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Autologous Whole-Blood Clot Therapy for Refractory Diabetic Foot and Ankle Wounds: Preliminary Clinical Findings

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1. ABSTRACT

1.1. Background: Topical autologous blood clot therapy is a localized care intervention that utilizes an autologous coagulum to promote reconstitution of the extracellular matrix within chronic or non-healing wounds. The clot functions as a bioactive scaffold enriched with intrinsic growth factors, cytokines, and chemokines that orchestrate cellular recruitment, angiogenic signaling, fibroblast activation, and regulated matrix deposition. In this study, we attempted to demonstrate the therapeutic effectiveness of ActiGraft in the management of complex chronic wounds occurring in patients with underlying diabetic comorbid conditions.

1.2. Methods: Chronic, non-healing diabetic foot wounds were treated using Red Dress ActiGraft technology. Local wound debridement was performed weekly in the outpatient clinic, followed by application of the autologous whole-blood clot. Wound-healing progression was documented through serial photographs, quantitative measurements of wound dimensions, and systematic recording of time to closure, continuing until complete epithelialization was achieved.

1.3. Results: The therapy demonstrated high clinical efficacy: five patients achieved complete wound closure, while the remaining two exhibited active epithelialization and a reduction in wound area exceeding 95%. No complications, infections, or adverse reactions attributable to the blood-clot application were observed in any patient.

1.4. Conclusions: ActiGraft offers an effective and safe therapeutic option for difficult-to-heal diabetic foot wounds, providing a biologically active scaffold that supports natural wound repair. These findings suggest that ActiGraft can play a valuable role in advanced diabetic foot wound management, particularly for patients who have not responded to conventional therapies.

Keywords: Blood clot; Diabetic foot; Chronic wound; ActiGraft

2. INTRODUCTION

The escalating global prevalence of diabetes mellitus, currently affecting more than 422 million individuals worldwide, has been accompanied by a corresponding rise in Diabetic Foot Ulcers (DFUs), ulcer progression,

and lower-extremity amputations, thereby profoundly diminishing patients' quality of life and increasing morbidity and mortality [1]. Consequently, diabetes mellitus continues to impose a substantial burden on healthcare systems worldwide and remains a leading etiological factor in the development of foot ulcers [2].

In parallel, over 2% of the global population is affected by chronic, non-healing wounds, defined as wounds that persist for more than one month without progressing through the normal phases of healing, with incidence increasing markedly in the geriatric population [2].

Up to one-third of diabetic patients will develop foot ulcers, and approximately 20% of these cases will ultimately result in partial or major foot or leg amputations [3].

Furthermore, DFUs account for more than 50% of non-traumatic lower-extremity amputations and are associated with a striking five-year mortality rate of approximately 30%, which is nearly double that observed among diabetic patients without DFUs [1,4].

Beyond their devastating clinical consequences, DFUs impose a significant economic burden, as reflected by nearly $4 billion in DFU-related healthcare expenditures incurred by the United States healthcare system in 2019. Collectively, these data underscore DFUs as a major public health challenge with profound clinical, societal, and economic implications that continue to intensify [1,5].

Recalcitrant wounds are frequently associated with unpredictable clinical trajectories and suboptimal outcomes. In the context of DFUs, delayed or failed healing is largely driven by profound cellular dysfunction within the wound microenvironment, leading to impaired immune responses and disrupted tissue repair mechanisms.

Similarly, dehiscence and infection in hard-to-heal wounds are associated with a markedly increased risk of postoperative complications and mortality. These chronic wounds are typically characterized by diminished concentrations of essential growth factors alongside elevated levels of inflammatory mediators, proteolytic debris, and matrix metalloproteinases, which collectively contribute to degradation of the Extracellular Matrix (ECM) and compromise tissue regeneration [6].

Physiological wound healing is a highly regulated, multistep process encompassing hemostasis, inflammation, proliferation, re-epithelialization, and remodeling. Central to this process is the regeneration of the injured ECM, which provides a critical structural scaffold for cellular migration, angiogenesis, and skin regeneration before ultimately being replaced by scar tissue.

Although inflammation is a necessary component of wound healing, serving to control bleeding, prevent infection, and stimulate cell proliferation, dysregulated or prolonged inflammation can be detrimental [7].

In patients with diabetes, chronic hyperglycemia alters ECM composition and elasticity, thereby disrupting normal wound healing dynamics. The combined loss of key ECM components within the wound bed and exposure of underlying structures significantly alters treatment pathways, prolongs healing time, and limits the capacity for recovery as ECM deterioration progresses. The formation of granulation tissue and subsequent epithelialization over vital structures becomes markedly delayed or may fail entirely [2]. When healing does not occur, continued soft-tissue breakdown may expose bone, tendon, or other deep anatomical structures, frequently necessitating escalation to advanced therapies and hospitalization [3].

This study evaluates a novel therapeutic approach: an autologous whole blood clot therapy, in order to address these structural and biochemical deficits. The efficacy of this system lies in its dual-action capability to reconstruct the wound microenvironment. First, it serves as a provisional scaffold that mimics the lost ECM,

providing a physical barrier against pathogen ingress. Second, it facilitates biological repair by delivering a concentrated surge of endogenous growth factors directly to the compromised wound bed, thereby reinitiating the stalled healing cascade.

3. MATERIALS AND METHODS

The study enrolled seven subjects with recalcitrant wounds exhibiting osseous, articular or tendinous exposure, five of whom had failed to respond to previous therapeutic interventions.

All participants provided written consent for the analysis and publication of their clinical data, including medical records, demographics, and wound imagery.

All seven subjects received care through a collaborative, multidisciplinary approach involving experts in orthopedics, internal medicine, infectious disease, and clinical nutrition.

Wound bed preparation was performed on all subjects prior to ActiGraft implantation. This regimen included three key components: total tissue debridement, restoration of bacterial equilibrium, and maintenance of an appropriate moisture content.

Debridement was accomplished *via* surgical or enzymatic means. Negative pressure wound therapy was also implemented in three cases. Infection management was initiated with intravenous antibiotics guided by sensitivity testing. The regimen was subsequently transitioned to oral antibiotics, maintained and adjusted according to clinical and laboratory findings.

Wound characteristics and healing progression were systematically assessed on a weekly basis. Parameters recorded included: wound dimensions, presence of epithelialization, quality and quantity of granulation tissue and fibrin, characteristics of the exudate (discharge), presence of odor, and level of localized pain and signs indicative of infection.

The ActiGraft preparation and application procedure, performed on a weekly basis, followed the established procedural manual. This began with the phlebotomy of approximately 18 mL of autologous blood into ACDA vacuum collection tubes. The collected blood was subsequently placed into a coagulation mold and mixed with both a calcium coagulant and kaolin to facilitate rapid coagulation, a process completed in approximately 8 minutes. Upon formation, the clot was directly placed into the wound bed and anchored by Steri-Strips. The wound was then covered with a non-adherent dressing and protective foam cover.

4. RESULTS

4.1. Case 1

A 66-year-old male with a history of type II diabetes mellitus developed a deep pressure ulcer overlying the right lateral malleolus, following a period of cast immobilization required after open reduction and internal fixation for a complex tibial plateau fracture.

The initial clinical presentation included purulent discharge and surrounding cellulitis originating from the ulcer, and critically, bony exposure of the lateral malleolus was observed. Initial management involved surgical debridement of the wound bed and empiric systemic antibiotic therapy, subsequently modified according to bacterial culture and sensitivity findings.

The necessity for an 18-day inpatient admission was directly attributed to the failure of the wound to progress and the ongoing presence of infection. The therapeutic regimen during hospitalization encompassed sustained systemic antibiotic administration combined with local applications of agents such as chlorine, povidone-iodine, and Flaminal Forte. Upon discharge, the patient continued outpatient local treatment with Flaminal Forte for an additional three weeks, again without significant clinical improvement. The vascular surgeon's assessment confirmed the presence of peripheral vascular disease; however, the condition was determined to be stable and did not necessitate immediate surgical or interventional treatment at that time. Prior to the initiation of the first ActiGraft application, the wound exhibited exposed lateral malleolus and presented as an elliptical defect with an approximate area of 5.9 cm^2 (Figure 1A).

The lesion was primarily covered with fibrin tissue but without overt clinical signs of active infection. The ActiGraft dressing was changed on a weekly basis.

The local wound bed preparation was performed as required during each dressing change, consisting mainly of cleaning with normal saline and septal scrub.

Minimal mechanical debridement was performed only when indicated. Full wound closure (complete epithelialization) was ultimately achieved following a total of six weekly treatment cycles (Figure 1B).

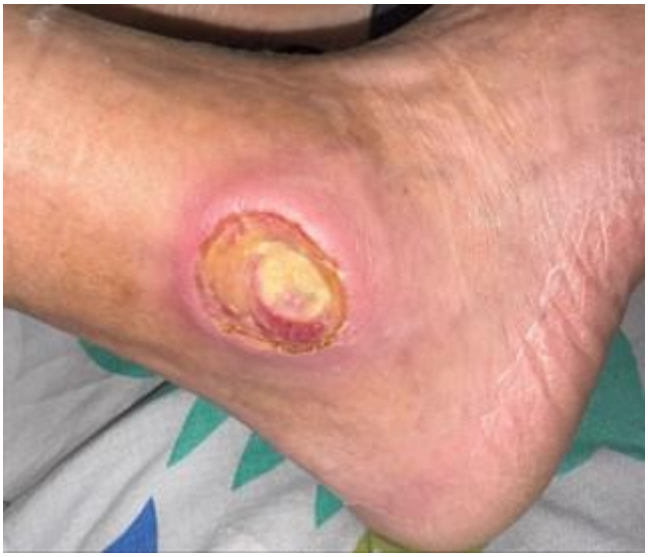

Figure 1A: Upon initial presentation, a circular wound measuring 2.5 cm × 3 cm is noted. The base of the fibrin-covered wound demonstrates the fully-exposed lateral malleolus and partial lateral collateral ligaments, all surrounded by a reactive process.

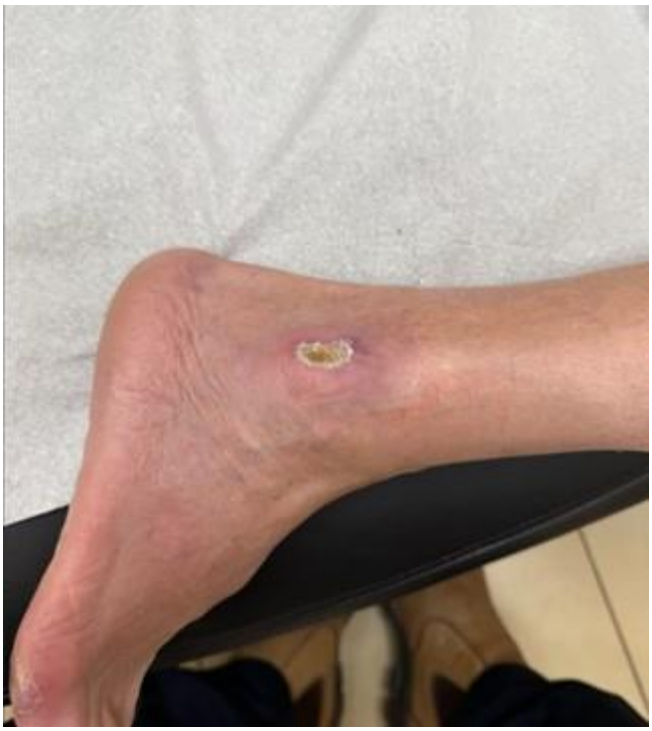

Figure 1B: Observe the complete lesion resolution following six weeks of targeted interventions.

4.2. Case 2

A 61-year-old male with a complex medical history, including type II diabetes mellitus, hypertension, gout, chronic congestive heart failure, chronic renal insufficiency, and treated coronary artery disease (status post stenting six years prior), presented with wet gangrene of the fifth digit on the left foot.

Radiography confirmed extensive osteolytic lesions extending to the head of the fifth metatarsus. The patient initially underwent a fifth ray amputation. Due to the rapid evolution of necrotic tissue, a subsequent surgical debridement was required three days post-amputation, following which negative pressure therapy was established.

Systemic antibiotic therapy was initiated and adjusted according to bacteriogram and resistogram results. Following consultation with an infectious disease specialist, and in consideration of the identified polymicrobial, multi-resistant flora within the wound, the final systemic antibiotic course spanned 12 weeks. A vascular assessment *via* angiography confirmed diffuse microvascular disease but determined that acute revascularization was unnecessary. Following multidisciplinary review and 15 days of inpatient management, the patient was discharged to continue local wound care with Negative Pressure Wound Therapy at home.

After 20 days of home care, the patient was readmitted due to a rapid elevation in systemic inflammatory markers. Local wound examination revealed predominantly granulation tissue; however, the proximal aspect of the wound exhibited necrotic tissue accompanied by purulent discharge. A third surgical intervention was performed, followed by the application of local antiseptic agents. Although the wound demonstrated partial progress in granulation, its overall size remained static.

Upon stabilization, treatment with the autologous blood clot was initiated during the second, two-week hospitalization. Pre-application assessment noted visualization of the joint capsule of the fourth

metatarsophalangeal joint distally, active granulation tissue centrally, and a subcutaneous tunnel extending proximally toward the middle aspect of the cuboid, partially exposing the fourth tarsometatarsal joint (Figure 2A). Following the second ActiGraft application, the patient was discharged, and weekly applications were continued in an outpatient setting. The wound required 16 weekly applications to reach the endpoint of complete closure (Figure 2B).

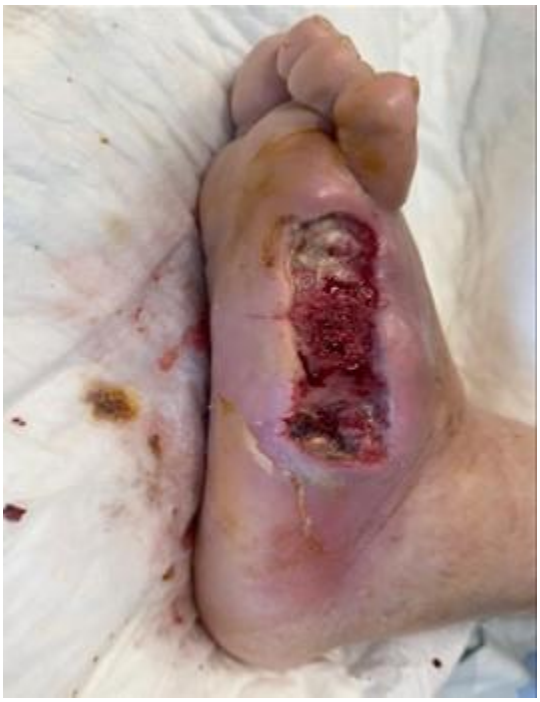

Figure 2A: Initial presentation, ovoid wound measuring 9 cm × 3 cm. At the distal aspect of the wound, observe the capsule of the fourth metatarsophalangeal joint; the proximal aspect of the lesion depicts the entrance to a subcutaneous tunnel extending proximally toward the middle aspect of the cuboid, partially exposing the fourth tarsometatarsal joint.

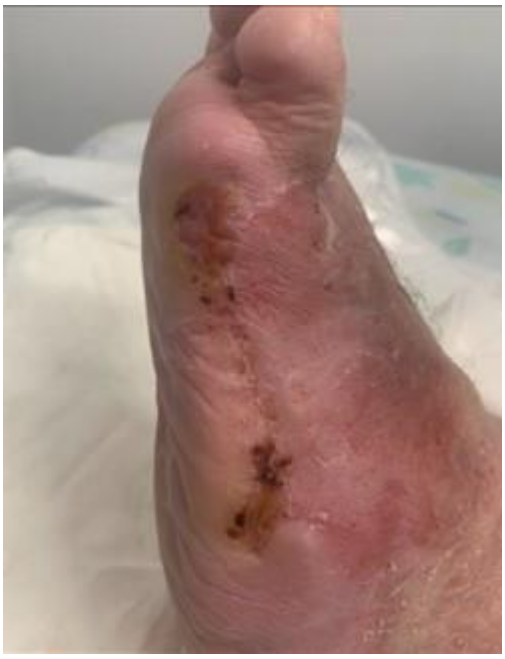

Figure 2B: Note the full restoration of tissue integrity.

4.3. Case 3

A 57-year-old male with a history of type II diabetes mellitus, dyslipidemia, coronary artery diseases (status post LAD stenting), and prior recurrent cerebrovascular events resulting in residual left-sided hemiparesis, presented as an active smoker with advanced diabetic foot disease. The patient's orthopedic history included a prior first toe amputation on the right side and a triple ray amputation (rays three, four, and five) on the left side, nine months preceding the current admission.

The patient was admitted exhibiting purulent discharge emanating from the second toe of the left foot, complicated by overriding of the hallux. Radiographic evaluation revealed complete osteolysis of the second toe, necessitating a transmetatarsal Lisfranc amputation, followed by stabilization of the infectious process with systemic antibiotics. Addressing the critical underlying vascular compromise, the patient underwent a diagnostic and therapeutic angiography involving Rotarex endarterectomy and balloon angioplasty across the entire length of the left superficial femoral artery, thereby achieving continuous arterial flow and optimizing the potential for subsequent wound healing.

Despite revascularization, the transmetatarsal amputation site subsequently developed necrosis, requiring further surgical debridement followed by the application of negative pressure wound therapy. After a five-week period of unsatisfactory healing progression, the wound, which was by then fully granulated (Figure 3A), was treated with the ActiGraft autologous blood clot product for one week; the patient's total hospitalization duration was six weeks.

Following the initiation of the autologous blood clot dressings, five additional outpatient applications on a weekly basis were sufficient to effect complete wound closure (Figure 3B).

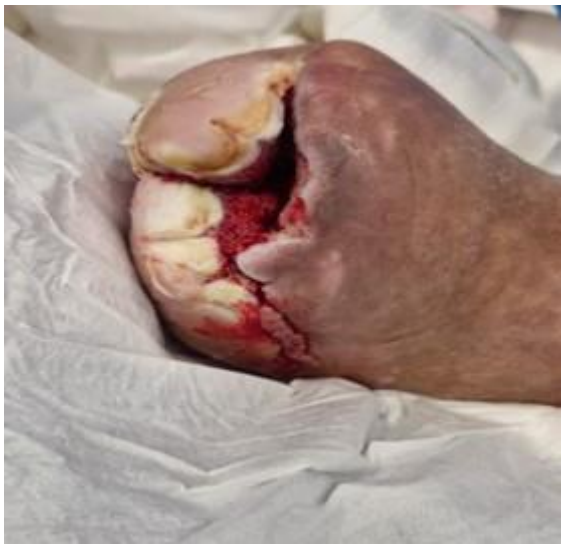

Figure 3A: Post-operative assessment following the Lisfranc amputation revealed wound dehiscence with significant peripheral maceration and centralized tunnelling within the wound bed.

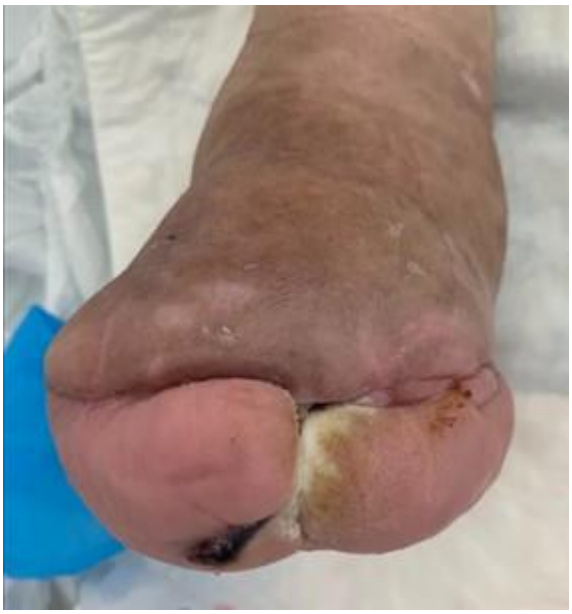

Figure 3B: Full closure of the tissue defect.

4.4. Case 4

A 63-year-old female with a long-standing history of type II diabetes mellitus and associated peripheral neuropathy presented to the emergency department with a persistent one-month infection of the second toe of the left foot. Despite a prior 10-day course of outpatient antibiotic therapy, the condition had failed to resolve.

Clinical examination upon admission revealed a "sausage digit" deformity of the second toe, characterized by significant edema, surrounding erythema, and a punctate ulcer. Although radiographic imaging showed no evidence of osteolytic changes, the failure of conservative management necessitated hospitalization for intravenous antibiotic therapy and second ray amputation (Figure 4A).

Vascular surgery consultation confirmed the absence of acute ischemia, and no urgent surgical revascularization was required. Six days post-amputation, the patient initiated regenerative therapy using an autologous blood clot and was subsequently transitioned to ambulatory care.

Following 11 cycles of this autologous blood clot therapy, complete epithelialization of the surgical site was successfully achieved (Figure 4B).

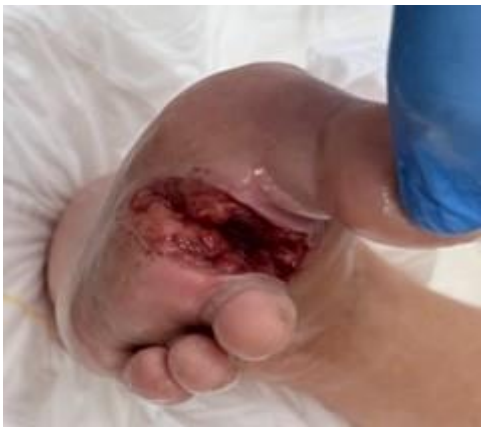

Figure 4A: Open wound post ray 2 amputation.

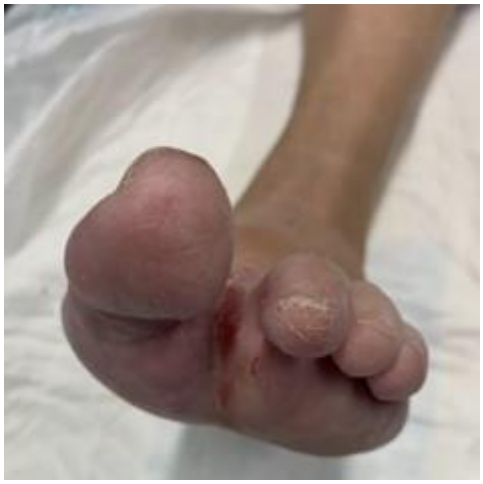

Figure 4B: Total re-epithelialization after 11 applications.

4.5. Case 5

A 52-year-old female with a medical history of type II diabetes mellitus and hyperlipidemia presented to the emergency department with a 10-day history of escalating left foot pain and systemic fever. Despite a prior course of outpatient antibiotic therapy for presumed cellulitis, her clinical status deteriorated, leading to an inability to bear weight.

Physical examination upon admission revealed significant edema, erythema, and localized hyperthermia of the left foot, accompanied by dorsal bullae and a 0.5 cm ulcer with purulent discharge overlying the third and fourth metatarsal heads. While radiographic imaging showed no evidence of acute osteolysis or osteomyelitis, the presence of subcutaneous gas near the metatarsal heads was noted. Laboratory investigations confirmed a severe systemic inflammatory response.

On the day of admission, the patient underwent urgent surgical debridement of the diabetic ulcers. Due to a lack of clinical and biochemical improvement, a second ray amputation was performed three days later. Persistent infection necessitated further surgical intervention, including first and third ray amputations with additional debridement. To address underlying peripheral artery disease, the patient underwent successful revascularization *via* balloon angioplasty.

Management included targeted intravenous antibiotic therapy based on culture sensitivities. Following a four-week hospitalization, the therapy was initiated using an autologous blood clot and the patient was transitioned to ambulatory care. A 95 % reduction in surface area over the course of 18 applications was achieved (Figure 5A,5B).

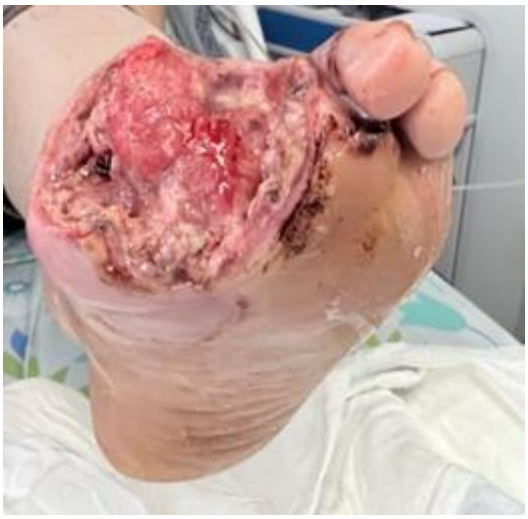

Figure 5A: Note extensive tissue exposure with visible osseous structures following amputations of the first through third digits.

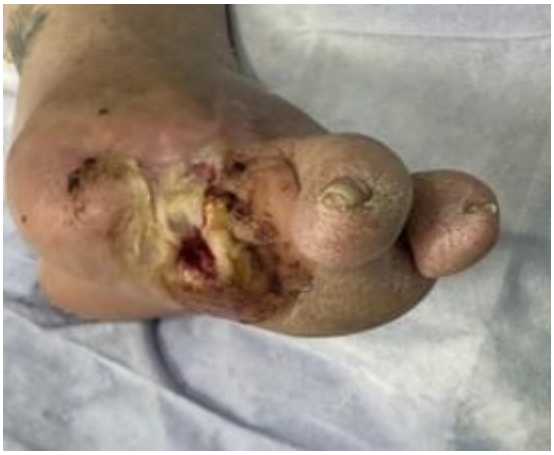

Figure 5B: Following a total of 18 sequential clinical applications, the extensive dorsal defect demonstrated remarkable epithelial regeneration, resulting in near-complete closure with a greater than 95% reduction in wound surface area.

4.6. Case 6

A 58-year-old female, with a significant medical history of type II diabetes mellitus, hypertension, and hyperlipidemia, presented following a traumatic fall involving the right ankle and forefoot. Clinical evaluation two weeks post-injury revealed diffuse edema, erythema extending to the hindfoot, and local hyperthermia.

Physical examination of the right second digit identified a wound at the distal-middle phalanx with serosanguinous discharge. Radiographic imaging confirmed extensive osteolytic changes involving the entire phalanx, consistent with acute osteomyelitis. Consequently, the patient underwent a second ray amputation on the right foot. Targeted antimicrobial therapy was initiated following the procedure.

After an unremarkable immediate postoperative course, the treatment regimen transitioned to regenerative wound care utilizing autologous blood clot technology. Following nine treatment cycles, the surgical site demonstrated complete epithelialization, characterized by the total absence of malodor or clinical signs of residual infection (Figure 6A,6B).

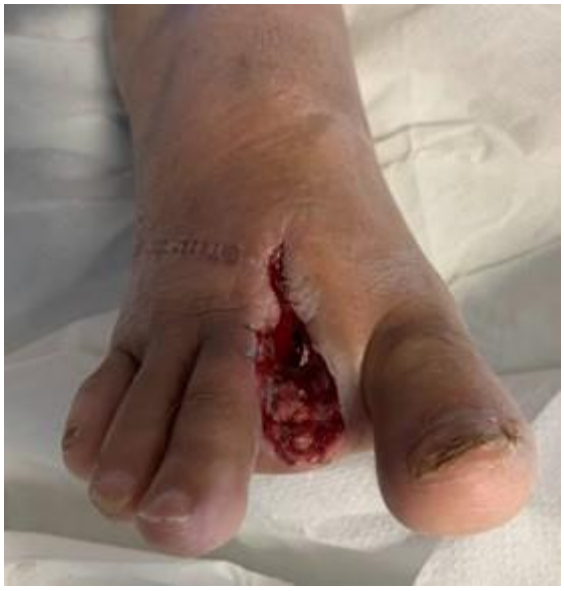

Figure 6A: Surgical defect secondary to second ray amputation.

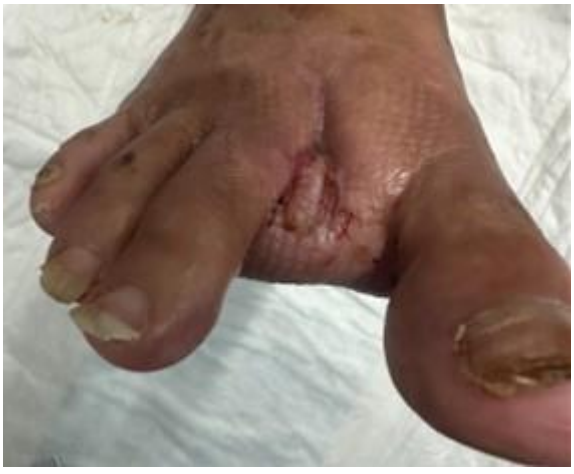

Figure 6B: Full integumentary integrity was re-established following nine clinical applications.

4.7. Case 7

A 56-year-old male with a complex medical history including poorly controlled type II diabetes, diabetic neuropathy, peripheral vascular disease, and an aortic aneurysm, presented to the emergency department with a two-month history of a non-healing wound on the fourth toe of his left foot. This wound had been previously treated at a community clinic without improvement and had progressed to a "mega toe" characterized by purulent discharge, surrounding erythema, and spreading cellulitis across the foot.

Radiographic imaging revealed cortical destruction and osteomyelitis affecting middle and proximal phalanx. The patient was managed by a multidisciplinary team, undergoing an initial fourth ray amputation. Despite this, the wound demonstrated no improvement, necessitating multiple subsequent debridements and further amputation of the third and fifth rays due to progression of the infection. During his hospitalization, the patient developed acute left-sided heart failure and acute kidney injury secondary to sepsis.

Multiple rounds of negative pressure therapy were also employed without significant success. The wound ultimately presented as a complex defect with a plantar circular ulcer approximately 2 cm in diameter and a large dorsal triangular wound measuring 10 cm × 9 cm × 11 cm, connected by a sinus tract. Targeted antibiotic therapy was administered based on bacterial culture sensitivities, and the patient underwent a therapeutic vascular catheterization.

Following extensive multidisciplinary discussions, below-the-knee amputation was considered as the next course of action due to the persistent non-healing nature of the wound and the patient's deteriorating condition. However, a decision was made to attempt limb salvage using blood clot therapy. Following 20 weeks of this novel treatment, the plantar wound achieved complete closure with full epithelialization. After a total of 23 treatment applications, the extensive dorsal wound demonstrated active epithelialization and a significant reduction in size, now measuring 2 cm × 2 cm × 1.5 cm (Figure 7A-7D).

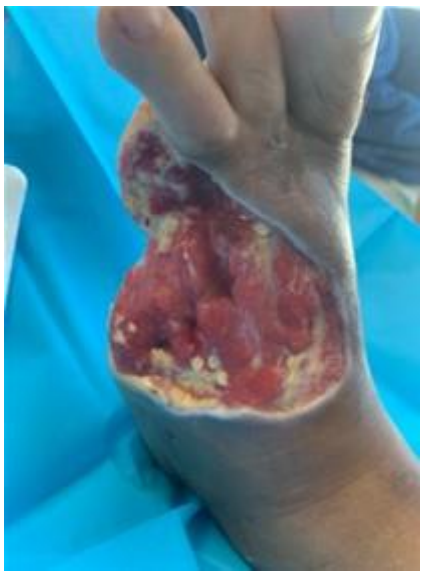

Figure 7A: Extensive dorsal triangular defect, measuring a formidable 10 cm × 9 cm × 11 cm.

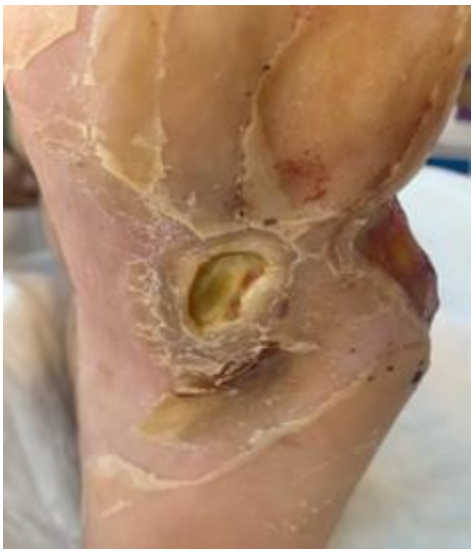

Figure 7B: A 2 cm circular ulcer compromises the plantar surface, presenting as a deep focal defect with frank exposure of the plantar fascia.

Figure 7C: Complete cutaneous restoration and definitive closure of the plantar wound were successfully attained following a 20-week therapeutic course.

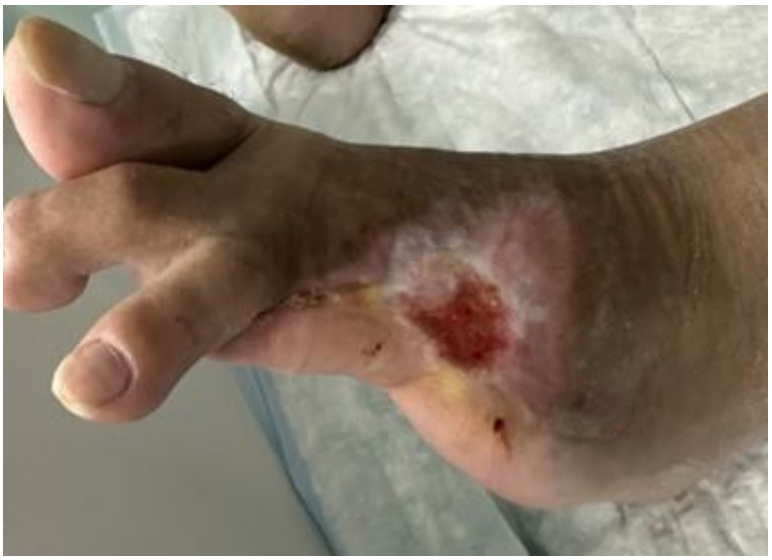

Figure 7D: Following a total of 23 clinical applications, the extensive dorsal defect exhibited robust epithelial proliferation achieving a greater than 95% reduction in size.

5. DISCUSSION AND CONCLUSION

DFUs are particularly challenging to manage due to the convergence of multiple risk factors, including infection, repetitive trauma, peripheral neuropathy, peripheral arterial disease, inadequate hygiene, and malnutrition [2]. Current standards of care for DFU management include wound dressings, debridement, pressure off-loading, vascular assessment, glycemic optimization, and rigorous infection control [8].

Both conventional and advanced wound dressings are designed to facilitate healing by maintaining a moist wound environment and managing exudate, while debridement, whether autolytic or surgical, aims to remove non-viable tissue and restore a wound milieu conducive to regeneration [5,9]. Importantly, within the DFU management paradigm, time represents a critical and independent prognostic variable. Prolonged periods of wound non-response or stasis are strongly associated with adverse outcomes, including progressive tissue necrosis and a significantly increased risk of limb loss [2]. This relationship underscores the urgent need for timely, aggressive, and effective therapeutic interventions to arrest pathological progression. Nevertheless, owing to the complex and multifactorial pathophysiology of DFUs, achieving durable wound closure and consistent, measurable wound size reduction remains a formidable clinical challenge.

Successful preservation of the affected extremity in patients with DFUs necessitates the implementation of an effective and biologically targeted therapeutic strategy that both initiates the wound-healing cascade and establishes a localized environment conducive to sustained re-epithelialization. Accordingly, optimal DFU management requires interventions that restore homeostasis within the wound microenvironment, correct underlying cellular dysfunction, and modulate the chronic pro-inflammatory state that perpetuates impaired healing and tissue breakdown [1].

Within this framework, ActiGraft (RedDress Ltd., Pardes-Hanna, Israel) represents a novel Autologous Whole Blood Clot (AWBC) therapy designed as a point-of-care, bedside treatment generated from the patient's own peripheral blood without the need for specialized equipment or advanced medical expertise [7]. This autologous

provisional wound matrix comprises viable endogenous cells and is inherently biocompatible, functioning as a metabolically active stromal scaffold that closely mimics the properties of the Native Extracellular Matrix (ECM) [1]. By providing a provisional fibrin-rich matrix, ActiGraft facilitates the transition of chronic, non-healing wounds into an acute healing phenotype, while simultaneously creating a protected microenvironment that enables endogenous repair processes to proceed in an organized and regulated manner [10].

Moreover, the scaffold promotes fibroblast recruitment, cellular infiltration, granulation tissue formation, angiogenesis, and subsequent tissue remodeling, thereby supporting progression from the inflammatory to the proliferative phase of wound healing [11]. In addition to its structural role, the autologous clot contributes to wound-bed optimization by maintaining appropriate moisture balance, reducing local pH toward an optimal physiological range, and delivering endogenous growth factors that stimulate keratinocyte and fibroblast migration, proliferation, collagen synthesis, and ECM turnover [12]. The AWBC also functions as a temporary ECM and physical barrier, protecting the wound from pathogen ingress, assisting autolytic debridement, and promoting rapid wound size reduction and durable closure [13]. Emerging evidence further suggests that AWBC therapy may facilitate macrophage phenotype transition toward a pro-regenerative profile, thereby supporting resolution of inflammation and tissue repair [7].

Clinical data demonstrate that autologous whole blood clot therapy is associated with accelerated healing times and superior outcomes compared with current accepted standard of care alone, highlighting its potential as a transformative approach for the management of hard-to-heal DFUs [1]. Kushner et al. [14], in their pilot study, reported that patients with various etiologies for their chronic wounds were effectively and safely treated with an autologous whole blood wound matrix [14]. Vallejo et al. [15], reported wound size reduction of 70% after two applications, 97.6% after three applications, and 90.9% after four applications [15]. Snyder et al. [1], reported that this innovative resulted in shorter healing time [1]. Snyder et al. also found that diabetic foot ulcers could be safely and effectively treated with an autologous blood clot product [11]. These case studies substantiate the clinical utility of ActiGraft for the management of complex, chronic wounds, with the overarching goal of providing safe and effective wound healing treatment, thereby substantially ameliorating the patient's health-related quality of life.

Safety is a critical feature, as the incorporation of the patient's autologous blood inherently minimized concerns regarding possible adverse immunological reactions or systemic complications. Furthermore, ActiGraft can be safely deployed across all anatomical sites, irrespective of the underlying presence of exposed osseous structures, tendons, or adjacent neurovascular bundles. Operationally, the feasibility of outpatient deployment further positions ActiGraft as a cost-effective solution that improves clinical outcomes while significantly reducing the healthcare burden.

The findings provide important preliminary evidence supporting the biological and clinical rationale of this intervention and justify its further evaluation. Collectively, these findings support the clinical utility of ActiGraft as a safe, effective, and biologically integrated therapy for complex diabetic wounds, with the overarching goal of enhancing limb preservation and improving patient quality of life [7]. The use of this product constitutes a meaningful shift from current standard treatment strategies for ulcer management.

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