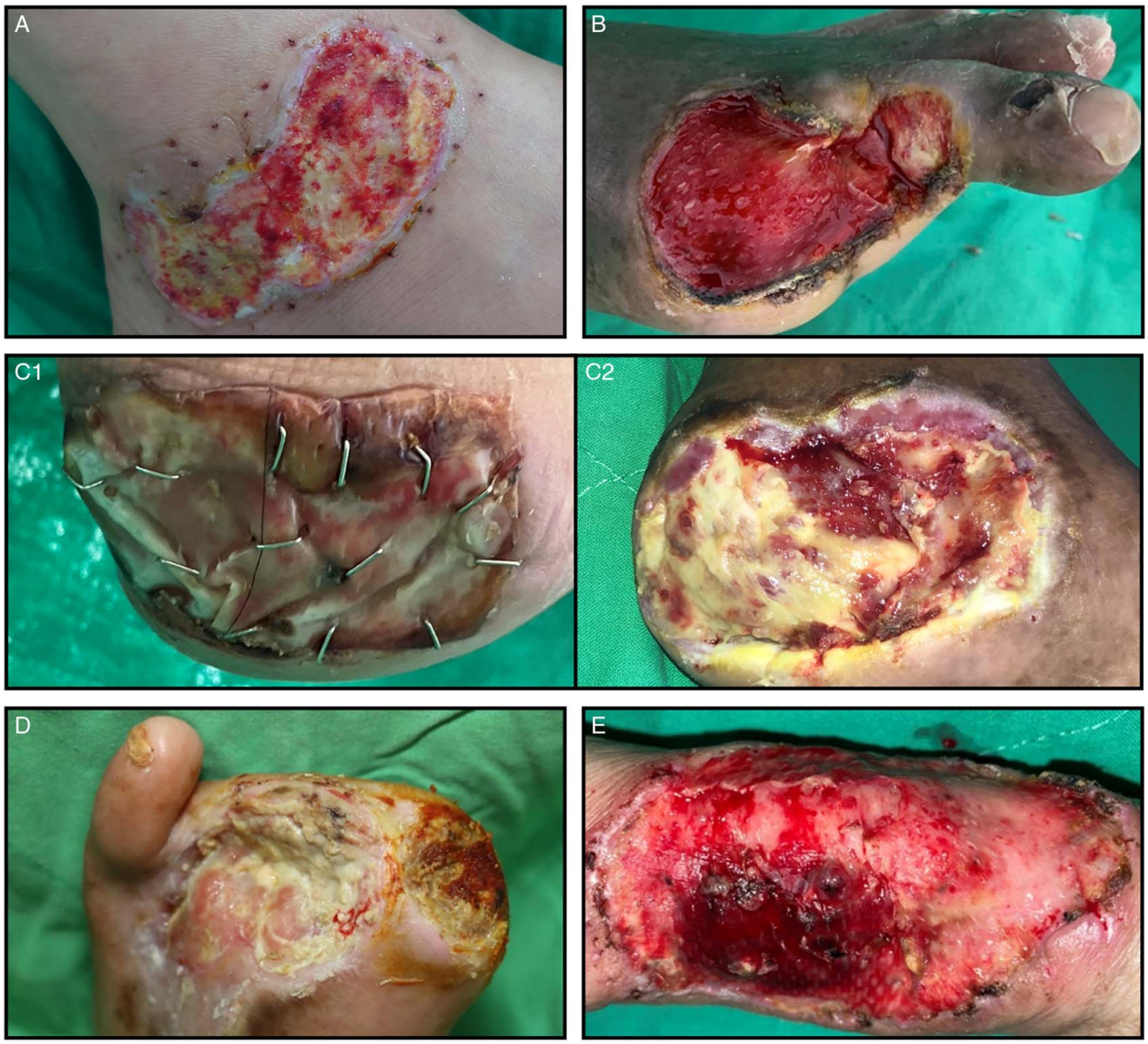

Figure 1. Poor and good graft take. Case A and B are good graft takes, showing well-adhered dermal regeneration templates (DRT) with different degrees of neovascularization and granulation. In cases with good graft take, the wounds usually present colors from light yellow and pink to red when the upper silicone layer is removed. However, if the graft was poorly taken, the wound will present colors in gray, pale, thick, and cloudy yellow and there may be massive drainage, pus, or hematoma under DRT. Cases C, D, and E are all poor graft takes. In the case of C, pus formation was found a few days after the application of DRT (C1). It was then removed to allow better pus drainage and infection control; however, slough and some pus could still be noted three weeks after the application (C2). Case D was a patient with a $TcPO_2$ level of 19 mmHg before applying DRT. Although the wound was completely debrided, the tissue became progressively necrotized after DRT was applied. The compromised vasculature was thought to be the main cause of persistent infection and poor graft take. Case E showed the presence of hematoma after removing the silicone sheet, causing poor graft take, especially at the hematoma site.

immunocompromised status (e.g. neutropenia, splenectomy, or acquired immunodeficiency syndrome) were excluded. Finally, patients who had not been followed up for at least 180 days from the DRT application were excluded from the analysis.

The definition and grouping of patients were determined carefully. Poor GT was defined as graft loss or deadhesion of the graft to the wound bed resulting from various factors (see Fig. 1). CH was defined as the reepithelialization of the entire wound without drainage (see Fig. 2). Patients were categorized into either the poor or good GT groups based on the status of their implanted DRT at D21. The relationship from each group with the CH rate at D180 was analyzed.

Our study employed a rigorous approach to ensure accurate and consistent wound/graft assessment. We carefully reviewed all available records from the patient's initial encounter with our plastic surgeon. For patients with missing written records, we relied on photographic records to maintain the integrity of wound assessment. Our plastic surgeon carefully reviewed these images, and those patients without a series of photographic records were excluded from our study.

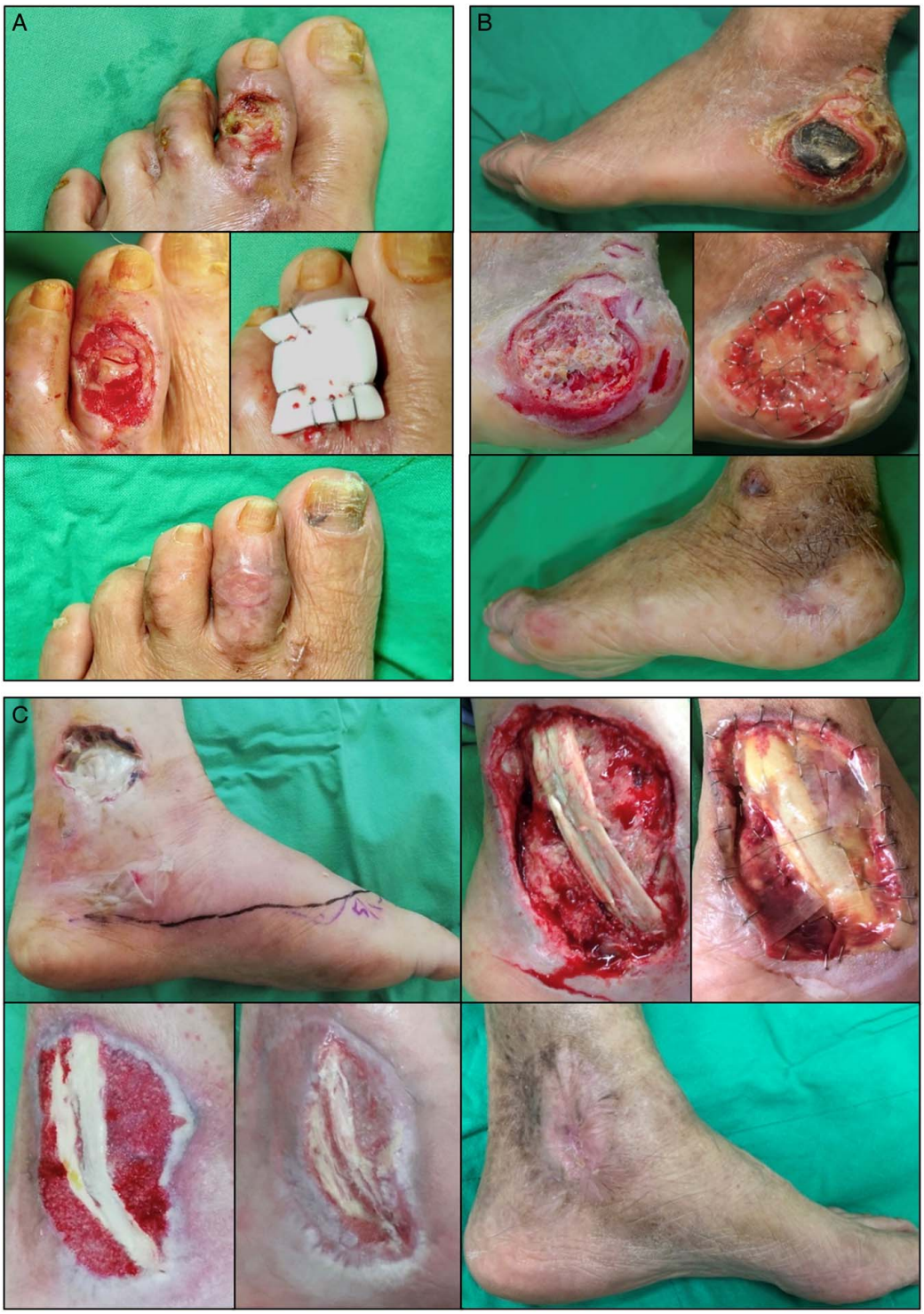

Figure 2. Complete healing after the application of dermal regeneration template at different wound locations and severity. In the three cases presented here, the final wound photographs all demonstrate thorough reepithelialization without drainage, aligning with our definition of complete healing (CH). Case A shows a 4 cm^2 wound with joint exposure on the left second toe. After the application of Terudermis, it achieved CH on day 57. Case B shows a 24 cm^2 wound with bone exposure on the left lateral heel. After the application of Integra, it achieved CH on day 112. Case C shows a 77 cm^2 wound on the left medial malleolus with initial necrotizing fasciitis and tendon exposure. After the application of Integra, it achieved CH on day 245.

We collected data, including patient characteristics, laboratory data, examination results for peripheral vasculature, wound characteristics and classifications, and treatment characteristics. All data points were considered as our potential RFs for poor GT at D21. Some of the peri-DRT outcomes were collected, but they were excluded from the analysis to identify independent RFs. The causes for poor GT were also recorded and analyzed. Additional data collected in this study, including the results of bacterial cultures of these wounds, changes in culture results between the initial encounter and before the application of the DRT, as well as a comparison of poor GT rates between DFUs and other wounds, are presented in Supplemental Tables 1–3 (Supplemental Digital Content 1, http://links.lww.com/JS9/B512). These parts are not discussed in the main text of this article.

The study protocol was submitted to the Joint Institutional Review Board of Taipei Medical University for ethical review and approved on 20 January 2022 (Protocol no. N202201021). This study was also registered at http://www.researchregistry.com (unique identifying number researchregistry9393), and was reported in line with the strengthening the reporting of cohort, cross-sectional and case–control studies in surgery (STROCSS) criteria[28].

DRT application protocol

The DRT application protocol for patients with DFUs at our hospital begins with the standard of care. Prophylactic/empiric antibiotics are administered and shifted to a definite regimen once culture evidence is obtained. Patients undergo ankle-brachial index (ABI) and $TcPO_2$ measurements to assess peripheral vasculature. Those with an ABI greater than 1.2 or less than 0.8, a $TcPO_2$ less than 30 mmHg, or a history of peripheral arterial disease (PAD) or end-stage renal disease (ESRD) undergo lower extremity computed tomography angiography to evaluate for PAD. For those requiring revascularization, we consulted a cardiologist or cardiovascular surgeon for percutaneous transluminal angioplasty, endovascular stenting, or bypass surgery. We consulted occupational therapists for custom-molded off-loading devices. We typically use removable cast walkers (RCWs) or therapeutic shoes/insoles as off-loading devices and rarely use irremovable casts. Patients who cannot use off-loading devices are encouraged to wear well-fitting footwear and socks to protect their wounds and prevent ulceration if appropriate. We consulted physiatrists to enhance the patients' daily exercises and create a long-term rehabilitation program. We consulted dietitians for nutritional support. Blood sugar was controlled frequently and rigorously, while hypoglycemic agent dosages were optimized. If the targeted blood sugar levels were difficult to achieve, we consulted an endocrinologist. The type of wound dressing applied depends on the wound's discharge and infection status. We typically use silver-containing dressings for infected wounds and prescribe negative pressure wound therapy to preliminarily controlled wounds. All patients underwent several surgical debridements.

DRTs are applied after all devitalized tissues were removed and after local infections were generally controlled. The DRT is fixed onto the wound using a chromic suture or skin staples. We used negative pressure wound therapy or tie-over bolster dressings to control the discharge and prevent mechanical shearing. After the operation, we routinely applied a short leg splint to protect and off-load the wound. The silicone sheet, that is, the upper layer of the DRT, will be removed at D14, which can be performed during hospitalization or outpatient clinic follow-up. We keep on secondary intentions after removing the silicone sheet usually but do not regularly apply STSGs.

At D21, the graft condition will be assessed again to identify poor GT and a main cause will be assigned. Based on the literature and our experience, we have concluded nine causes for poor DRT take, namely: 1) residual necrotic tissue, 2) hematoma, 3) seroma, 4) mechanical shearing, 5) excessive pressure, 6) other surgeon error, 7) infection, 8) vascular compromise, and 9) systemic complication[27,29–31]. Patients with acute wounds receiving DRT will be regularly followed for 1–2 weeks within 1–2 months after reconstruction, while patients with chronic wounds, such as DFUs, will be followed up for at least 6 months until complete wound healing and without evidence of recurrence.

Outcomes

The primary outcome was the overall CH rate at D180 for each group. Other outcomes at D180, including vascular stenosis, minor and major amputation, and mortality, were also presented. Identifying independent RFs for poor GT at D21 was our secondary outcome. The causes for poor GT were also determined.

Statistical analysis

We used either Pearson's χ^2test or Fisher's test for categorical variables and presented the results as numbers and percentages. Continuous variables are presented as means with SDs in parentheses and were analyzed using independent *t*-tests. We defined statistical significance as a *P*-value <0.05. We used the Kaplan–Meier analysis and log-rank test to present the actual time to CH in each group. To identify independent RFs for poor GT, potential RFs that showed statistical significance in univariable analysis were put into multivariable analysis using logistic regression with forward stepwise conditional selections. We reported the odds ratios (OR) for each independent RF. We conducted all statistical analyses using IBM SPSS software (version 25.0; IBM SPSS).

Results

Study cohort and univariable analysis

Between 2016 and 2021, a total of 265 operation schedules were recorded for the application of DRT in the reconstruction of all wound types (Fig. 3). After excluding 185 schedules (121 patients without DFU, 49 with insufficient records, and 15 patients who underwent subsequent DRT), 80 patients with a mean age of 68.3 years and a female population of 47.5% were included in the study. These patients were divided into poor and good GT groups, with 38 and 42 patients, respectively.

The two groups had similar demographic data, except for the use of diabetic medications, with a lower proportion of oral hypoglycemic agents (50.0 vs. 73.8%, $P = 0.028$) and a higher proportion of insulin or combination therapy (44.7 vs. 19.1%, $P = 0.013$) in the poor GT group (Table 1). Only the difference in the percentage of $TcPO_2$ less than 30 mmHg before DRT was statistically significant (36.8 vs. 2.4%, $P < 0.001$) in the lab data and examination results (Table 2). Even HbA1c levels did not exhibit a statistically significant difference between the two

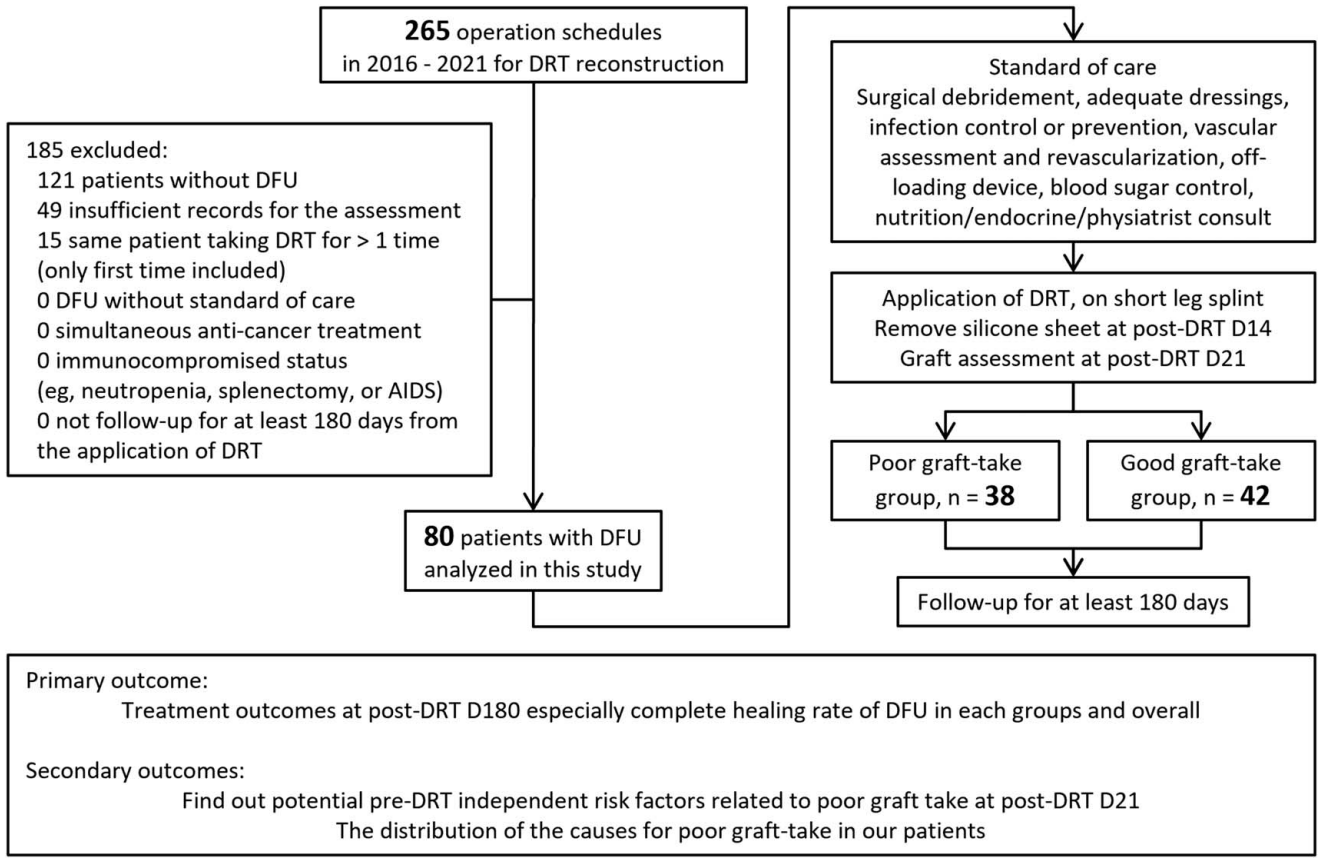

Figure 3. Study protocol, including the standard of care in treating patients with diabetic foot ulcers, the number of patients enrolled in the analysis and each group, and the outcomes of our study. DRT, dermal regeneration template; DFU, diabetic foot ulcer; D14, day 14; D21, day 21; D180, day 180.

groups. Patients in the poor GT group had a significantly larger wound size (53 cm^2 vs. 22 cm^2, $P = 0.006$) and a higher percentage of wounds greater than 30 cm^2 ($P = 0.025$; Table 3). Patients with wounds on their toes were significantly more likely to have good GT, while those on their heels were more likely to have poor GT. A higher proportion of patients with diabetic neuropathy (DN) was noted in the poor GT group (84.6 vs. 54.8%, $P = 0.011$).

At the initial encounter, 80% of all wounds were classified as University of Texas wound classification system (UTWCS) 3D and 86.3% of all wounds were M–W grades 3 or 4. Before applying DRT, 66.3% of all wounds were UTWCS 2B or higher and 58.8% of all wounds were M–W grades 3 or 4. The poor GT group had more wounds classified as UTWCS 3D and M–W grade 3 but fewer wounds as UTWCS 2A and M–W grade 2 before reconstruction, which was statistically significant. Patients in the good GT group showed significant improvement in UTWCS from the initial encounter to the time before reconstruction and downstaging in UTWCS.

Among our treatment characteristics, off-loading was significantly lower in the poor GT group (18.4 vs. 76.2%, $P < 0.001$) and others showed no significant difference (Table 4). Peri-DRT outcomes showed an average operation time of 31.1 min and a length of hospital stay after DRT of 11.5 days. Thirteen patients in the poor GT group received a subsequent DRT for the target wound (one of them had a third DRT), which was a proportion significantly higher than in the good GT group (only one patient and not for the target wound). Overall, 20.0% of our patients received a subsequent STSG after DRT.

Primary outcome

CH at D180 reached 86.3% overall and was significantly lower in the poor GT group (76.3 vs. 95.2%, $P = 0.021$; Table 5). Kaplan–Meier analysis revealed a mean time to CH of 119.2 days for all 80 patients, with the poor GT group taking significantly longer time (157 days vs. 85 days) and $P < 0.001$ in the log-rank test (Fig. 4). Furthermore, vascular stenosis was significantly more prevalent in the poor GT group (71.1 vs. 23.8%, $P < 0.001$). The poor GT group had a higher proportion of patients with minor amputation, major amputation, and mortality, but none was statistically significant.

Secondary outcomes

Fourteen binary variables reached statistical significance in our univariable analysis, including the use of OHA or insulin/combination therapy for diabetes mellitus control, $TcPO_2$ less than 30 mmHg before DRT, wound greater than 30 cm^2, wound located on the toe or heel, the presence of DN, the use of off-loading device, UTWCS 2A or 3D and M–W grades 2 or 3 before DRT, improvement in UTWCS, and downstaging UTWCS. After conducting logistic regression with forward stepwise analysis,

Table 1

Patient characteristics.

Variables	Poor graft-take group ($n=38$)	Good graft-take group ($n=42$)	P
Mean age, year (SD)	68 (11.8)	68 (13.9)	0.980
Female, no. (%)	16 (42.1)	22 (52.4)	0.358
Mean BMI, kg/m^2 (SD)	24.1 (4.53)	25.2 (5.34)	0.308
Smoking, no. (%)	6 (15.8)	6 (14.3)	0.851
DM status			
HbA1c			
% (SD)	7.4 (1.64)	7.4 (1.92)	0.976
> 8%, no. (%)	7 (18.4)	13 (31.0)	0.196
DM meds			
OHA, no. (%)	19 (50.0)	31 (73.8)	0.028
Insulin/combination, no. (%)	17 (44.7)	8 (19.1)	0.013
Other comorbidities			
Hypertension, no. (%)	37 (97.4)	38 (90.5)	0.362
Dyslipidemia, no. (%)	7 (18.4)	3 (7.1)	0.180
Stroke, no. (%)	10 (26.3)	9 (21.4)	0.608
Coronary artery disease, no. (%)	24 (63.2)	23 (54.8)	0.446
Peripheral arterial disease, no. (%)	36 (94.7)	39 (92.9)	1.000
CKD/ESRD, no. (%)	27 (71.1)	21 (50.0)	0.055
ESRD alone, no. (%)	17 (44.7)	15 (35.7)	0.411
Cancer, no. (%)	1 (2.6)	1 (2.4)	1.000
Glucocorticoid use, no. (%)	3 (7.9)	0 (0.0)	0.103
Other immunosuppressant, no. (%)	1 (2.6)	0 (0.0)	0.475

CKD, chronic kidney disease; DM, diabetes mellitus; ESRD, end-stage renal disease; OHA, oral hypoglycemic agent.

four variables remained statistically significant: $TcPO_2$ less than 30 mmHg, off-loading device usage, DN presence, and wounds on the toe (Table 6). The OR for these variables were 154.14, 0.03 (1/35.71), 6.51, and 0.20 (1/4.98), respectively.

Infection was the most frequent cause of poor GT, accounting for 44.7% of cases, followed by vascular compromise at 21.1% and hematoma at 15.8% (Fig. 5). Given the high odds ratio of $TcPO_2$ observed in the multivariable analysis, we have used it to describe the distribution of causes of poor GT in Supplementary Figure 1 (Supplemental Digital Content 1, http://links.lww.com/JS9/B512).

Discussion

The current study demonstrates the effectiveness of combining DRT with standard care for larger and higher-graded DFUs. Our results show an 86.3% CH rate after 180 days, with a mean time to CH of 119 days. DRT offers benefits over traditional reconstruction methods like skin grafts or flaps by reducing overall costs, shortening hospital stays, and enabling outpatient treatment[23,32,33]. Our findings support the use of DRT as a viable treatment option, potentially treating a wider range of wound types and severity than its previous clinical applications.

The evidence of DRT used in complex DFUs must be made more extensive. For instance, Clerici *et al*.[34] treated 30 patients with DFUs involving tendon or bone exposure with DRT, followed by STSG after 21 days. Their study reported an average healing time of 74.1±28.9 days and an 86.7% healing rate.

Table 2

Lab data and examinations.

	Overall				
Variables	($n=80$)	P	Poor graft-take group ($n=38$)	Good graft-take group ($n=42$)	P
Lab data					
Albumin before DRT, g/dl (SD)	3.6 (0.67)		3.5 (0.69)	3.7 (0.65)	0.245
WBC					
Initial, kcell/μl (SD)	8.80 (3.184)	0.061	9.36 (3.166)	8.30 (3.153)	0.137
Before DRT, kcell/μl (SD)	8.30 (3.042)		8.75 (2.976)	7.89 (3.078)	0.207
WBC decrease, no. (%)	39 (48.8)		17 (44.7)	22 (52.4)	0.495
Hb					
Initial, g/dl (SD)	11.4 (2.01)	< 0.001	11.3 (2.01)	11.4 (2.03)	0.757
Before DRT, g/dl (SD)	10.8 (2.25)		10.5 (2.29)	11.0 (2.21)	0.373
PLT					
Initial, kcell/μl (SD)	261 (108.8)	0.003	283 (122.4)	241 (92.0)	0.090
Before DRT, kcell/μl (SD)	237 (88.6)		253 (92.9)	222 (82.8)	0.113
CRP					
Initial, mg/dl (SD)	4.32 (6.172)	0.206	4.58 (6.114)	4.09 (6.289)	0.729
Before DRT, mg/dl (SD)	3.48 (5.135)		3.99 (5.346)	3.02 (4.954)	0.400
CRP decrease, no. (%)	30 (37.5)		13 (34.2)	17 (40.5)	0.563
Examination results (peripheral vascular assessment)					
ABI					
Initial (SD)	0.95 (0.284)	0.210	0.99 (0.283)	0.90 (0.281)	0.140
Before DRT (SD)	0.96 (0.238)		0.99 (0.237)	0.94 (0.238)	0.313
$TcPO_2$					
Initial, mmHg (SD)	22 (14.2)	< 0.001	24 (14.2)	22 (14.3)	0.552
Before DRT, mmHg (SD)	36 (12.0)		34 (14.6)	38 (8.9)	0.192
Initial, <30 mmHg, no. (%)	58 (72.5)		25 (65.8)	33 (78.6)	0.290
Before DRT, <30 mmHg, no. (%)	15 (18.8)		14 (36.8)	1 (2.4)	< 0.001

ABI, ankle-brachial index; CRP C-reactive protein; DRT, dermal regeneration template; Hb hemoglobin; PLT platelet; $TcPO_2$, transcutaneous oxygen pressure; WBC, white blood cell.

Table 3

Wound characteristics and classifications.

Variables	Poor graft-take group ($n=38$)	Good graft-take group ($n=42$)	*P*
Wound characteristics			
Pre-DRT size			
cm^2 (SD)	53 (61.7)	22 (22.9)	**0.006**
>25 cm^2, no. (%)	18 (47.4)	12 (28.6)	0.083
>30 cm^2, no. (%)	16 (42.1)	8 (19.1)	**0.025**
Initial location			
Toe, no. (%)	12 (31.6)	28 (66.7)	**0.002**
MTP joint, no. (%)	1 (2.6)	2 (4.8)	1.000
Dorsal foot, no. (%)	5 (13.2)	2 (4.8)	0.248
Plantar foot, no. (%)	1 (2.6)	5 (11.9)	0.204
Heel, no. (%)	8 (21.1)	2 (4.8)	**0.041**
Malleolus, no. (%)	7 (18.4)	2 (4.8)	0.078
Others, no. (%)	4 (10.5)	1 (2.4)	0.185
Diabetic neuropathy, no. (%)	31 (81.6)	23 (54.8)	0.011
Charcot deformity, no. (%)	4 (10.5)	2 (4.8)	0.416
Wound classification (UTWCS), %			
Initial			
2A/2B/2C/2D	0.0%/2.6%/ 0.0%/7.9%	0.0%/2.4%/ 0.0%/16.7%	1.000/1.000/ 1.000/0.318
3A/3B/3C/3D	0.0%/7.9%/ 0.0%/84.2%	0.0%/4.8%/ 0.0%/76.2%	1.000/0.664/ 1.000/0.370
Pre-DRT			
2A/2B/2C/2D	15.8%/2.6%/ 7.9%/2.6%	50.0%/0.0%/ 0.0%/2.4%	**0.001**/0.475/ 0.103/1.000
3A/3B/3C/3D	10.5%/50.0%/ 0.0%/10.5%	16.7%/31.0%/ 0.0%/0.0%	0.525/0.082/ 1.000/**0.047**
Improved in UTWCS			
Overall, no. (%)	32 (84.2)	42 (100.0)	0.009
Downgrading, no. (%)	8 (21.1)	14 (33.3)	0.219
Downstaging, no. (%)	31 (81.6)	41 (97.6)	0.024
Wound classification (Meggitt–Wagner), no. (%)			
Initial			
Grade 2	2 (5.3)	9 (21.4)	0.051
Grade 3	26 (68.4)	23 (54.8))	0.21
Grade 4	10 (26.3)	10 (23.8)	0.796
Pre-DRT			
Grade 2	11 (29.0)	22 (52.4)	**0.033**
Grade 3	27 (71.1)	19 (45.2)	**0.020**
Grade 4	0 (0.0)	1 (2.4)	1.000
Improved Meggitt–Wagner	18 (47.4)	21 (50.0)	0.814

DRT, dermal regeneration template; MTP joint, metatarsophalangeal joint; UTWCS, University of Texas wound classification system.

Table 4

Treatment characteristics and peri-dermal regeneration template outcomes.

Variables	Poor graft-take group ($n=38$)	Good graft-take group ($n=42$)	*P*
Treatment characteristics			
Antibiotics, no. (%)	38 (100.0)	42 (100.0)	1.000
Silver-containing dressings, no. (%)	32 (84.2)	37 (88.1)	0.614
NPWT, no. (%)	29 (76.3)	35 (83.3)	0.433
Off-loading device, no. (%)	7 (18.4)	32 (76.2)	**<0.001**
Prior debridement, no. (SD)	2.5 (1.5)	2.4 (1.1)	0.618
Revascularization			
PTA, no. (%)	18 (47.4)	27 (64.3)	0.128
Bypass, no. (%)	2 (5.3)	2 (4.8)	1.000
Both, no. (%)	1 (2.6)	0 (0.0)	0.475
Overall, no. (%)	21 (55.3)	29 (69.1)	0.203
Post-DRT short leg splint, no. (%)	38 (100.0)	42 (100.0)	1.000
Peri-DRT outcomes			
DRT operation time, min (SD)	33.0 (14.70)	29.4 (14.71)	0.275
Post-DRT LoHS, day (SD)	14 (14.2)	9 (11.0)	0.099
Subsequent DRT, no. (%)	13[a] (34.2)	1[b] (2.4)	**<0.001**
Subsequent STSG, no. (%)	9 (23.7)	7 (16.7)	0.433

DRT, dermal regeneration template; LoHS, length of hospital stay; NPWT, negative pressure wound therapy; PTA, percutaneous transluminal angioplasty; STSG, split-thickness skin graft.

[a]One of the patients received three times of DRT for target wound reconstruction and achieved complete wound healing at post-DRT day 126.

[b]This patient received a second DRT for treating another wound and the target wound achieved complete wound healing at post-DRT day 88.

However, the study had limitations, such as the exclusion of heel ulcers and the low percentage of patients with critical limb ischemia. In another study by Iorio *et al.*[35], DRT achieved an 83% successful limb salvage rate in high-risk wounds compared to a 46% success rate in the control group ($P=0.001$) after an average follow-up of 309 days. However, the study did not report the time to CH. In 2020, Hicks *et al.*[36] reported a cohort with 93.5% of wounds classified as wound, ischemia, and foot infection (WIfI) stages 3 or 4. Some differences existed in patient characteristics between their study and ours, with our study having a higher proportion of PAD (69.4 vs. 93.8%) and larger wounds (30.9 cm^2 vs. 38 cm^2). In contrast, their study had a higher glycosylated hemoglobulin (HbA1c) level (8.94 vs. 7.4%) and more patients with DN (98.8 vs. 67.5%). Our study reported a mean time to CH of 119.2 days overall and 157 days in the poor GT group, which was shorter than the outcome in their study (198 days). Both studies support using DRT as an effective treatment option for complex diabetic foot wounds, with favorable healing outcomes.

To the best of our knowledge, our study is the first to propose identifying poor GT on the 21st day after the application of DRT, thus anticipating the acknowledgment of the possibility of delayed wound healing. DRT has been histologically demonstrated in its four distinct phases: namely, imbibition, fibroblast migration, neovascularization, and final remodeling and maturation[37]. According to our experience, these post-DRT DFUs would have different degrees of neovascularization at D21. In previous DRT studies, GT assessments were either not mentioned or poorly defined. Some studies used the degree of granulation for graft assessment, while others performed biopsies for histological analysis[38,39]. In our study, the definition of poor GT avoided the need for biopsies and also eliminated the need to wait for 100% granulation, which varies from wound to wound and can result in an inaccurate actual time during longer follow-up intervals. We chose to perform a graft assessment on D21 based on our clinical experience, but further research is needed to establish stronger evidence.

We identified four independent RFs associated with poor and good GT at post-DRT D21, including $TcPO_2$ less than 30 mmHg and DN, which were associated with poor GT, and using an off-loading device and toe wound, which were associated with good GT.

$TcPO_2$ has been used to evaluate the microcirculatory function and prognosticate wound healing, which makes it valuable in monitoring revascularization efficacy. It is often studied together

Table 5

Outcomes at post-DRT day 180.

Variables	Overall ($n=80$)	Poor graft-take group ($n=38$)	Good graft-take group ($n=42$)	*P*
Complete wound healing	69 (86.3%)	29 (76.3%)	40 (95.2%)	**0.021**
Vascular stenosis, no. (%)	37 (46.3)	27 (71.1)	10 (23.8)	**< 0.001**
Post-DRT minor amputation (below the ankle), no. (%)	13 (16.3)	8 (21.1)	5 (11.9)	0.268
Post-DRT major amputation (above the ankle), no. (%)	7 (8.8)	5 (13.2)	2 (4.8)	0.184
Mortality, no. (%)	6 (7.5)	5 (13.2)	1 (2.4)	0.097

DRT, dermal regeneration template.

with other diagnostic tools such as ABI, skin perfusion pressure, and toe pressure[40–46]. In the studies by Thottiyen *et al*. and López-Moral *et al*., the cut-off values of 27.5 mmHg and 28.5 mmHg were found to have an optimal sensitivity/specificity of 84.7%/81.6% and 91%/100%, respectively, for distinguishing between healed and nonhealed DFUs[47,48]. In our study, we used a cut-off value of 30 mmHg and observed a significant difference between poor and good GT groups. We recommend that all patients with DFUs undergo $TcPO_2$ examination prior to DRT application. In addition, revascularization should be performed to restore $TcPO_2$ levels, which increases the likelihood of treatment success.

In more than half of patients with diabetes, DN is a prevalent complication and is a significant RF for foot ulceration and recurrence[49–53]. Patients with DN may have limited physical mobility, which leads to other comorbidities. There is no known cure for DN and management strategies focus on preventing progression, treating glycemia, and other metabolic RFs, as well as providing symptomatic treatment of neuropathic pain and foot care, including pressure relief to prevent and control foot ulcerations or further deformity, and dietary supplements and lifestyle modifications[54]. Various supplements have been combined with other therapies to reduce oxidative stress and regulate nerve growth factors[54,55]. In our hospital, we recommend vitamin B complex for its safety and tolerability. Exercise therapy has gained increasing attention in the management of DN. Researchers suggest that various exercises, including weight-bearing exercises, improve DN if prescribed at the appropriate intensity and do not cause injury[56–58]. We usually collaborate with physiatrists to help maintain the physical mobility of patients with DFUs.

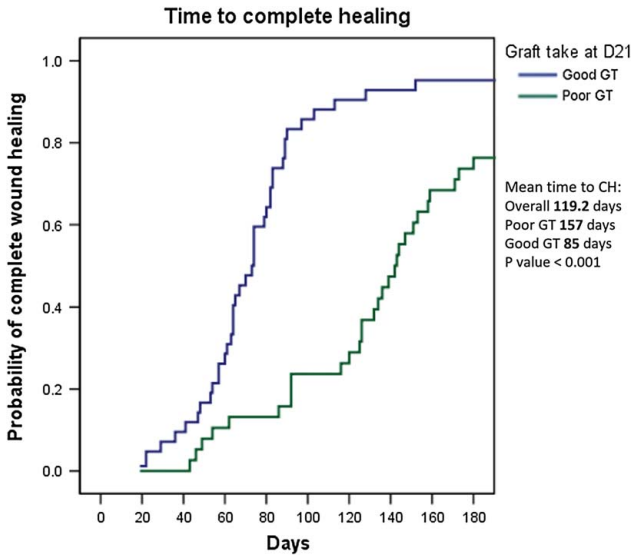

Figure 4. Kaplan–Meier curve for complete wound healing after dermal regeneration template in the two groups (with *P*-value <0.001 in the log-rank test). The mean time to complete healing was 119.2 days overall, 157 days in the poor graft-take group, and 85 days in the good graft-take group. D21, the 21st day after applying the dermal regeneration template; GT, graft take.

Off-loading is a crucial intervention for neuropathic plantar DFU healing. It is considered one of the most important treatments for wound healing and is highly effective in uncomplicated wounds[59–64]. The gold standard for off-loading noninfected or nonischemic wounds is the total contact cast (TCC), showing superior effectiveness in promoting wound healing of all different off-loading devices[64–69]. However, in our hospital, we prefer to use RCWs concerning daily activity, self-care, and hygiene. RCWs offer better step activity than TCC and can be made irremovable by wrapping composite fibers around a TCC, providing the best of both worlds[70–72]. Custom-made footwear and off-loading insoles play a role in preventing foot ulceration in diabetes and reducing recurrence rates compared with regular footwear[73,74]. We provide them to patients who decline TCC or RCW, patients with healed wounds for protection, and those with DFUs located in nonpressure areas. Although off-loading devices offer benefits, improper use may result in poor wound healing or an increased risk of recurrence[75,76]. Thus, educating patients and caregivers on the proper usage of off-loading devices and regularly checking the feet for signs of abnormal pressure, trauma, or ulceration is necessary[77].

Our study faced challenges in accurately categorizing wound locations because of the relatively large wound sizes. As a result, we used the initial wound site instead of the location before DRT, which was often larger and composed of multiple location categories. Previous research has typically divided wound location in DFU into forefoot, midfoot, and hindfoot categories. Outcomes are better in the forefoot and worse in the hindfoot, with a shorter median time to healing, lower rates of incomplete 6-month wound healing, major amputation or mortality, and all-cause mortality[78–81]. In our study, univariable analysis found that both toe wounds in the good GT group and heel wounds in the poor GT group were statistically more frequent, but only the wound located on the toe remained significant in multivariable analysis. We believe such results still reflect the importance of wound location for GT. While applying DRT to a toe wound may have a lower risk of poor GT, physicians should still carefully prepare the wound bed, and should also be vigilant when applying DRT to areas with higher risks of poor GT. A prompt

Table 6

Multivariable analysis of variables related to poor graft take.

Variables	*P*	Odds ratio (95% CI)
DM meds, OHA	0.205	–
DM meds, insulin/combination	0.377	–
$TcPO_2$ < 30 mmHg before DRT	**< 0.001**	154.139 (9.354–2540.002)
Wound size > 30 cm^2	0.922	–
Wound located on the toe	**0.039**	0.201 (0.044–0.924)
Wound located on the heel	0.544	–
Diabetic neuropathy	**0.037**	6.510 (1.331–31.834)
Off-loading device	**< 0.001**	0.028 (0.005–0.147)
UTWCS 2A before DRT	0.596	–
UTWCS 3D before DRT	0.125	–
M–W grade 2 before DRT	0.720	–
M–W grade 3 before DRT	0.715	–
Improved in UTWCS	0.113	–
Downstaging UTWCS	0.136	–

DM, diabetes mellitus; DRT, dermal regeneration template; M–W, Meggitt–Wagner ulcer classification grade; OHA, oral hypoglycemic agent; $TcPO_2$, transcutaneous oxygen pressure; UTWCS, University of Texas wound classification system.

adjustment of the treatment plan may prevent potential delayed wound healing.

Our study included an elderly patient population with multiple chronic diseases, such as stroke, coronary artery disease, PAD, and chronic kidney disease or end-stage renal disease. To avoid the risks associated with additional surgeries and anesthesia, the creation of a new wound, and the potential for future complications, we performed subsequent STSG only in selected patients. As a conventional method for reconstructing DFUs, STSG has been associated with higher healing rates and shorter healing times[82]. The use of STSG is often a subsequent step following the application of a DRT in the reconstruction process. In a prospective study involving wounds classified as UTWCS 1A and 2A, conducted by Jeon *et al.*[83], it was observed that the experimental group, which used Matriderm (MedSkin Solutions Dr. Suwelack AG) in conjunction with STSG, exhibited a significantly shorter CH period (8.61 weeks vs. 12.94 weeks, $P = 0.010$) and a higher elasticity ratio (affected/nonaffected side 0.72 vs. 0.19, $P = 0.030$) compared to those solely treated with STSG. On the other hand, our approach of not routinely performing STSG is aligned with some studies as well, including a retrospective study by Scalise *et al.*[84], which found that advanced dressings are a viable alternative to STSG in elderly patients with high comorbidity scores. Another study by Uccioli *et al.*[85] showed that using Nevelia (Symatese Aesthetics) DRT without subsequent STSG effectively treats ischemic diabetic foot postsurgical wounds, with 51% of patients achieving CH after an average of 6.7 months. Likewise, a retrospective cohort study by Cottone *et al.*[86] revealed that more than half of the patients in their study received only secondary intention after DRT application, and the rate of healing for surface wounds 30 days after the removal of the silicone sheet ranged from 19 to 52% between groups. These studies suggest that avoiding STSG in certain patient populations can still be a viable and effective treatment option, despite the potential for a longer healing process.

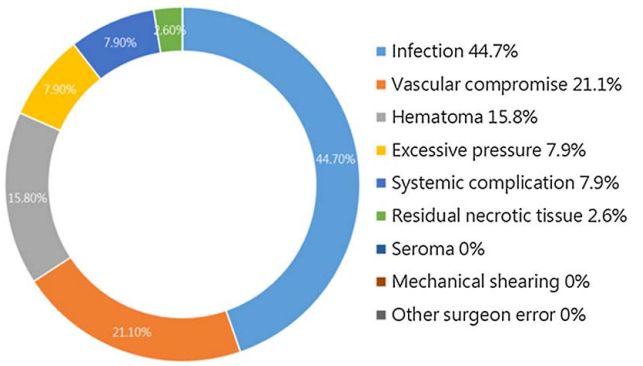

Figure 5. Distribution of the causes for poor graft take. Nine main causes of poor graft take were concluded in our study, which were: 1) residual necrotic tissue, 2) hematoma, 3) seroma, 4) mechanical shearing, 5) excessive pressure, 6) other surgeon error, 7) infection, 8) vascular compromise, and 9) systemic complication. The figure demonstrates the distribution of the causes, and the top three causes in our cohort were infection (44.7%), vascular compromise (21.1%), and hematoma (15.8%).

We identified several limitations that must be considered when interpreting our results. First, our study was limited by its retrospective design and being conducted at a single-center. As a result, we cannot establish definitive causality between our identified RFs and poor GT at D21 or delayed wound healing. Second, because of the sample size and data collection biases, some variables were not included or were not statistically significant in our RF analysis. Although we attempted to control for potential confounding factors, the limitations affected our results. Third, the large variation in wound size may have influenced the outcomes of our study. The differences in wound characteristics may have contributed to the variability in healing times and GT rates. Fourth, while we found that infection was the most common cause of poor GT, we did not provide a detailed classification or description of the severity of infection. Classifications that include the severity of infection, such as the WIfI or Infectious Diseases Society of America classification system, may provide better insight into the impact of infection on GT[87,88]. Within the confines of this limitation, we have devised a randomized controlled trial aimed at elucidating in greater depth the influence of bacteria on DFUs.

Conclusion

Our study introduces the novel concept of poor GT on D21 after DRT application, which is associated with delayed wound healing. We identified four independent RFs, including $TcPO_2$ less than 30 mmHg and DN for poor GT and off-loading devices and toe wounds for good GT. Physicians should assess these RFs before applying DRT and consider appropriate interventions to mitigate their effects. If poor GT is identified on D21, treatment plans should be adjusted to prevent unfavorable outcomes. DRT represents a viable alternative to address DFU. Despite the anticipated extended healing time, it can be performed without subsequent STSG and can also achieve CH, offering an encouraging research direction for the future.

Ethical approval

The study protocol was submitted to the Joint Institutional Review Board of Taipei Medical University for ethical review and approved on 20 January 2022 (Protocol No. N202201021).

Consent

Not applicable. We obtained general consents for research purposes without patient identifying details upon initial presentation of all patients at our hospital, in line with ethical standards.

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Author contribution

T.-Y.T., K.-J.L., MD (co-first author): conceptualization, data curation, formal analysis, investigation, methodology, software, visualization, writing – original draft, and writing – review and editing; H.-Y.C., MD: conceptualization, data curation, formal analysis, investigation, and methodology; Y.-C.W., MD: data curation, investigation, and methodology; C.-U.H., HN: investigation and methodology; X.-Y.L., RN: data curation, investigation, and methodology; F.-C.T., MD, PhD: conceptualization, data curation, investigation, methodology, and writing – review and editing; C.-S.T., MD: conceptualization, data curation, investigation, methodology, and writing – review and editing; Y.-H.C., NP: data curation and investigation; F.-Y.W., RN: formal analysis, methodology, software, and writing – review and editing; S.-C.C., MD (corresponding author): conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, supervision, validation, and writing – review and editing.

Conflicts of interest disclosure

All authors in this research have no financial or personal relationships with other people or Organizations that could inappropriately influence our work.

Research registration unique identifying number (UIN)

Research Registry UIN: researchregistry9393.

Guarantor

Shun-Cheng Chang, takes full responsibility for the work, the conduct of the study, and the decision to publish. He has direct access to the study data and exercised control over the process leading to publication.

Data availability statement

The datasets utilized in this study are available for verification purposes to the journal during the submission process. Additionally, if the study successfully undergoes the review process and is accepted for publication, we commit to providing the data upon specific and reasonable requests. In the event that the study is declined for publication, data sharing would not be applicable.

Provenance and peer review

Not commissioned, externally peer-reviewed.

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