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A long-term follow-up study of diabetic foot ulcer using micronized acellular dermal matrix

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Abstract

Treating a diabetic foot ulcer (DFU) extending to the tendon or bone can be a challenge for physicians. Recent studies have shown positive results of micronized acellular dermal matrix (ADM) treatment for treating DFU. However, studies on such ADM with a long-term follow-up are rare. Thus, the objective of this study was to retrospectively analyse patients treated with micronized ADM with a long-term follow-up to assess the effectiveness of the treatment and determine the recurrence rate. The rate of success of complete healing was 62.96% and the time of complete healing was 86.96 days in this study. The recurrence rate of DFUs was 41.17% in the overall group. However, it was only 23.52% in the micronized ADM group. The average duration of recurrence was 720.50 ± 505.12 days. The recurrence rate was 50% in weight bearing areas such as the plantar and heel. It was 12.5% in toes and non-weight bearing areas. In conclusion, micronized ADM can be used to effectively treat DFUs that have invaded ligaments or bones. A close follow-up of weight bearing area wounds will allow us to identify and treat recurrence early.

KEYWORDS

acellular dermis, diabetic foot, recurrence, therapeutics, ulcer

Key messages

- paste-type acellular dermal matrix (ADM) is effective in treating diabetic foot ulcer (DFU) that invades ligaments or bones
- outcomes and recurrence rates of patients with DFU treated with micronized ADM were reviewed with a long-term follow-up
- micronized ADM significantly improved recovery rates and shortened treatment periods
- micronized ADM does not seem to decrease the recurrence period or recurrence rate, especially on weight bearing area

1 | INTRODUCTION

With increasing prevalence of diabetes worldwide, the prevalence of diabetic feet is also increasing. Diabetic foot ulcer (DFU) is one of the complications of diabetes. It is associated with infections or ulcers of deep tissues, peripheral neuropathy, and problems with blood vessels. In severe cases, it is associated with amputation of extremities or even death, making it an important issue worldwide.[1] Treatment of DFU remains challenging. Effective treatment of DFU requires revascularization, debridement, offloading, proper antibiotic selection, and wound dressing. Various wound dressing materials have been developed for diabetic foot, ranging from foam dressing material to enzymes, growth factors, and negative pressure wound therapy.[2,3] In the case of a hard to heal wound in which ligaments or bones are exposed, it is difficult to expect recovery from a conservative treatment. If the recovery takes too long, surgical treatment such as skin graft and flap coverage might be considered.[4]

This is where the dilemma arises. In a patient with a hard to heal DFU who has a slow or unresponsible recovery from a conservative treatment, surgery is often difficult given the general condition and wound environment. When decisions are difficult to make, advanced wound care materials are needed to accelerate wound healing. Among various materials used for DFU, several studies have shown that acellular dermal matrix (ADM) is effective for hard to heal DFU.[3,5] ADM is a biomaterial derived from autologous and allogenic tissues that undergo a process to remove cells while keeping the bioactive dermal matrix consisting of collagen, elastin, and fibronectin.[6] Several forms such as sheet type and gel type of ADM have been developed. They provide various proteins and structural support necessary for wound healing, showing good results in the treatment of DFU.[7] Among them, injectable micronized ADM is a one new option for treating diabetic wounds.[8] Conventional ADM sometimes requires preparation before applying. In addition, it is difficult to perform trimming or moulding to apply it to irregular surfaces, thus requiring additional procedures for fixation and adjusting the thickness. In addition, it is difficult to apply conventional ADM to tunnelled or undermined cavitary wounds. On the other hand, micronized ADM has several advantages: (1) it is easy to adjust the dose, (2) it does not require preparation before applying, (3) it is easy to perform shape control, and (4) it is applicable to irregular, undermined, cavitary, or tunnelled wounds. Thus, the authors of this study selected micronized ADM.

With 7 years of experience, the authors have achieved positive results in the treatment of DFU with a micronized ADM. However, recurrence rates and stability of micronized ADM treatment with a long-term follow-up have not been reviewed yet. Therefore, the authors performed a retrospective study of patients with diabetic ulcers of Wagner grade II or higher that had tendon or bone exposure who were applied with a micronized ADM. Test results and history of patients treated using micronized ADM were charted and reviewed. Healing rate, length of hospitalisation, total duration of treatment, recurrence rate, and recurrence location were then evaluated with a long-term follow-up.

2 | PATIENTS AND METHODS

This was a 7-year retrospective cohort study to evaluate the efficacy and safety of a micronized ADM for treating DFU. From January 2015 to December 2018, 91 patients with DFU intended secondary healing rather than surgery due to various reasons. Their medical records were reviewed. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki. It was approved by Soonchunhyang University Hospital (Cheonan, South Korea) human research review committee and the Institutional Review Board (IRB) of Soonchunhyang University Cheonan Hospital (IRB FILE No.: 2015-10-028-008). All participants provided written informed consent for the publication before study.

Micronized ADM (CG Paste; Daewoong Pharmaceutical, Seoul, Korea) is an undifferentiated, cell-free dermis matrix that is safely used in clinical practice currently. It is a free-flowing, acellular allogeneic dermis. It contains collagen, elastin, fibronectin, laminin, and proteoglycans known to be components of the human skin. Thus, it can aid in the interaction between normal cells and the extracellular matrix.

TABLE 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• Patients >19 years old • Type 1 or 2 diabetes patients • Wound size >4 cm^2 • Full thickness skin defect to bone exposure wounds (> Wagner's Grade 2, except Grade 5) • Wound without uncontrolled infection • Free of necrotic tissue • Adequate blood circulation	• Superficial or partial thickness skin defects • Suspected local skin malignancy • History of radiation therapy or chemotherapy • Active infection wound • Started wound healing prior to the study • Received biomedical or topical growth factors within the previous 30 days

Note: Wagner's Grade 2: Deep ulcer, penetrating down to ligament and muscle, but no bone involvement or abscess formation; Grade 3: Deep ulcer with cellulitis or abscess formation, often with osteomyelitis; Grade 4: Localised gangrene; Grade 5: Extensive gangrene involving whole foot.

Inclusion criteria were: (1) patients over 19 years old, (2) type 1 or type 2 diabetes, (3) wound size larger than 4 cm^2, (4) full thickness skin defect to bone exposure wounds (over Wagner's DFU classification[9] Grade 2, except Grade 5), (5) wound without uncontrolled infection, (6) adequate blood circulation, and (7) free of necrotic tissue. Exclusion criteria were: (1) superficial or partial thickness skin defects, (2) suspected local skin malignancy, (3) history of radiation therapy or chemotherapy, (4) uncontrolled active infection state, (5) start of wound healing prior to the study, and (6) those who had received biomedical or topical growth factors within the previous 30 days (Table 1).

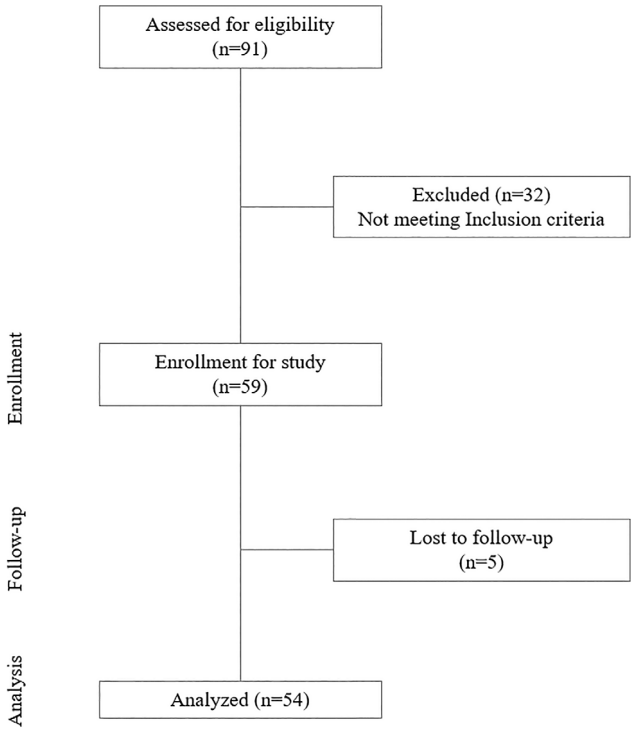

FIGURE 1 Patient classification method and analysis used in the study. Among 91 patients, 59 people who met the inclusion criteria were enrolled. Excluding those who were lost to follow-up, a retrospective chart review was ultimately conducted for 54 patients

Based on the above criteria, 32 people who did not meet the inclusion criteria were excluded from 91 subjects. As a result, 59 patients who used a micronized ADM were enrolled in this study. During the follow-up process, five patients in the micronized ADM group were excluded from tracking. Finally, medical records of 54 patients in the micronized ADM group were analysed (Figure 1).

The following data were collected during the study period: age, gender, location of ulcers, tendon exposure, bone exposure, smoking history, hypertension, body mass index (BMI), diabetes mellitus, glycosylated haemoglobin (HbA1c), haemoglobin/haematocrit (Hb/Hct), blood urea nitrogen creatinine ratio (BUN/Cr), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, estimated glomerular filtration rate (eGFR), serum albumin level, ankle-brachial index (ABI), hospitalisation period, treatment period, and recurrence. Ulcer location, tendon exposure, and bone exposure were assessed through chart reviews, photographs, and X-rays. Depending on smoking history, subjects were divided into those who never smoked and smokers. Smoking pack years were sought for smokers. Hypertension was defined as systolic blood

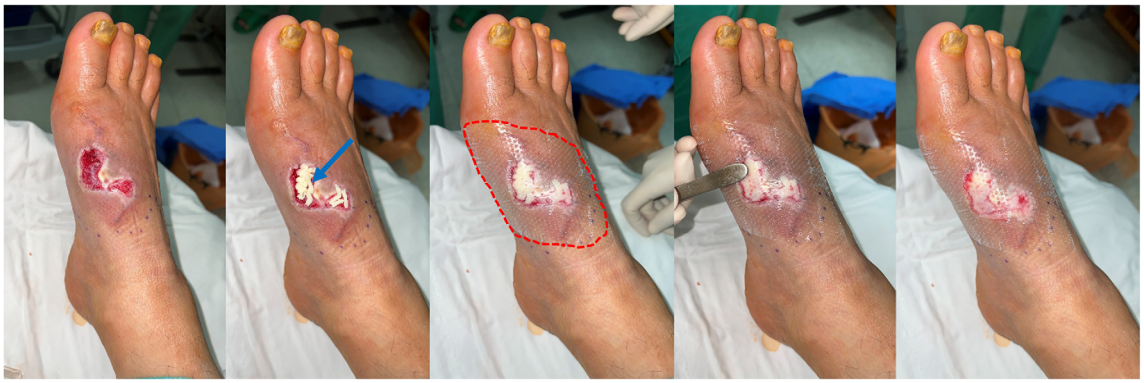

FIGURE 2 A schematic of how to apply micronized ADM with silicone sheet. Micronized ADM was filled without a dead space on the raw surface. Its upper boundary was filled to the surrounding dermis level. It was difficult to apply micronized ADM on irregular surfaces, especially at areas that could flow down. Also, there were concerns about unpredictable separation of micronized ADM from wounds and absorption into negative pressure wound therapy devices. Therefore, the micronized ADM was covered with a silicone barrier (Mepitel® One; Mölnlycke Health Care, Sweden) to spread the micronized ADM evenly and to minimise loss by sheering or friction. The blue arrow indicated micronized ADM covering the raw surface. The red dotted line is the outline of a silicone sheet. The author preferred to mould micronized ADM using the back of forceps while the silicone barrier was covered. It is much easier to mould and spread micronized ADM evenly with a silicone sheet covered on it

TABLE 2 Demographics of patients with complete healing

	Healed with mADM (N = 34)
Age	60.06 ± 12.06
Gender	
Male	26
Female	8
ABI	1.10 ± 0.12
BMI	24.91 ± 2.33
HbA1C	7.63 ± 1.60
Hypertension	
With	23
Without	11
Smoking History	8.05 ± 16.24
Smoker	9
Non-smoker	25
Haemoglobin	11.02 ± 1.51
Haematocrit	32.47 ± 4.13
eGFR	64.54 ± 35.37
Albumin	3.61 ± 0.61
ESR	57.92 ± 29.48
CRP	64.58 ± 75.88
Tendon	
Exposure	28
Non-exposure	6
Bone	
Exposure	18
Non-exposure	16

Note: Data are presented as mean ± standard deviation.
Abbreviations: ABI, ankle-brachial index; BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; HbA1C, glycosylated haemoglobin A.

TABLE 3 Recovery rate, hospitalisation period, treatment period, recurrence rate, and recurrence period

	Heald with mADM (N = 34)
Recovery rate	62.96%
Hospitalisation period	32.93 ± 27.42 days
Treatment period	86.96 ± 50.56 days
Recurrence rate	23.52% (Overall, 41.17%)
Recurrence period	720.50 ± 505.12 days

Note: Data are presented as mean ± standard deviation.

pressure greater than 140 mmHg or diastolic blood pressure greater than 90 mmHg. BMI was a measure of body fat based on height and weight. Diabetes mellitus was defined as a fasting blood glucose of greater than 126 mg/dl, random blood glucose of greater than 200 mg/dl, and HbA1c of greater than 6.5%. HbA1c, Hb/Hct, BUN/Cr, ESR, CRP, and eGFR levels were measured with standard blood tests. ABIs were recorded for normal and sick sides. The hospitalisation period was defined as the period from the date of hospitalisation to the date of discharge. The treatment period was defined as the period from the date of hospitalisation to the date of completion of treatment. Recurrence was defined as the case of a new DFU after the end of treatment. The recurrence period was defined as the period from the date of completion of treatment to the date of recurrence. The location of recurrence was assessed through chart reviews, photographs, and X-rays.

For patients assigned to this study, injectable micronized ADM was filled without a dead space on the raw surface. Its upper boundary was filled to the surrounding dermis level. Therefore, the dose of micronized ADM was used in accordance with the wound condition without a fixed dose. The application interval was implemented in weeks 1, 2, and 3. Major concerns regarding this method were unpredictable separation from wounds and absorption into negative pressure wound therapy devices. Therefore, the micronized ADM was covered with a silicone barrier (Mepitel® One; Mölnlycke Health Care, Sweden) to minimise loss by sheering or friction. In a state where infection and bleeding were controlled. Discharge was controlled through conventional foam dressing or negative pressure wound therapy. The number and interval of replacements of foam dressing and negative pressure wound therapy were determined according to the amount of exudate (Figure 2). Wound evaluation was performed using progress record. Photograph and X-ray were used to evaluate treatment effect, duration of treatment, and recurrence of the course after starting the treatment.

Among clinical and laboratory parameters, categorical variables are expressed as mean with standard deviation (SD) or medians. Categorical variables are expressed as frequencies with percentage (%). They were compared with chi-squared test or Fisher's exact test, as appropriate. Whether weight bearing area, whether tendon and bone exposure were evaluated. And the recurrence rate was performed using regression analysis. Univariable logistic regression analysis was performed to examine associations of recurrence with weight bearing area, exposure of tendon, bone, gender, and smoking. For each factor, odds ratio (OR) was calculated to determine the likelihood of recurrence. Variables considered clinically meaningful were also included in the multivariable logistic regression. A 10-fold cross-validation was used to improve reliability. Forward selection was used as a variable selection method. All statistical analyses were performed using

TABLE 4 Locations of ulcer and recurrence

	Ulcer location	Recurrence	Recurrent on same site	Recurrent on different site
1	Left toes	X		
2	Left 1st toe	X		
3	Left 1st toe	O	Left 1st toe & foot plantar	
4	Left 1st toe	O		Left 5th toe
5	Left 1st toe	O		Left 4th toe
6	Left 2nd toe & foot dorsum	O	Left 2nd toe	Left 4th toe
7	Left 2nd toe	X		
8	Left 2nd web space	O		Left 5th toe
9	Left 3rd toe	X		
10	Left 5th toe	X		
11	Left heel	O	Left heel	
12	Left heel	X		
13	Left foot dorsum	X		
14	Left foot dorsum	X		
15	Left foot plantar	O	Left foot plantar	
16	Right 1st toe	X		
17	Right 1st toe	X		
18	Right 1st toe	O		Right foot plantar
19	Right 2nd toe	O		Right 1st toe
20	Right 2nd toe	X		
21	Right 2nd toe	X		
22	Right 5th toe	O	Right 5th toe	Right 2nd toe
23	Right 5th toe	O		Left 2nd toe
24	Right 5th web space	X		
25	Right lateral malleolus	X		
26	Right foot plantar	O	Right foot plantar	
27	Right foot plantar	X		
28	Right foot plantar	X		
29	Right foot plantar	X		
30	Right foot plantar	X		
31	Right foot plantar	O	Right foot plantar	
32	Right foot plantar	O	Right foot plantar	
33	Right 5th MTP joint	X		
34	Right toes	X		

Rex Pro software version 3.6.3 (Rex soft, Seoul, South Korea). Statistical significance was considered when *P*-value and t-score were less than .05.

3 | RESULTS

Excluding cases with amputation or failure of limb salvage and cases when recovery from surgical treatment such as graft or flap surgery could not be evaluated, the number of successful treatments with secondary intention was 34 (62.96%) out of 54 who received micronized ADM treatment. For all 34 patients who recovered with secondary intention, the following demographic variables were recorded: age, gender, ankle-brachial index (ABI), BMI, HbA1c, HTN, smoking history, Hb/Hct, eGFR, albumin level, ESR, and CRP (Table 2). In this study, the average length of time it took to use micronized ADM after admission was 20.34 days (range, −19.34 to 77.66 days), the average time it took to apply micronized ADM to discharge was 12.68 days (range, −11.68 to 37.32 days), and the average time it took to end treatment after applying micronized ADM was 66.40 days (range, −41.40 to 110.60 days). The average hospitalisation period taken from the start of admission to the discharge date was 32.93 days (range, −25.93 to 82.07 days). The average duration of treatment from the start of admission to the end of treatment was 86.96 days (range, −54.96 to 150.04 days). Of the 34 patients with DFUs, 14 patients had recurrence, with an overall recurrence rate of 41.17%. Eight (23.52%) of 34 patients with DFUs had recurrence at previous wound where micronized ADM was applied. The average duration of recurrence was 720.50 ± 505.12 days (Table 3). The location of the ulcer was 15 on the left foot and 19 on the right foot.

TABLE 5 Factors associated with recurrence

	Healed with mADM (N = 34)	*P*-value	OR
Weight bearing area	10	.0379*	7.0
Non-weight bearing area	24		
Tendon			
Exposure	28	.0975	0.21
Non-exposure	6		
Bone			
Exposure	18	.1623	1.94
Non-exposure	16		
Gender			
Male	26	.914	0.9
Female	8		
Smoking			
Smoker	9	.0894	4.2
Non-smoker	25		

Note: Wald confidence intervals were calculated. A regression analysis was conducted with all covariates. Coefficients from the analysis were summarised. **P*-value was computed using regression analysis.

Among them, 18 developed ulcers on their toes, 8 developed ulcers on the plantar side of foot, 3 developed ulcers on the dorsum of foot, and 7 developed ulcers on web space and malleolus. The recurrence rate of ulcer was 16.66% (3/18) in the toe, 50% (4/8) in the plantar side of foot, 50% (1/2) in the heel, and 0% on the dorsum of the foot or web space. In other words, the recurrence rate was 50% (5 of 10) at weight bearing area and 12.5% (3 of 24) at the non-weighted bearing area (Table 4). In this study, weight bearing was significantly associated with recurrence after cure ($P = .0379$, OR = 7.0), whereas tendon ($P = .0975$, OR = 0.21) and bone exposure ($P = .1623$, OR = 1.94) was not associated with recurrence after cure. Smoking history ($P = .0894$, OR = 4.2) and gender ($P = .914$, OR = 0.9) showed no significant association with recurrence either (Table 5).

3.1 | Case 1

A 54-year-old male patient with a medical history of diabetes and hypertension visited the hospital. Two years ago, he had a surgical history of amputation of his right third toe and distal part of third metatarsal bone. The patient showed an ulcer of 3 × 7 cm in size that occurred 2 weeks ago on the right foot plantar accompanied by a foul odour. X-rays confirmed osteomyelitis of the 3rd metatarsal bone exposed to ulcers. After removing the necrotic tissue, negative pressure water therapy was performed. After that, 2 cc of micronized ADM was filled in the defective area three times at weekly intervals. After 5 weeks, the wound showed improvement. X-ray also confirmed improvement of osteomyelitis. However, 2 years later, the wound recurred and the patient visited the hospital again. The ulcer occurred again just in front of the previously treated area, although the bone was not exposed (Figure 3).

3.2 | Case 2

A 65-year-old male patient with a history of diabetes, hypertension, and gastric cancer was hospitalised with skin necrosis and redness of the left foot that occurred 2 weeks ago. After removal of the dead skin, necrosis of the 2nd and 3rd toe extensor tendons and necrosis of soft tissue were identified. A photo was taken after sharp debridement and negative pressure wound treatment. The metatarsophalangeal joint of the 2nd toe was exposed. A defect of skin tissue measuring 3 × 6 cm on the dorsum of the foot was connected to the 1st webspace and a tunnel under the skin. No osteolytic part was seen on the foot anteroposterior X-ray. At 1, 2, and 3 weeks, 3 cc of a micronized ADM was applied. After 4 weeks of treatment, the patient was discharged from the hospital and followed up with outpatient treatment. After 12 weeks, the wound had healed completely without any complications (Figure 4).

3.3 | Case 3

A 42-year-old female patient with a history of diabetes and hyperlipidemia presented with an abscess on her right 5th toe and extensive induration on her right foot that occurred 2 weeks ago. The patient was treated by incisional drainage with Penrose drain insertion. A 2 × 2 cm skin defect and multiple small skin defect were observed after drainage. Through the wound, infected bone and ligament tissue were seen. Foot anteroposterior X-rays also showed osteomyelitis findings on the proximal and middle phalanx of the fifth toe. After removing necrotic ligaments and bones and controlling the infection, 2 cc of micronized ADM was injected into the dead space. A micronized ADM was reapplied at one and 2 weeks. After 3 weeks of treatment, the patient was discharged from the hospital and followed up with outpatient treatment. After 12 weeks, the wound had healed completely without any complications. A year and a half later, an ulcer developed at the distal phalanx of the fifth toe. It was connected along the extensor tendon to the previous ulcer location (Figure 5).

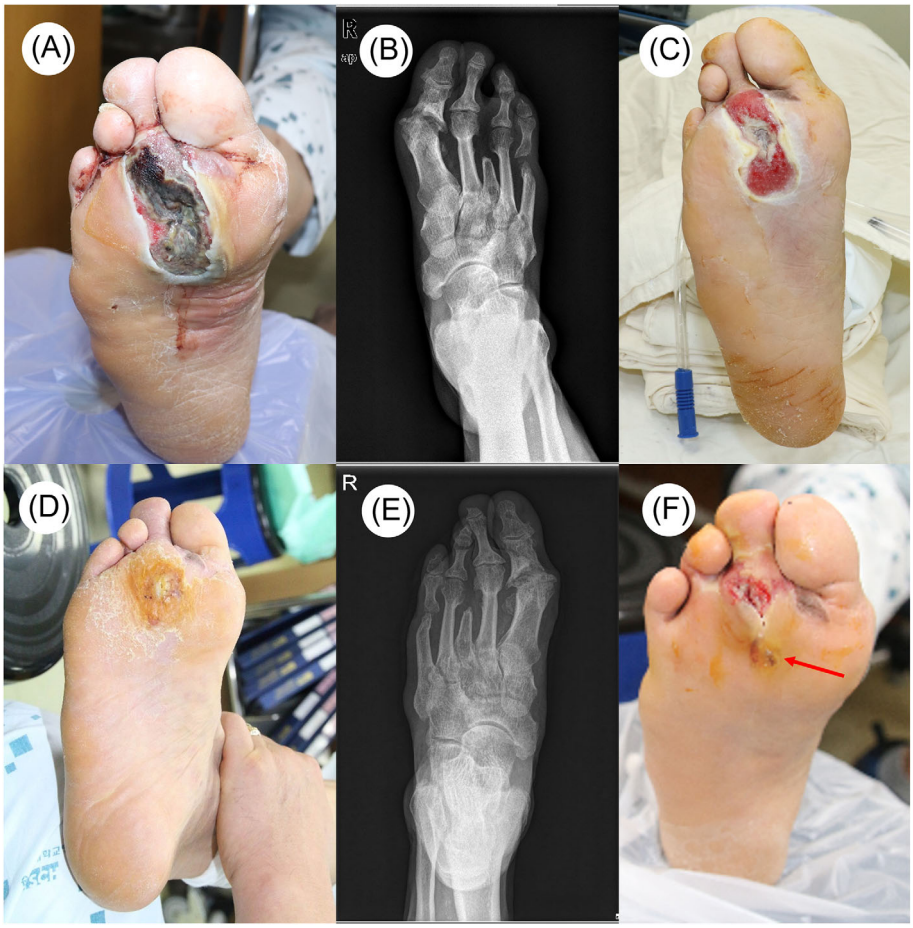

FIGURE 3 Case 1. A 54-year-old male patient who had amputated his third toe visited the hospital. (A) Two weeks ago, a 3 × 7 cm ulcer with a foul odour developed on the plantar of the right foot, and the remaining third metatarsal bone that had been amputated was touched through ulcer. (B) There was osteomyelitis on the remaining third metatarsal bone and head of fourth metatarsal bone. Other toe deformations have also been observed. (C) Bed preparation was performed through removal of necrotic tissues, IV antibiotics and NPWT treatment. 2 cc of micronized ADM was used to fill in the defective area three times at weekly intervals. (D) After 5 weeks, the inflammation and swelling around the wound decreased and the wound was almost completely closed. (E) X-rays showing improvement of the osteomyelitis of the third metatarsal bone and the head of fourth metatarsal bone. (F) The ulcer recurred 2 years later and the red arrow remained scarred at the location where the previous ulcer was. Recurrent ulcers developed in more distal portion where they had been previously treated. There was no bone exposure

3.4 | Case 4

A 57-year-old male patient with a history of diabetes and hypertension was admitted for a skin defect in his right foot that occurred after trauma 2 months ago. There was a skin and soft tissue defect of 2 × 2 cm in size on the right foot and exposure to the 5th metatarsal bone. On foot anteroposterior X-rays, inflammation of the 5th metatarsal bone and metatarsophalangeal joint was shown. Debridement was performed to remove dead bones and joints. The swelling and redness gradually improved. A healthy wound bed was prepared. At weeks 1, 2, and 3, 2 cc micronized ADM was applied. After 3 weeks of treatment, the patient was discharged and followed up with outpatient treatment. At week 11, a contracture occurred slightly. However, treatment was completed without any major complications (Figure 6).

3.5 | Case 5

A 44-year-old female patient with a medical history of diabetes and hypertension was hospitalised with ulcers in the left second toe and dorsum of foot. The patient had previously had a surgical history of amputation of the second toe. The ulcer occurred in the metatarsophalangeal joint area of the second toe, which was previously amputated. It was connected to the ulcer of the dorsum of foot along the extensor tendon. Necrotic ligaments and tissues

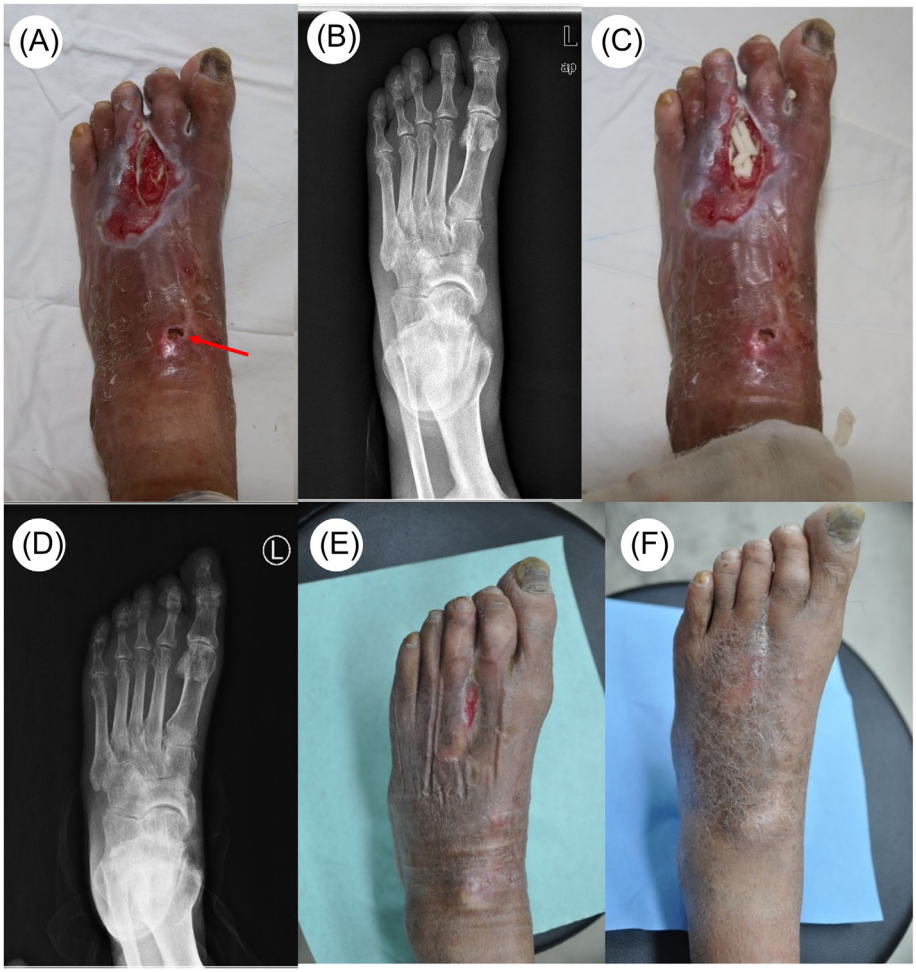

FIGURE 4 Case 2. A 65-year-old male patient was hospitalised with an ulcer that exposed the ligaments and joints of his left foot. (A) The abscess was spread along the extensor tendon of the foot to the front of the ankle. The red arrow indicates the hole through which the Penrose drain was inserted to drain the discharge. Ligaments of the second and third toes were necrosis, the second metatarsophalangeal joint was exposed, and there was a tunnel into the web space of the great toe. The size of the defective area was 3 × 6 cm. (B) Fortunately, it did not progress to osteomyelitis. (C) Micronized ADM (3 cc) was used three times a week to fill exposed defects, the tunnel under the dorsum of the foot, and the tunnel to the great toe. (D) On X-rays, there was no significant change compared to previous foot x-ray. (E) The patient was discharged from the hospital and treated on an outpatient basis. At 6 weeks after micronized ADM application, almost all wounds were recovered except for a slight raw surface on the dorsum of the foot. (F) After 12 weeks, the wound fully healed. The ulcer has not recurred since

were removed and 2 cc of micronized ADM was used to fill the defective area. After a month of treatment, she was discharged from the hospital and the defective area was cured through outpatient treatment. However, 2 years later, she visited the hospital with an ulcer accompanied by osteomyelitis in the proximal phalanx of the fourth toe of the left foot. Previously, wounds of the dorsum of foot and second toe filled with micronized ADM had been recovered without complications. However, when she visited the hospital again another 1 year later, ulcers recurred on the second toe. She had to be hospitalised with inflammation and oedema, although the wound was not as deep as bones or ligaments (Figure 7).

3.6 | Case 6

A 76-year-old male patient with a medical history of diabetes and chronic renal failure was hospitalised for an ulcer in the right second toe. The head of the second metatarsal bone was exposed through ulcer accompanied by necrotizing tissue and biofilm. The head of the second metatarsal bone and the unviable tissue were removed. After serial debridement, 1.5 cc of micronized ADM was applied to the defective area at weekly intervals. Three weeks later, the patient was discharged from the hospital and received outpatient treatment. Eight weeks later, the wound recovered without any complications. Diabetic ulcers have not recurred since then (Figure 8).

3.7 | Case 7

A 53-year-old man with a history of diabetes was hospitalised with an ulcer in his left great toe. The ulcer was located on the medial side of the proximal phalanx of the great toe. On X-rays, bones of the middle phalanx were almost lost. Only a few of the distal phalanx and proximal phalanx remained. Osteomyelitis was quite advanced. Ligaments were also accompanied by inflammation. Dead bones and ligaments were removed and the cavitary defect was filled with 2 cc micronized ADM. Osteomyelitis improved after antibiotic treatment and micronized ADM. Recovery of distal phalanx and proximal phalanx was confirmed on X-rays. After 6 weeks of treatment, the wound was healed almost completely. Outpatient treatment was

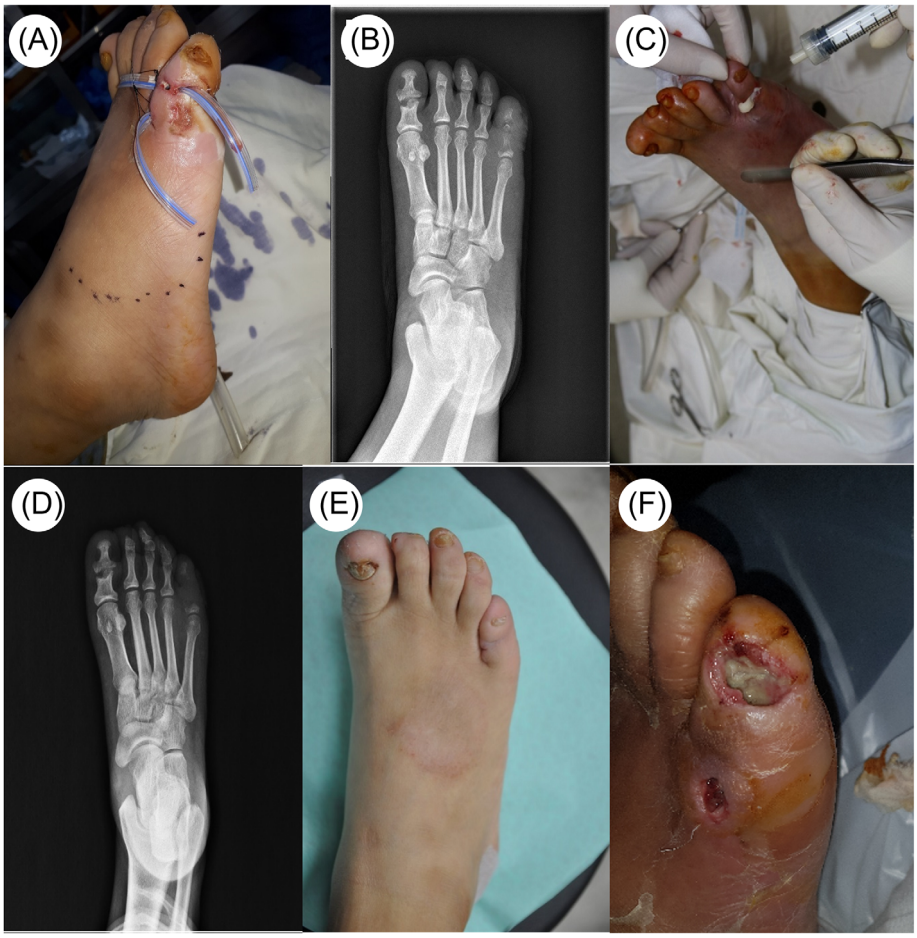

FIGURE 5 Case 3. A 42-year-old female patient with a history of diabetes and hyperlipidemia presented with an abscess on her right fifth toe and extensive induration on her right foot that occurred 2 weeks ago. (A) At the time of admission, there were redness and swelling up to the area marked by a black dotted line. There was an abscess in the metatarsophalangeal joint of the right fifth toe that invaded the phalangeal bone. Penrose drain was inserted after drainage was performed. (B) Initial X-rays showing osteomyelitis findings in the middle phalanx and proximal phalanx of the fifth toe. Necrotic tissues including ligaments, middle phalanx bone, and distal portion of proximal phalanx bone were removed. (C) Micronized ADM (2 cc) was used to fill a 2 × 2 cm wound with exposed bones that occurred after debridement. (D) X-ray also showed that the middle phalanx bone and distal portion of proximal phalanx bone were removed. (E) After using 2 cc of micronized ADM at weeks 1, 2, and 3, the wound was fully healed without any complications at 12 weeks. (F) A year and a half later, an ulcer developed at the distal phalanx of the fifth toe, which was connected along the extensor tendon to the previous ulcer location

performed, and wound was closured completely. However, a year later, his great toe showed necrosis and amputation was performed at other hospital. He was hospitalised again 2 years later due to ulcers in the amputated area, heels, and soles of his feet (Figure 9).

3.8 | Case 8

An 81-year-old male patient with a history of diabetes and arteriosclerosis obliterans was hospitalised for an ulcer in the left great toe that occurred spontaneously a month ago. The tip of the toe was mummified. X-ray showed bone erosion. Therefore, only the proximal head of the proximal phalanx was preserved. The mummified toe was amputated, including the rest of osteolytic bones and tissues. Bed preparation was performed using NPWT. Then 1 cc of micronized ADM was applied three times at weekly intervals. Eleven weeks after applying micronized ADM, the wound recovered without complications. There has been no recurrence since then (Figure 10).

4 | DISCUSSION

Proper wound regeneration begins when a well-coordinated process between the surrounding microenvironment and the cell is activated. Among them, the extracellular matrix (ECM) is the key element of wound healing, providing structural support as the largest

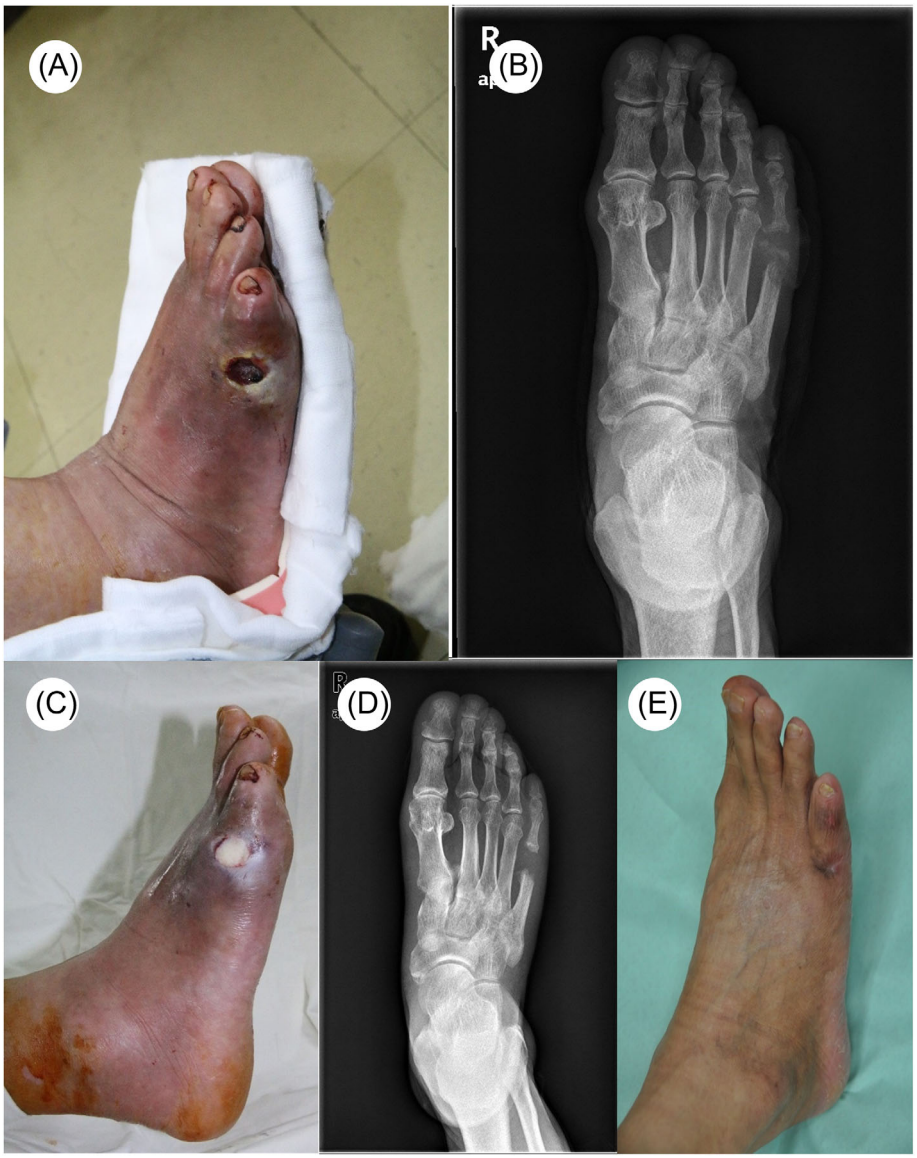

FIGURE 6 Case 4. A 57-year-old male patient with a history of diabetes and hypertension was admitted for a skin defect in his right foot that occurred after trauma 2 months ago. (A) There was redness on the feet and general swelling in the lower extremities with skin and soft tissue defect of 2 × 2 cm in size on the right foot and exposure to the fifth metatarsal bone. Pus was drained from inside the bone. (B) Foot X-rays showed osteomyelitis of the fifth metatarsal bone and metatarsophalangeal joint. Debridement was performed to remove dead metatarsal bones and metatarsophalangeal joints. (C) The swelling and redness gradually improved with IV antibiotics and proper wound management. A healthy wound bed was prepared. Then 2 cc micronized ADM was applied at weeks 1, 2, and 3. (D) Foot X-rays also showed that the fifth metatarsal bone and part of the metatarsophalangeal joint had been removed. After 3 weeks of treatment, the patient was discharged and treated on an outpatient basis. (E) At week 11, a contracture occurred slightly. However, the treatment was completed successfully without any major complications

component of the dermal layer.[10,11] It also provides signalling proteins needed for cell adhesion and signalling, promoting effective wound healing.[12-14] In chronic wounds, sometimes ECM is dysfunctional or deficient, making wound healing difficult. Replacing and restoring damaged ECMs can accelerate wound healing.[6,15] Acellular dermal matrix (ADM) helps wound healing by stimulating angiogenesis, acting as a chemoattractant for endothelial cells, providing growth factors, and permitting a substrate for fibroblasts to attach.[10,16,17] The current standard for using micronized ADM (CG Paste) in wound healing is direct application over wounds. Micronized ADM has been shown to be effective for various wounds, including DFU, pressure ulcers, ischemic ulcers, and burn treatments.[6,18-22]

In previous studies,[18] the authors have found that micronized ADM in various wounds in combination with NPWT can significantly ($P < .0001$) reduce the size of the wound from 15.48 ± 22.38 cm^2 in 20 patients to 8.97 ± 17.73 cm^2 after 4 weeks. Among those 20 patients, the depth of the wound could be assessed for 12 patients. In these 12 patients, the depth of the wound was significantly ($P = .013$) decreased from 9.8 ± 8.3 mm to 5.4 ± 4.9 mm after 2 weeks. After 4 weeks of treatment, 13 (65%) of 20 patients had wound closure through secondary intention healing using only micronized ADM with NPWT.[18] In a recent prospective randomised controlled multicentre clinical trial, 81 patients in the group using a micronized ADM and the control group also showed significant differences in wound area reduction rate from week 2 to study endpoint, with granulation tissue and epithelization rates being significantly increased in the study group compared with those in the control group.[19] In the study group, 29 (76.32%) of 38 wounds

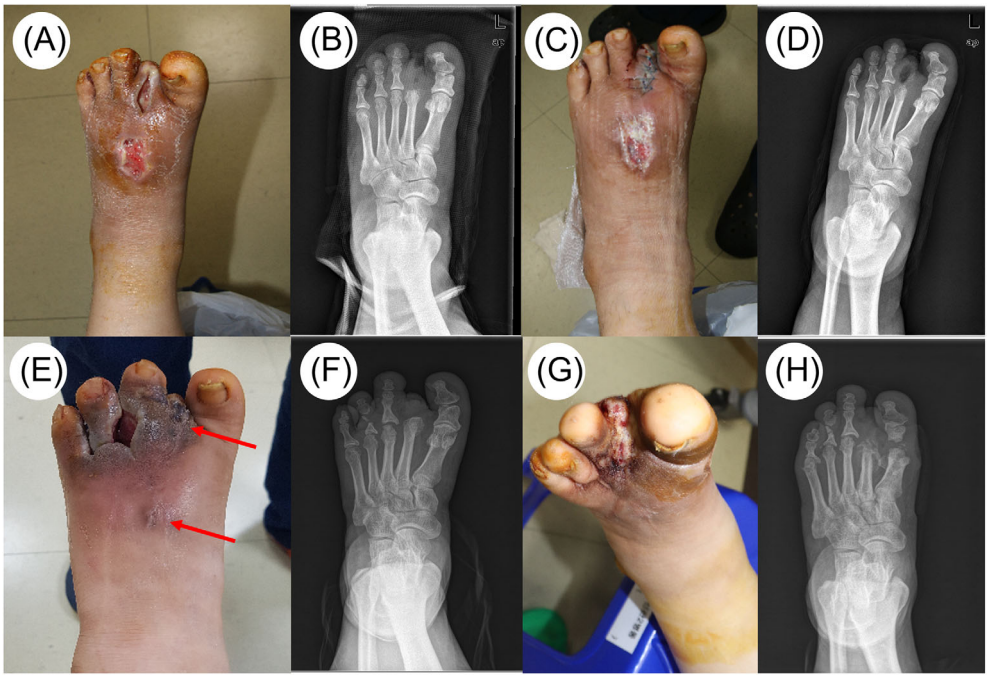

FIGURE 7 Case5. A 44-year-old female patient who had been amputated her second toe was hospitalised with ulcers in the left second toe metatarsophalangeal area and the dorsum of foot. (A) The ulcer located in the metatarsophalangeal joint of the second toe had the head of the second metatarsal bone exposed. It was connected along the extensor ligaments to the dorsum of the foot. (B) There was no osteolytic lesion around ulcers on the X-ray. (C) 2 cc of micronized ADM was used to fill in defective areas of dorsum of the foot and second toe. The second toe was closed using Nylon. (D) However, as shown in the X-ray, free air was identified in the defect area. Treatment was continued using micronized ADM with NPWT. A month later, she was discharged from the hospital for outpatient treatment. (E) However, 2 years later, she visited the hospital with an ulcer accompanied by osteomyelitis in the proximal phalanx of the fourth toe of the left foot. The red arrow points to a scar due to a previous ulcer treated with ADM. (F) X-rays confirmed the osteomyelitis of the fourth toe. (G) However, after 1 year, an ulcer with pus on the second toe recurred. (H) X-ray also confirm necrosis of the second metatarsal bone

were healed by 12 weeks, whereas only 11 (30.56%) of 36 wounds were healed in the control group ($P = .001$). In another meta-analysis, the complete healing rate of the ADM group was higher than that of the control group (RR at 12 weeks: 1.73, 95% CI: 1.31-2.30, $P = .0001$; RR at 16 weeks: 1.56, 95% CI: 1.28-1.91, $P < .00001$).[20] The time taken to complete healing was shorter in the ADM group than in the control group (MD = −2.41, 95% CI: −3.49 to −1.32, $P < .0001$), with ADM being significantly effective in improving wound depth, wound area, and quality of life.[20]

In the present study, healing occurred in 34 (62.96%) of 54 patients in the micronized ADM group. The healing rate of the study group was similar to those in other prospective studies or meta-analysis studies.[21] Unlike other studies,[5-8,16-20,22,23] an analysis was also conducted for the timing of application or recurrence of micronized ADM. It was found that micronized ADM was applied on an average of 20.34 days after hospitalisation. Most patients began wound management by undergoing vascular examinations immediately before or immediately after hospitalisation with sharp debridement in the absence of blood flow abnormalities. In the case of impaired blood flow, wound management was initiated at 3–4 weeks after implementing percutaneous transluminal angioplasty at a time when reperfusion injury was minimized,[24,25] which was found to be at an average of 20.34 days after initiating wound management. It was worth noting that it took an average of 12.68 days to be discharged from the hospital when using a micronized ADM and that treatment was terminated after an average of 66.40 days on an outpatient basis. This meant that most patients succeeded in complete healing within a short time through outpatient treatment after a quick discharge. This has significant benefits in terms of decreasing the time and cost of treatment as well as hospitalisation length, material costs, and quality of life of patients.

In a study using rats, 18 animals were tested 6 animals in each group: a control group, a micronized ADM scrub dressing group, and a micronized ADM subcutaneous injection group.[23] Rats in each group were assessed for the size of the wound every 3, 5, 7, 10, and 14 days. A histoimmunological examination was then performed. In

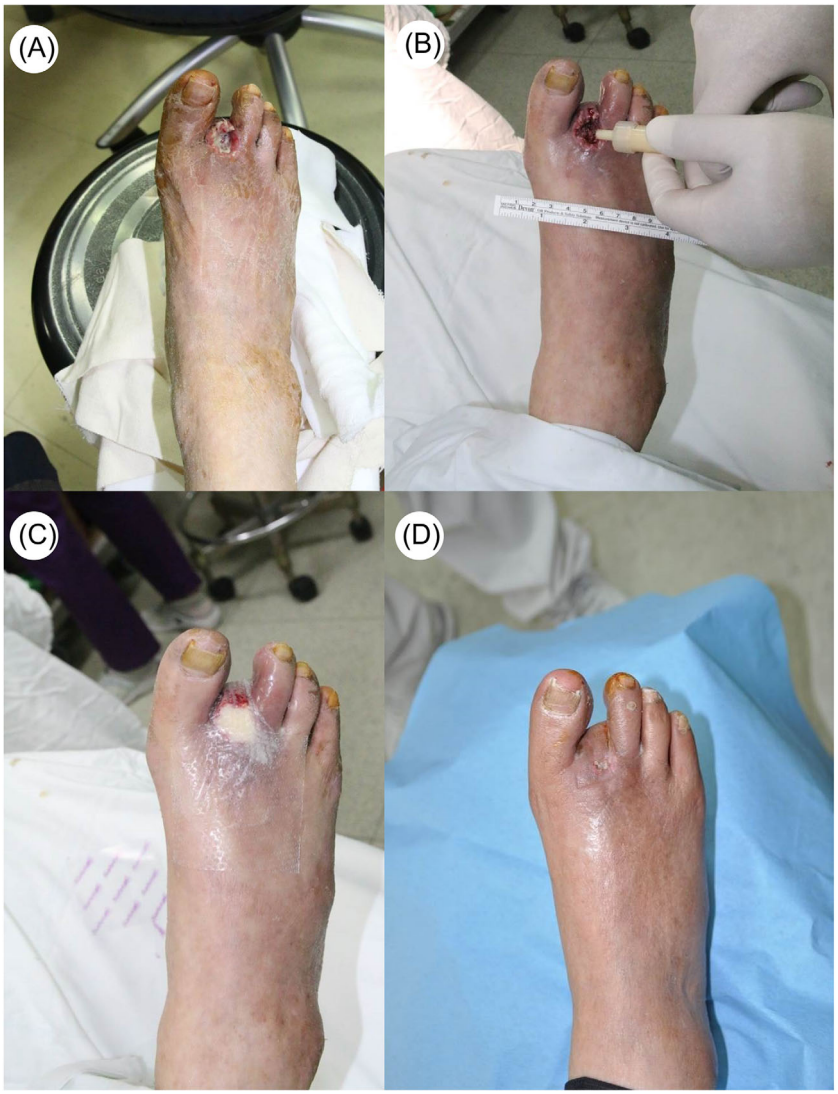

FIGURE 8 Case 6. A 76-year-old male patient with a medical history of diabetes and chronic renal failure was hospitalised for an ulcer in the right second toe. (A) The second toe was removed due to total necrosis. The metatarsophalangeal joint of the second toe remained defective, exposing dead tissues and the head of the second metatarsal bone. (B) After serial debridement, 1.5 cc of micronized ADM was applied to the defective area at weekly intervals. (C) The micronized ADM was covered with a silicone sheet. (D) After a total of 11 weeks of starting micronized ADM treatment, the wound recovered without any complications. No recurrence occurred

that study, the micronized ADM scrub dressing group and the micronized ADM subcutaneous injection group showed significant reduction of wound sizes at days 10 and 14 than the control group. Histioimmunological analysis confirmed that the number of vessels was significantly increased in the study group.[23] In addition, ADM can induce migration of fibroblasts by providing an extracellular matrix, enabling it to survive as autogenous tissue. ADM is known to help heal wounds through mechanisms such as acting as a scaffold for granulation tissue formation, providing receptors for fibroblast attachment, stimulating angiogenesis, functioning as a chemoattractant of vascular endothelial cells, and including growth factors.[26,27] Histological and histomorphometric analyses after applying ADM to a 20 × 20 mm size skin defect in a rabbit revealed no statistically significant ($P > .05$) difference in healing rate. However, significant increases were found for epidermal thickness ($P < .05$), dermal thickness ($P < .05$), and Type I & III collagen ($P < .05$).[22]

In previous animal studies,[22,23,26,27] the principle of ADM for accelerating the recovery of soft tissues has been revealed, showing several positive results. However, in a real clinical setting, a small wound set up in an animal experiment can be treated with other materials sufficiently without using ADM. Animal and clinical studies on whether ADM can be applied in complex wounds accompanied by tendon or bone exposure are lacking. In our study, a micronized ADM was applied to DFU (Wagner grade II or higher) in which tendon or bone was exposed. Our results confirmed that a micronized ADM was effective for tendon and bone exposed DFUs. After a long-term observation, the healing rate and complete healing rate were significantly high in the patients who treated with micronized ADM. However, micronized ADM did not appear to have a significant effect in reducing the recurrence rate or preventing recurrence in our study. The recurrence rate at the location where micronized ADM was applied was 23.52%. The overall recurrence rate was

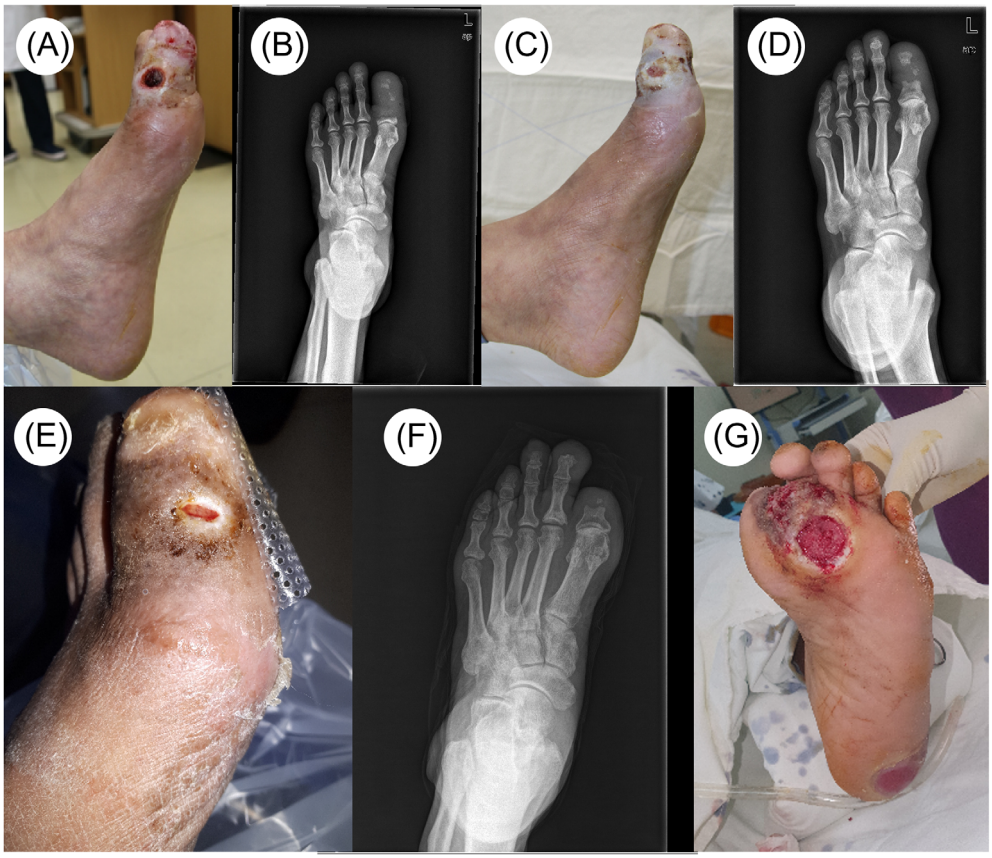

FIGURE 9 Case 7. A 53-year-old man with a history of diabetes was hospitalised with an ulcer in his left great toe. (A) The patient had an ulcer in the medial side of the left great toe that occurred 3 weeks ago with necrotic bones and ligaments identified. (B) X-rays confirmed osteomyelitis of the left great toe. (C) After removing dead bones, infected ligaments, and necrotic tissues, 2 cc micronized ADM was filled in the area of a 2 × 2 cm defect that occurred. (D) After 6 weeks, the osteomyelitis improved on X-rays. (E) The defect on the great toe also seemed to have healed almost fully, leaving only a few raw surfaces. (F) However, a year later, his great toe was necrosis and amputation was performed in another hospital. (G) Ulcers on the amputated great toe and the plantar of the foot were hyperkeratosis. There were also ulcers on the heel

41.17%. Recurrences occurred within an average of 3 years, similar to the results of other studies.[28] Armstrong et al. reported a 40% chance of recurrence in 1 year, a 60% chance of recurrence in 3 years, and a 65% chance of recurrence in 5 years.[29] Overall, the recurrence rate of our study was similar to those of other studies, although the recurrence rate of the weight bearing area was significantly higher than that of the non-weight bearing area.

Cho et al. have found that neovascularization and active granulation can occur due to collagen deposition when micronized ADM is applied in porcine wound models.[30] Carvalho-Junior et al. have found a statistically significant increases in thickness and density of epidermis, dermis, collagen I and III in studies on ADM in rabbits.[22] Although a histological study was not performed after the wound healed in our study, a similar healing phenomenon might have occurred in DFUs. However, after treatment with micronized ADM, the thick thickness of the epidermis and dermis and the high collagen density did not prevent the recurrence of diabetic foot in the weight bearing area. In a non-weight bearing area, on the other hand, micronized ADM showed a good course of treatment and an exceptionally low recurrence rate. After months of using ADM, ADM can turn into fibroblasts, myofibroblasts, and various other free cells (lymphocytes, macrophages, granulocytes, mast cells) of the connective tissue,[31] which may not have been sufficient for the mechanical property to hold on to the weight. In addition, neuropathy or deformed bony structure may contribute to recurrence. If similar results are obtained in future large-scale prospective studies, further research is needed to identify histology/pathological causes of recurrence of micronized ADM. In addition, weight bearing wounds may require close continuous clinical observation as recurrence may occur even if the wound is completely cured using a micronized ADM. Among risk factors such as gender, smoking history, poor glycemic control, peripheral arterial disease, neuropathy, and

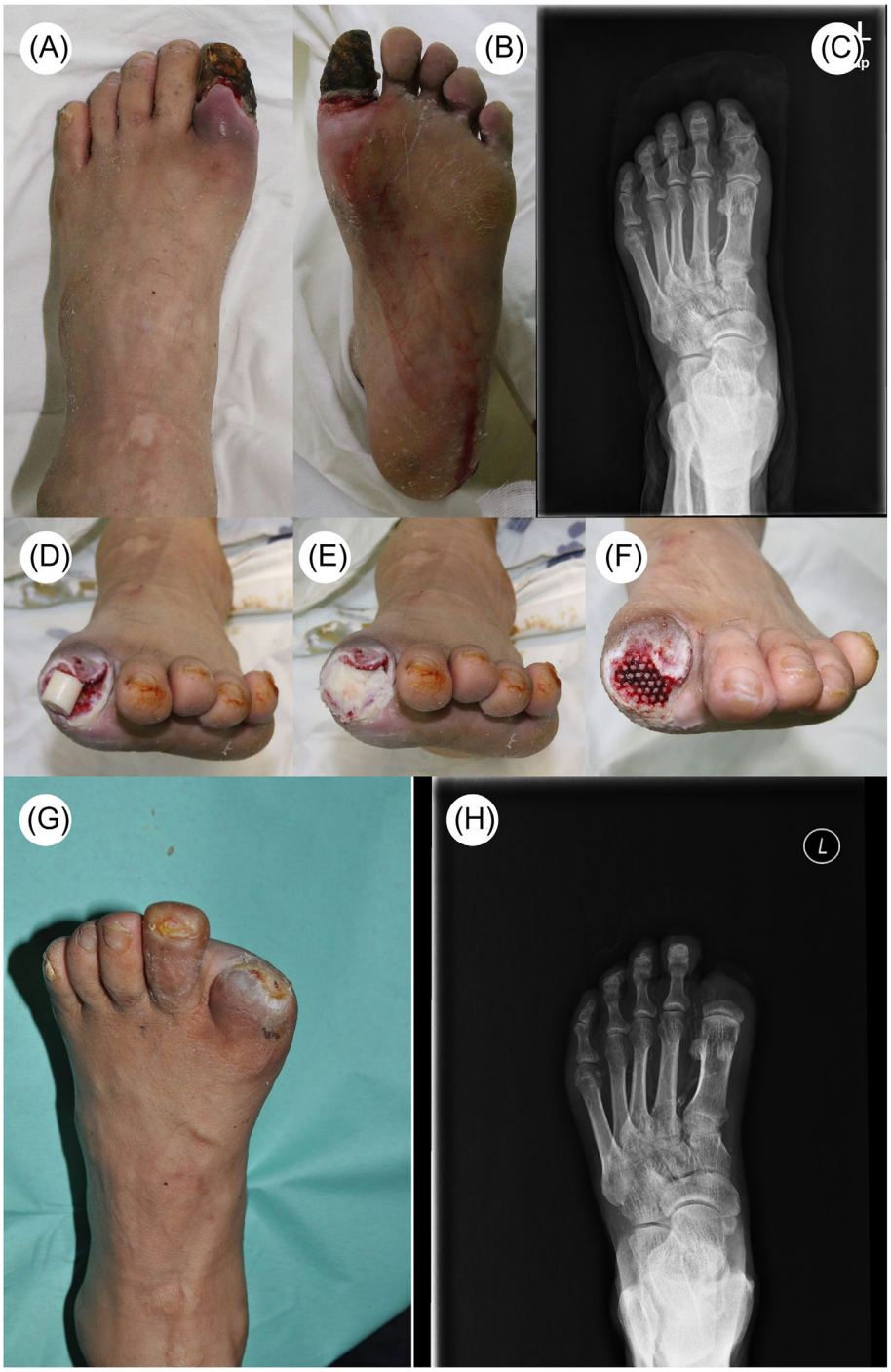

FIGURE 10 Case 8. An 81-year-old male patient with a history of diabetes and arteriosclerosis obliterans was hospitalised for an ulcer in the left great toe that occurred spontaneously a month ago. (A, B) The left great toe was mummified, accompanied by swelling and inflammation. (C) X-rays also confirm osteolytic lesion in the distal portion of the proximal phalanx of the great toe. (D, E) Only the proximal portion of the proximal phalanx was preserved. The rest part was completely amputated, including bones and ligaments. The bed preparation was performed through NPWT and 1 cc of micronized ADM was applied to a 2×2 cm sized defect of the great toe. (F) A week after applying micronized ADM, the micronized ADM was turned into granulation tissues. (G, H) Eleven weeks later, the wound recovered without complications. There has been no recurrence since then

chronicity of ulcer known to be associated with recurrence of DFU,[28,32] smoking and gender were less strongly associated with DFU recurrence. Reasons for this were: (1) smoking history was not thoroughly investigated; and (2) male and female population ratios were not matched. The chronicity of ulcers, blood glycemic control, neuropathy, and the degree of occlusion of arteries were not evaluated in this study.

In this study, a long-term follow-up after the application of micronized ADM confirmed the efficacy, stability, and recurrence rate of micronized ADM. However, this study has some limitations. First, although we had a significant number of patients from different physicians, there might be a selection bias. In addition, bias might have occurred during treatment and application of micronized ADM during treatment. Therefore, a long-term study with a prospective randomised controlled multicentre clinical trial is needed. Second, even if a wound corresponding to the same Wagner grade was chosen within each group, some evaluations might have

been omitted because the size, location, and depth of individual wounds were not completely unified. Progress sheets and photographs were then used to evaluate subsequent course of treatment. Third, there was no control over the duration of applying NPWT during hospitalisation, making it difficult to clearly determine and control how different dressing methods and dressing cycles affected the course of treatment. Although not all patients in this study used NPWT, NPWT could accelerated wound healing. It might have synergies when it is used in combination with micronized ADM.[30]

In conclusion, micronized ADM induced secondary healing in tendon/bone exposed DFUs commonly known to be hard-to-heal wounds. When adequate recovery of blood flow and wound bed preparation preceded the procedure, proper use of micronized ADM could reduce total healing time and the number of hospitalisation dates. It also dramatically increased the healing rate and enabled early outpatient therapy. Thus, it is cost effective. In this long-term follow-up experience, micronized ADM showed sufficient stability, although it did not lower the recurrence rate or prevented recurrence. A close follow-up of weight bearing area wounds will allow us to identify and treat recurrence early.

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DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

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