Actigraft™ Topically Applied Blood Clot Therapy

With the results of this study, it is anticipated that wound healing over bone and tendon in patients with DFUs will improve.

BY ROBERT J. SNYDER, DPM, MSC, MBA

Introduction

Actigraft™ is a unique topically applied blood clot therapy supported by evidence of efficacy in treating foot ulcers in patients with diabetes. Current research supports improved wound healing in full thickness ulcerations. However, there is potential for many other innovations. The problem in investigating these advances is the feasibility of study design and funding. Many researchers are often stifled by the process of applying to an agency such as the National Institutes of Health (NIH) to procure resources.

Foot ulcers in patients with diabetes (DFUs) represent a marker for death and are as serious as some cancers.

The following example outlines a template and an example of how an application could be organized and prepared. It is hoped that these insights will make a rather daunting process less onerous.

Rationale

Foot ulcers in patients with diabetes (DFUs) represent a marker for death and are as serious as some cancers.[1] There are many advanced therapies which may aid in wound healing, however, most evidence involves full-thickness wounds and there remains a paucity of research surrounding ulcers where bone and tendon are exposed. This represents an unmet medical need, especially in the outpatient setting. Exposure of these vital structures is common yet is a serious complication that could lead to osteomyelitis, sepsis, limb loss and death; the need for additional studies remains critical. Therefore, the global aim is to explore a potential therapy that could accelerate wound healing while expediting coverage of tendon and bone.

Point of Care autologous blood clot tissue is a safe unlimited resource that has been shown to promote epithelization, and mediate cell migration, while augmenting the extracellular matrix, fostering growth factor production, angiogenesis, and collagen synthesis.[2,3] Furthermore, this therapy could prevent infection via macrophages and potentially stimulate pluripotent stem cell recruitment.[3] A multi-center, prospective randomized controlled trial will be undertaken to determine if the application of topically applied autologous blood clot tissue accelerates wound healing and the coverage of vital structures in patients with diabetes.

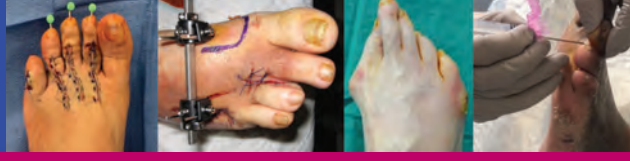
Specific Aim 1: Assess the predictive value of a 4-week surrogate endpoint to determine successful coverage of bone and tendon of wounds in patients with diabetes (DFU) utilizing topical autologous blood clot tissue.

Snyder, et al.[4] posit that wound healing can be predicted by assessing a 4-week surrogate endpoint. In this study, data was dichotomized by percent area reduction (PAR) of $< 50\%$ or $\geq 50\%$ by week 4 to assess the association of PAR with DFU closure by 12 weeks. If the ulcer failed to reach 50% within this time frame, the chances of healing within two groups were 2% (n = 133) and 5% (n = 117), respectively ($P <= 0.001$).[4] Our research seeks to determine if bone and tendon will be covered at the

Continued on page 82

Clinical Innovations

Clinical Innovations is PM's ongoing series of articles dedicated to introducing new concepts, technologies and studies to the podiatric community. Readers should be aware that Podiatry Management does not specifically endorse any of the technologies, concepts, or products being discussed.



surrogate endpoint as outlined. The hypothesis proposes that in patients with diabetes suffering from foot ulcers where bone and tendon are exposed, the adjunctive utilization of topical blood clot tissue will facilitate coverage of these vital structures within four weeks.

Specific Aim 2: Distinguish whether foot wounds in patients with diabetes will completely re-epithelize by week 12 utilizing topical blood clot tissue.

Of the 15 required endpoints including pain reduction, physical function, infection control and percent area reduction—the U.S. Food and Drug administration (FDA) views complete re-epithelization/time to heal as the primary outcome to receive approval.[5] Warriner, et al.[6] analyzed 120 patients that reached the threshold of at least 50% PAR at week 4 yet failed to heal by week 12. The study concluded that wounds that failed to progress or that worsen between weeks 4 to 6 ($p = 0.001$) and those that failed to achieve 90% PAR by week 8 ($p = 0.001$) remained unlikely to heal by week 12.[6] Our research hypothesizes that DFUs treated adjunctively with topically applied autologous blood clot tissue will re-epithelialize in this expected time frame.

Specific Aim 3: Determine the durability of ulcer healing.

Armstrong, et al. posit an approximate 40 % ulcer recurrence rate within one year.[10] The FDA is, therefore, keenly interested in the durability of ulcer healing, particularly in those patients where the study vehicle was utilized. Delphi studies of clinicians and patient surveys reveal the overwhelming importance of reducing the chance of wound recurrence.[7] It is our hypothesis that DFU healing will be maintained 3 and 6 months after complete re-epithelialization.

Background and Significance

Problem

There are 34.2 million patients with diabetes in the United States representing 10.5% of the population[7], approaching healthcare costs of $15 billion.[8] Foot ulcers are one of the most common complications with an annual incidence of approximately 4% and a lifetime risk approaching 34%.[9,11] Fifteen percent of diabetic foot ulcers result in lower extremity amputations.[10] Although peripheral neuropathy is the catalyst for ulceration, peripheral vascular disease and infection are the elements that lead to sepsis, limb loss, and death.[10] In 2016, the Centers for Disease Control (CDC) reported "130,000 lower extremity amputations: 5.6 per 1000 adults with diabetes".[11]

Complex ulcerations with exposed tendon and bone pose the greatest risk of osteomyelitis; more than half of these ulcerations become infected.[12] However, most studies regarding ulcer healing in this group include full thickness wounds with a paucity of evidence surrounding complex ulcerations. Performing innovative randomized controlled clinical trials on complex DFUs represents an unmet medical need, especially in the outpatient clinical setting. For example, research could postulate that complex DFUs treated adjunctively with topically applied autologous blood clot tissue could completely re-epithelialize by week 12.

What We Know

Osteomyelitis is of great significance in DFUs and could lead to catastrophic consequences. Compared with non-diabetics, diabetics are four times more likely to develop bone infection and have a two-fold risk of sepsis and death linked to these infections.[13] Unfortunately, due to the immunosuppressed state of patients with this disease, clinical signs and symptoms of infection are often absent and early radiological tests are negative.[14] Grayson, et al. analyzed the use of a sterile probe (Probe-to-Bone Test) through the ulcer on 75 hospitalized patients to determine if bone could be elicited. They posited an 89% positive predictive value and a 56% negative predictive value for osteomyelitis.[15] However, Lavery, et al. conducted a similar study on 247 patients in an outpatient clinical setting and noted contradictory results with a 57% positive predictive value and a 98% negative predictive value.[16]

Complex ulcerations with exposed tendon and bone pose the greatest risk of osteomyelitis; more than half of these ulcerations become infected.

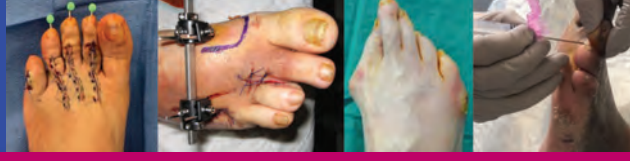
This dichotomy raises the level of suspicion for bone infection; therefore, the probe to bone test may need to be augmented with clinical, radiologic (i.e.: serial x-rays, MRI), and laboratory data including bone biopsy. To forestall escalation to infection and the need for invasive procedures, coverage of bone in the outpatient setting would be prudent, however evidence is lacking as to the efficacy and effectiveness of topically applied matrices.

Studies Involving Matrices that Could Adjunctively Cover Bone and Tendon:

In 2010, a study by Clerici, et al. "analyzed 30 patients who underwent dermal regeneration template grafting to cover exposed bone status post extensive surgical debridement. This was followed by a skin graft leading to complete wound closure of 86.7%".[17] However, this valiant attempt to cover bone with a topical matrix required an operating room intervention and would not be practical in the outpatient setting.

In 2017, Frykberg, et al. performed a prospective, multicenter, open-label, single arm trial of a cryopreserved placental membrane in DFUs with exposed bone and/or tendon (ITT: 31; PP: 27). "96.3% of patients achieved 100% granulation at 16 weeks with a mean DFU reduction

Continued on page 84



of 92.3% during the same time frame, and 59.3% achieved complete closure".[18] Although this study was promising, there was no control, the small sample size was under-powered, and results could fall prey to selection bias.

Fortunately, there are several innovative matrices currently under investigation that could aid in the coverage of bone and tendon, representing a potential paradigm shift. One such trial is a phase III double-blind study involving a hydrogel sheet containing allogenic adipose delivered mesenchymal stem cells vs. a hydrogel sheet without these cells in patients with Diabetic Wagner Grade 2—foot ulcers (Identifier: NCT04569409).[19] We are currently participating in an observational clinical Registry (Identifier: NCT04699305) evaluating topical autologous blood clot therapy in complex DFUs.[20] These innovations could make a substantial long-term impact on quality of life and amputation prevention.

TABLE 1

Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
Wound is present for at least 4 weeks but no longer than 2 years.	Signs of clinically significant infection, active osteomyelitis, or malignancy at the target ulcer
Target wound surface area is in the range of 2-30 cm^2 (calculated length x width) and has bone and/or tendon exposed **(Wagner Grade 2; ulcer extends to ligament, tendon, joint capsule and bone with no abscess or osteomyelitis)**.[23]	A significant decrease in the arterial blood flow of the target extremity (Ankle Brachial Index < 0.7 mmHg or monophasic Doppler wave forms of major foot vessels)
Patient understands the nature of the procedure, can adhere to the protocol regimen, and provides a signed informed consent form prior to any study procedure.	Patients undergoing hemodialysis or peritoneal dialysis
Hba1c< 12 %	Patients with poor nutritional status (low Pre-albumin) or poorly controlled Diabetes Mellitus (HbA1c > 12%)

Feasibility and Preliminary Data

Three sites are planned and will be overseen by key opinion leaders in the wound research field with significant clinical trial experience and vast databases from which to evaluate the feasibility of screening patients. As Lead Principal Investigator, this physician researcher has performed over 50 randomized controlled trials and is assisted by a seasoned certified Research Coordinator, a research assistant and two well-trained sub-investigators. Collaboration with referral sources (i.e.,primary care physicians, podiatrists, vascular surgeons) and other research professionals remains pivotal to success. Therefore, although always challenging, recruitment should not be problematic. However, if an unanticipated and significant drop-out rate occurs (for example), more sites and additional patients may have to be considered.

Kushnir, et al. performed a pilot study on seven patients with multiple etiologies utilizing 35 autologous blood clot matrices and gleaned a 78% healing rate with no adverse effects.[21]

Our group performed a 12-week open-label, perspective, multicenter clinical trial on 20 patients utilizing topically applied autologous blood clot matrix on full thickness DFUs.

This analysis revealed 65% healing in the Intent to Treat (ITT) group vs. 72% in the Per Protocol (PP) group.

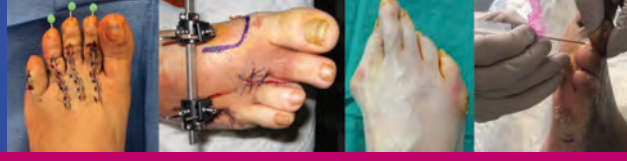
This analysis revealed 65% healing in the Intent to Treat (ITT) group vs. 72% in the Per Protocol (PP) group.

This pilot study posits that the treatment modality is safe and efficacious in treating foot ulcers in patients with diabetes.

The PAR at 4-weeks in the ITT group was 61.6 % vs. 60.3% in the PP group. A Kaplan-Meier analysis of the ITT group revealed a mean healing time of 59 days (95% CI, 48.3-69.3) vs. the PP of 56 days (95% CI, 45.1-66.9). Mean Adverse Events (AE) for the ITT and PP groups were 1.6 (SD, 1.50; 95% CI, 0.90-2.30) and 1.7 (SD, 1.53; 95% CI, 0.90-2.43), respectively.

This pilot study posits that the treatment modality is safe and efficacious in treating foot ulcers in patients

Continued on page 85



with diabetes.[22] However, these cohorts were small with limited power and it may be difficult to extrapolate these results to more complex wounds with exposure of vital structures. Furthermore, no control was utilized in the previous studies potentially leading to selection bias. Our new proposal will address therapeutic efficacy of this treatment in more deleterious circumstances and will include a standard of care control arm.

Research Design and Methodology

Study design: We propose to conduct a multicenter, prospective randomized controlled trial in three stages.

(Specific Aim 1): To posit a 4-week surrogate endpoint phase to assess the cohort of DFU patients that exhibit coverage of bone and tendon in the topically applied blood clot tissue group vs. the control group. *(Specific Aim 2):* To analyze the rate of complete wound closure (Time to Heal) in DFU patients at the end of 12 weeks. *(Specific Aim 3):* To evaluate the durability of DFU closure at 3 and 6 months. See Inclusion and Exclusion above (Table 1).

Study Protocol: (Chart 1)

Screening: Initial screening will include informed consent, a complete history and physical and vascular evaluation (ABI; Hand-held Doppler) CMP, CBC, Blood glucose and HbA1c, foot x-ray and wound measurements utilizing eKare 3D digital imaging (eKare, Inc, Fairfax, VA 22031).

2-week Run-in Period: Each patient will be treated with standard of care (Debridement, offloading with boot, and moist wound healing) for 2-weeks. Those who are deemed noncompliant or reach 30% PAR will be excluded from the trial.

4-week Surrogate Endpoint Phase: Patients will be randomized to either the topically applied autologous blood clot tissue arm or standard of care with moist wound healing. In each case, offloading and debridement will be continued, and the patient will be evaluated weekly including eKare imaging.

12-week Endpoint Phase: Patients that have not exhibited complete healing will be followed as per randomization until healed or at the end of week 12. Those who reach complete re-epithelization will immediately move to the durability phase.

Durability Phase: Patients who have completely healed will continue offloading and will be followed at 3 months and 6 months to assess recurrence

Study population: Since approximately 34% of all patients with diabetes will develop a foot ulcer in their lifetime,[10] patients can be gleaned from the databases of each research site as well as referrals from other clinicians. The study will include 88 consecutive patients with diabetes (44 in each group; 1:1); ages 18 and older and from any gender. The trial will commence on January 1, 2022 and end on July 31, 2023 (Table 1).

A power analysis was conducted using G*Power 3.1.9.6 to identify the required sample size for this study.

Statistical Approach and Power Calculations

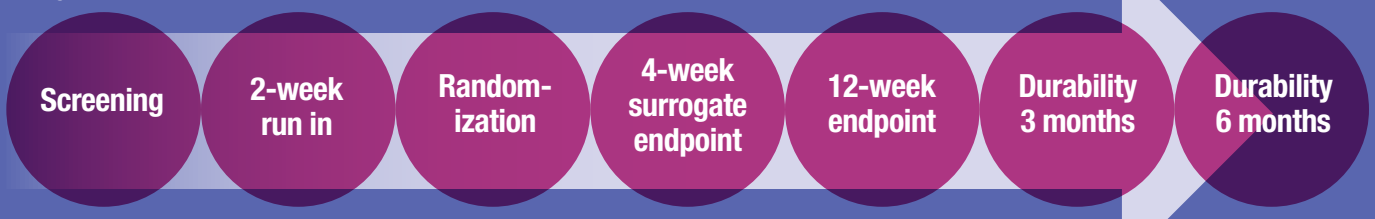
A power analysis was conducted using G*Power 3.1.9.6 to identify the required sample size for this study. The anticipated effect size was sourced from preliminary data on topical autologous blood clot tissue effectiveness. The odds ratio for achieving the primary endpoint of 50% or greater PAR by week 4 is 2.84, indicating that those who are treated with this therapy are 2.84 times as likely to achieve the endpoint of 50% or greater PAR. According to Chen, et al., "this is midway between a small and medium effect size".[24] A significance level of $P <= 0.05$ was selected.

This study will evaluate percent area reduction as a continuous measure using ANCOVA with baseline wound size as the covariate. Secondary outcomes will include the proxy measure of complete healing as identified by 50% or more PAR by week 4. Given the effect size identified in preliminary data, to evaluate the primary outcome with an alpha of .05 and power of .9, the minimum required sample size is 88 (44 per group). To account for loss to follow-up and loss of fidelity, the sample size could be inflated by 5%, resulting in a need for 92 patients.

A Kaplan-Meier Curve between two groups with con-

Continued on page 86

CHART 1



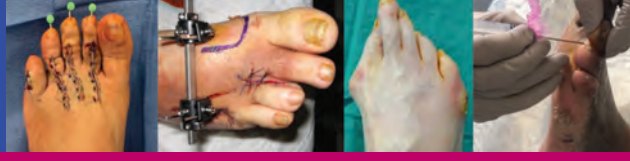


CHART 2

tinuous outcome variables will create a visual representation of treatment effectiveness utilizing the Log-rank test and the Mantel-Haenszel formula. Linear regression will be performed regarding age, gender, and hemoglobin a1C to analyze the potential effects these covariates may have on the model to determine if they represent co-founders. STATA v16 will be utilized to analyze the data.

Limitations, Anticipated Results and Timeline

One of the potential challenges is sample size. Although this has been accommodated for in the statistical model, the drop-out rate may be more significant than anticipated, particularly in the durability phase. Patient compliance/concordance could be augmented with reimbursement for transportation. Additionally, this therapy is contraindicated in patients with infections, active osteomyelitis, and skin cancers; therefore, results may not be generalizable.

With the results of this study, it is anticipated that wound healing over bone and tendon in patients with DFUs will improve.

Due to concerns relating to COVID-19, patients may still be reluctant to visit clinicians in their offices.[25] Therefore, enrollment goals may not be met, necessitating extension of the study. Mitigation may also require additional sites.

Furthermore, there may be additional co-founders that remain unidentified such as blood pressure, dyslipidemia, and BMI requiring additional analysis. Due to topical application of an autologous blood clot with the need for blood draws in patients only randomized to the vehicle arm, blinding is not practical. Nonetheless, with the results of this study, it is anticipated that wound healing over bone and tendon in patients with DFUs will improve. This will fulfill a cost-effective unmet medical need particularly in the clinic environment.[26] A manuscript of findings and analysis utilizing the CONSORT design may help facilitate a change in clinical practice, increase knowledge in the field, and foster the betterment of public health. (See timeline Chart 2). **PM**

References

[1] Armstrong DG. Wrobel J, Robbins JM. Guest editorial: are diabetes-related wounds and amputations worse than cancer? International Wound Journal. 2007; 4(4): 286-287.

[2] Emre O, Chinenye WM. The use of Actigraft, an autologous skin graft, in the treatment of complex diabetic foot ulcers: a case study. Annals of Reviews and Research. 2021;6(2): 555.

[3] Snyder RJ, Schultz G, Wachuku C, Rashid AN, Ead, JKK. Proposed mechanism of action of topically applied autologous blood clot tissue: a quintessential cellular and tissue-based therapy. Journal of the American Podiatric Medical Association. 2020; 13: 20: 140.

[4] Snyder RJ, Cardinal M, Dauphinee DM, Stavosky. A post-hoc analysis of reduction in diabetic foot ulcer size at 4 weeks as a predictor of healing by 12 weeks. Ostomy Wound Management. 2010;56(3): 44-50.

[5] Driver VR, Gould LJ, Dotson P, Allen LL, Carter MJ, Bolton LL. Evidence supporting wound care end points relevant to clinical practice and patients' lives. part 2. literature survey. Wound Repair and Regeneration. 2018; 1-10.

[6] Warriner RA, Snyder RJ, Cardinal MH. Differentiating diabetic foot ulcers that are unlikely to heal by 12-week following achieving 50% percent area reduction at 4 weeks. International Wound Journal. 2011;8(6): 632-637.

[7] Gould LJ, Liu J, Wan R, Carter MJ, Dotson M, Driver VR. Evidence supporting wound care end points relevant to clinical practice and patients' lives. Part 1. Wound Repair and Regeneration. 2020;1-10.

[8] Rice JB, Desai U, Cummings AKG, Birnbaum HG, Konicki M, Parsons NB. Durden of diabetic foot ulcers for Medicare and private insurers. Diabetes Care. 2014;37(9): 651-658.

[9] Centers for Disease Control and Prevention. National Diabetes Statistics Report, 2020. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Dept of Health and Human Services; 2020.

[10] Husers J, Hafer G, Heggermann J, Wiemeyer S, John SM, Hubner U. Predicting the amputation risk for patients with diabetic foot ulcerations-a Bayesian decision support tool. BMC Medical Informatics and Decision Making. 2020;20: 200.

[11] American Diabetes Association. Classification and diagnosis of diabetes. Diabetes Care. 2019 Jan; 42(Supplement 1): S13-S28

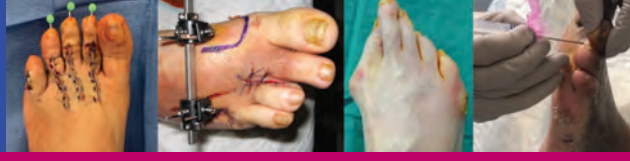
[12] Armstrong DG, Boulton, AJM, Bus SA. Diabetic foot ulcers and their recurrence. New England Journal of Medicine. 2017;376: 24.

[13] Shank CF, Feibel JB. Osteomyelitis in the diabetic foot: diagnosis and management. Foot and Ankle Clinics. 2006;11(4): 775-89.

[14] Lipsky BA, Berendr AR, Cornia PB, Pile JC, Peters EJG, et al. Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clinical Infectious Disease. 2012, 54(12): 132-173.

[15] Grayson ML, Gibbons GW, Balough K, Levin E, Karchmer

Continued on page 88



AW. Probing to bone in infected pedal ulcers. A clinical sign of underlying osteomyelitis in diabetic patients. Journal of the American Medical Association. 1995; 273(9): 721-3.

[16] Lavery LA, Armstrong DG, Peters EJ, Lipsky BA. Probe-to-bone test for diagnosing diabetic foot osteomyelitis: reliable or relic? Diabetes Care. 2007;30(2): 270-274.

[17] Clerici G, Caminiti M, Curci V, Quarantiello A, Faglia E. The use of a dermal substitute to preserve maximal foot length in diabetic foot wounds with tendon and bone exposure following urgent surgical debridement for acute infection.2010;7(3): 176-183.

[18] Frykberg RG, Gibbons GW, Walters JL, Wukick DK, Milstein FC. A prospective, multicenter, open label, single arm clinical trial for treatment of chronic complex diabetic foot wounds with exposed tendon and/or bone: positive clinical outcomes of viable cryopreserved human placental membrane. International Wound Journal. 2017;14(3): 569-577.

[19] Clinical study to evaluate efficacy and safety of Allo-ASC-DFU in patients with wagner grade 2 foot ulcers. Clinicaltrials.gov: Identifier NCT04569409.

[20] An observational clinical registry to collect safety and efficacy data on wound care in medical centers. Clinicaltrials.gov: Identifier NCT04699305.

[21] Kushnir I, Kushnir A, Serena TE, Garfinkel D. Efficacy, and safety of a novel autologous wound matrix in the management of complicated, chronic wounds: a pilot study. Wounds. 2016; 28(9): 317-327.

[22] Snyder RJ, Kasper MA, Patel K, Carter MJ, Kushnir I, Kushnir A. Safety, and efficacy of an autologous blood clot product in the management of Texas 1a or 2a neuropathic diabetic foot ulcers: a prospective, multicenter, open label pilot study. Wounds. 2018; 30(7): 205-212.

[23] Wagner FW. The diabetic foot. Orthopedics. 1987. 10(1): 163-172.

[24] Chen H., Cohen P, Chen S. How big is a big odds ratio? Interpreting the magnitudes of odds ratios in epidemiological studies. Communications in Statistics—Simulation and Computation. 2010; 39: 860-864.

[25] Rogers LC, Snyder RJ, Joseph WS. Diabetes-related amputations: a pandemic within a pandemic. Journal of the American Podiatric Medical Association. 2020; 20: 248.

[26] Snyder RJ, Ead K. A comparative analysis of the cost effectiveness of five advanced skin substitutes in the treatment of foot ulcers in patients with diabetes. Annals of Reviews & Research. 2020;6(1): 555678.

Dr. Snyder is Dean, Professor and Director of Clinical Research at Barry University School of Podiatric Medicine. He has served as Principal Investigator on more than 60 randomized clinical trials. In 2018, Dr Snyder was inducted as Faculty Fellow of Podiatric Medicine, Royal College of Physicians and Surgeons (Glasgow).