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The Use of Active Coagulation Whole Blood—An Innovative Treatment Strategy for Hard-To-Heal Wounds

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Abstract

Background: Deep and tunneling wounds are a challenge to apply and maintain most advanced wound dressings to promote effective healing. An autologous whole blood clot is a topical treatment and has been found to be safe and effective in healing cutaneous wounds. The active coagulation whole blood (ACWB) clot treatment, using the patient's own blood, is used to treat deep and tunneling wounds, by mixing the blood with coagulation components and applying it into the wound cavity allowing the clot to re-form inside the wound. We aimed to explore ACWB treatment in hard-to-heal wounds.

Methods: 5 patients with multiple comorbidities, exhibiting surgical abdominal wound, chronic pilonidal sinus, stage 4 sacral pressure ulcer with exposed bone, post-amputation surgical site wound, and non-healing wound dehiscence at the site of a prior hip replacement, were all treated with the ACWB clot treatment.

Results: Complete wound healing was observed in 4/5 cases. In the fifth case, there was a 70% reduction in the depth and surface area of the abdominal surgical wound.

Discussion: The ACWB treatment was found to be effective in deep wounds with cavities and exposed structures. ACWB, in its flowable form, can effectively provide coverage of the deepest interstices of the wound's cavities by virtue of its liquid properties, forming a fibrin matrix, mimicking the role of the extracellular matrix. The flowable formulation of ACWB treatment safely and efficiently provides coverage of the entirety of the wound surface to improve the time and process of complex wound surface healing.

Keywords
acute care surgery, wound healing

Introduction

Wounds that are normally mild and easily curable are known as acute wounds. Acute wounds follow a systematic healing sequence and heal within a 3-to-4-week period.[1] However, when a wound persists after 4 weeks, it is defined as a chronic wound[2] or otherwise known as a hard-to-heal wound.[1] Such wounds may be deep, long-lasting, and may require specialized treatments and care to initiate, facilitate, or complete the healing process. Unlike acute wounds, hard-to-heal wounds fail to progress through one or more of the four different phases of wound healing: homeostasis, inflammation, proliferation, and remodeling or maturation.[3] Furthermore, it is recommended that any hard-to-heal wound that has not healed by 40%-50% after 4 weeks of adequate standard-of-care (SoC) treatment should prompt alternative strategies, often via referral to a wound care specialist or multidisciplinary wound care team.[1]

The most common hard-to-heal wounds are leg ulcers, diabetic foot ulcers (DFUs), pressure ulcers (PUs), and arterial ulcers,[4] but other less common hard-to-heal wounds include neuropathic ulcers, surgically debrided wounds, traumatic wounds, and skin tears.[5] Hard-to-heal wounds cause large financial and social challenges to the patients, adding a huge burden to the healthcare system.[6]

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As such, it is critical to ensure appropriate and timely treatments for those with hard-to-heal wounds, to increase their quality of life and decrease the short- and long-term financial costs not only to the individual but also to the health care system.[6]

Albeit shown to be efficacious in many wounds, often conventional treatment options prove ineffective in healing some chronic,[7] especially deep and tunneling, wounds as well as wounds with exposed structures.[8,9] Those wound characteristics, often irregularly shaped or deep, are challenging to treat, requiring treatment that is not only applied on the surface of the wound, as a sheet-like structure,[8,9] but also has an effect on the deeper layers of the wound. An alternative treatment option with the ability to fill or penetrate the entire wound allowing direct contact with the deep areas of the wound may have a better effect on the healing process of the wound.[9,10] This approach has been shown to achieve greater and faster healing in deep or tunneling wounds, whereas conventional treatments have in many instances not been able to heal smaller areas involving deeper structures.[11]

Autologous whole blood clot therapy is a novel wound treatment that makes use of a patient's own peripheral blood to form a "clot-like tissue."[5] The autologous whole blood clot was found to be safe and effective in the complete healing of cutaneous wounds[11,12] initiating the wound healing process in hard-to-heal wounds. This allows for the wound to progress from the inflammatory phase to the proliferative phase. An autologous whole blood clot is suggested to have a pivotal role in promoting cell granulation and reducing bacteria bioburden.[10] The autologous whole blood clot effect has been suggested to be attributed to its extracellular matrix (ECM) properties, creating homeostasis in the wound area.[11] The clot creates a mechanical barrier preventing bacterial infiltration and movement into the wound and inhibits bacterial proliferation[13] attributing to the wound's infection control. The autologous whole blood clot attracts to the wound necessary growth factors which enhance and accelerate the wound into subsequent phases of the wound healing process.[14] The autologous whole blood clot is a firm matrix placed topically on the wound. It has been hypothesized that deep wounds, canals or tunneling wounds, exposed structures (eg, bone and tendon), and extensive or very large wound surface areas will benefit from the flowable formulation of the clot matrix which provides coverage all interstices of these complex wounds by the liquid followed by clot formation on the entire wound surface regardless of surface terrain. This ability to cover the entire convoluted wound surface is very challenging for many advanced wound dressings currently.

Active coagulation whole blood (ACWB) treatment uses kaolin and calcium gluconate for an ex vivo activation of the blood and applies it to the patient's wound cavity to allow the completion of the activation process. The ACWB is used to treat deep or tunneled wounds and wounds with exposed structures.

In this study, we have explored the efficacy of ACWB treatment in challenging to dress wounds, with cavities and exposed structures.

Materials and Methods

Patients

Patients, 18 years or older, that presented with the following hard-to-heal wounds: surgical abdominal wound, chronic pilonidal sinus, stage 4 pressure ulcer (PU) with exposed bone, post-amputation surgical wound, and non-healing dehiscence hip replacement wound, were invited to participate and signed an informed consent form.

ACWB Clot Application and Procedure

Up to 18 mL of peripheral blood was withdrawn from the patient and collected into sterile Acid Citrate Dextrose Adenine (ACDA) vacuum tubes. The blood was then withdrawn from the tube and placed in an activation mold containing calcium gluconate and kaolin (RedDress, Pardes-Hanna, Israel) for an ex vivo activation of the blood, initiating the coagulation process. The blood was gently mixed with the coagulation agents for 20 seconds and withdrawn using a safety needle to a 20 mL sterile syringe. The needle was discarded and the blood was immediately applied to the cavity of the wound, allowing the blood to form a clot inside the wound, effectively flowing to provide coverage to the entire wound surface regardless of terrain. The clot formation was completed within 8-12 minutes, followed by a wound dressing with a non-adherent pad to protect the area but without need to provide dressing coverage of the entire wound surface (Figure 1). A weekly re-application of the ACWB took place.

Results

Case Study 1

A 63-year-old female with a medical history of obesity, mosaic variegated aneuploidy (MVA), and prior attempts to repair a ventral hernia, was ultimately complicated by enterocutaneous fistulas which had resulted after the treatment of an acute small bowel obstruction, in a previous incisional hernia site. Fascial closure was accomplished with polypropylene (PP) mesh, and the skin/subcutaneous layers were closed primarily.

A superficial wound infection resulted in dehiscence of skin and subcutaneous layers and exposure of the polypropylene mesh and adjacent fascia. The patient was hospitalized to try and control the infection. Advanced dressings were used at this point, including MediHoney

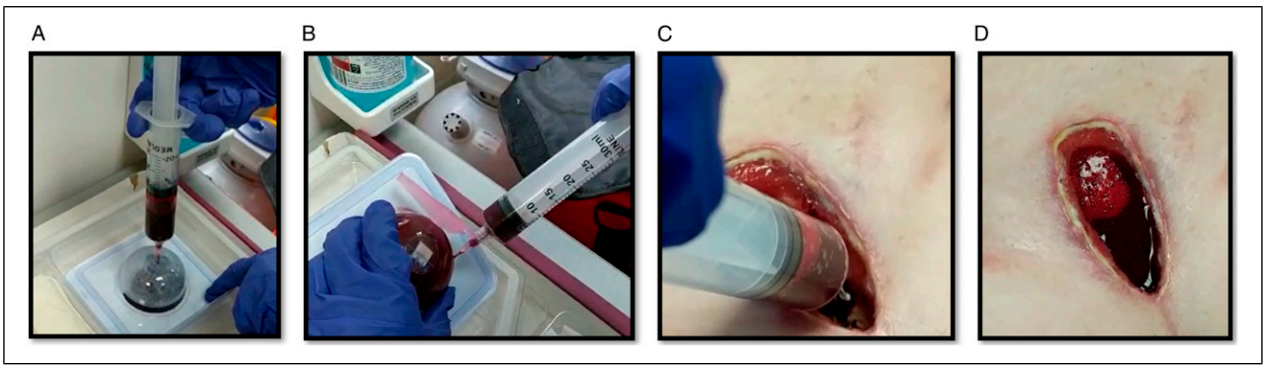

Figure 1. ACWB procedure. (A) Following the blood draw, an ACWB kit was employed, and the collected blood was carefully injected into an activation mold. (B) The blood was then combined with coagulation agents and drawn into a syringe using a safety needle. (C) Subsequently, the safety needle was detached, and the blood mixture was applied into the wound cavity, filling it entirely. (D) Finally, the blood was left to naturally clot inside the wound cavity, facilitating the healing process.

(Derma Science, Princeton, NJ, USA). However, after 4 weeks of treatment, healing was not progressing. The wound size was 20 cm^2 and revealed fully exposed mesh and fascia, which was deemed colonized by bacteria and lacked any granulation tissue. Small intestine loops were visible beneath the mesh due to dehiscence of the fascial closure, and the bowel was coated with fibrin deposits but no evidence of granulation tissue due to chronic inflammation and bacterial colonization of the open wound surface. Active coagulation whole blood flowable clot treatment was instilled into the wound to address the poor wound healing of this very challenging to dress wound surface. Initial ACWB application was preceded by gently wound cleansing with saline and gently rubbing with gauze, without formal wound debridement. Following the procedure, the patient was discharged and continued to receive care at the outpatient clinic. A non-adherent absorbent dressing was loosely applied to the wound cavity and then a polyvinyl chloride (PVC) film was used to cover the wound to avoid further outside contamination of the surgical site. The absorbent bandage was replaced on the second day and a new PVC film was applied to cover the dressing, without disturbing the ACWB covering the entire wound surface. The wound was gently cleansed with saline, and the flowable ACWB was reapplied on postoperation day 3 and as an outpatient on postoperation day 6. The wound exhibited the formation of granulation tissue upon the mesh and within the interstices with previously exposed intestine by day 7. Active coagulation whole blood was applied twice a week and following the seventh application, exposed and unincorporated mesh was excised. The same process of gentle wound cleansing followed by ACWB application was continued weekly with change of the loose overlying absorbent dressing, performed at 3-4 day intervals. After a total of 10 applications of ACWB, the patient continued standard-of-care treatment with dry dressing. The patient's last follow-up visit occurred at week 12, in which the wound had a massive granulation tissue with, and the wound depth was dramatically reduced.

Case Study 2

A 52-year-old female, a heavy smoker with a history of recurrent pilonidal sinus (PNS) with abscess, was previously treated by failed excision and antibiotics. Treatment of the chronic non-healing PNS excision site consisted of silver nitrate (Bray Healthcare, Faringdon, Oxfordshire, UK) application to the poorly granulating chronically inflamed wound surface. Three months following the excision, the patient reported increased pain in the wound area and was treated with Aqua cell (Convatec, Reading, Berkshire, UK), saline, and a steroid dressing which had reduced the wound size in both area and depth. Further treatment involved applying topical creams containing steroids, which led to some granulation, but minimal signs of healing progress. As a result, the patient was referred for Negative Pressure Wound Therapy (NPWT) for a duration of 2 weeks which further reduced wound dimensions, but healing remained incomplete despite the ongoing topical application of steroids was resumed to further aid in the healing process. Four (4) months after the NPWT treatment, the wound increased in size demonstrating a wound of 20 mm long and 10 mm deep. The patient refused another NPWT treatment and was referred to a plastic surgeon for consideration of a skin grafting or rotation flap closure. The application of ACWB was proposed to the patient as a final attempt at healing prior to performance of a complex surgical wound excision and closure procedure. The sinus tract was irrigated with saline and flowable ACWB was instilled into the sinus cavity, allowing the blood to coagulate inside the wound for 8 minutes. The sinus tract external opening was dressed with a non-adherent covered with absorbance

foam leaving the ACWB clot. Active coagulation whole blood was reapplied weekly for 4 consecutive weeks to a total of 4 applications. At the 1-week follow-up, the wound appeared superficial in depth and was reduced in size (10 mm length and 3 mm width). The wound continued to decrease in size in the next 3 weeks, achieving a complete wound closure.

Case Study 3

A 57-year-old male with a medical history of severe peripheral vascular disease (PVD), type 2 diabetes mellitus (T2DM), arterial insufficiency, severe Chronic Obstructive Pulmonary Disease (COPD), post-Cerebrovascular Accident (CVA), Coronary Bypass, renal insufficiency, and upper gastrointestinal bleeding was presented. The patient was a right-above the knee amputee who incurred a left femur fracture requiring open reduction and internal fixation. During convalescence of the femur fracture, he developed a pressure ulcer of the left heel extending full thickness to the calcaneus bone. The patient was treated for 2 months with NPWT, advanced dressings, that is, hydrophilic polyurethane foam, Flaminal (Flen Health, Luxembourg), protozoan (B. Braun, Melsungen, Germany), and ultrasonic and surgical debridement, with no signs of significant improvement. The wound was also treated surgically for osteomyelitis and underwent partial calcanectomy of the left posterior tibial nerve due to gangrene and osteomyelitis of the bone. At this point, ACWB was offered as an alternative to amputation which the patient had refused.

Active coagulation whole blood treatment was applied to the complex wound surface in order to try and prevent further deterioration of the wound. On day 1 of the ACWB clot treatment, the wound consisted of a fibrogranular base with necrosis and exposed bone and tendon, and hyperkeratotic tissue surrounded the wound bed. Surgical debridement was performed prior to ACWB application. Active coagulation whole blood was applied weekly for 3 weeks, resulting in reduction of the wound by 34.6% showing a major improvement in the wound characteristics. Intermittent debridement of focal superficial necrotic areas was required during the first 4 weeks of ACWB therapy, but thereafter no further necrosis occurred and fibrin deposition was reduced. However, after the sixth treatment application, the patient experienced upper gastrointestinal bleeding, leading to the interruption of the ACWB treatment. Instead, the patient received treatment with NPWT during his hospitalization. The seventh treatment application took place four weeks after the sixth application, and subsequent applications were administered weekly. By week 12, following the eighth application, the wound had progressed to advanced healing stages, and the wound has reduced by 88.77%. At the end of the 14-week treatment period, the patient tested positive for COVID-19 and was not able to return for 2 months. Upon the patient's return, the wound was treated by additional ACWB clot application and showed complete coverage of exposed vital structures and was considered to be healed by the treating physician.

Case Study 4

A 76-year-old male, with a medical history of diabetes type II, hypertension, hyperlipidemia, and cerebrovascular accident (CVA), was presented with a diabetic vasculopathy with gangrene on the great big toe. The patient suffers from limb-threatening peripheral vascular disease (PVD) of the right lower extremity. The patient underwent an endovascular procedure to the right leg for limb salvage due to gangrene on his great toe. Despite the intervention, treatment of the gangrene ultimately required toe amputation and open management of the amputation wound. Despite use of advanced dressings and a wound NPWT, the amputation site failed to heal. The wound bed measured area was 7.2 cm^2 and 1.2 cm in depth with signs of fibrotic tissue and minimal granulation evident, consistent with the findings of necrosis.

Active coagulation whole blood clot treatment was introduced following a sharp debridement. At the end of the first week, the wound had reduced in size, measuring 6.08 cm^2 and 1 cm in depth, with an increase in granulation tissue. The patient underwent 18 weeks of treatment, which involved 15 weekly applications of ACWB. During the initial five weeks, this treatment was combined with hyperbaric oxygen therapy. Before each ACWB application, sharp debridement was performed. By the second week of ACWB treatment, the wound size measured 4.2 cm^2 with a depth of .8 cm. Although the patient was instructed not to put weight on the foot, he was non-compliant and started walking without assistance. Despite the non-compliance, the wound continued to progress positively. By the 10th week of ACWB application, the wound size had reduced to 2.45 cm^2 with no depth, and there was no fibrotic tissue present. By the end of week 18, the wound had fully epithelized (Figure 2).

Case Study 5

A 48-year-old female, with diabetes type II, obesity, hyperlipidemia, asthma, and hypertension, underwent hip replacement surgery after experiencing a subcapital femur fracture complicated by a deep and superficial surgical site infection due to Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria in the surgical site. The patient was treated with intravenous antibiotics, along with NPWT treatments, and advanced dressings. Despite several courses of antibiotics, wound debridements, and advanced wound dressing therapy, the wound remained unhealed and chronically inflamed. The patient

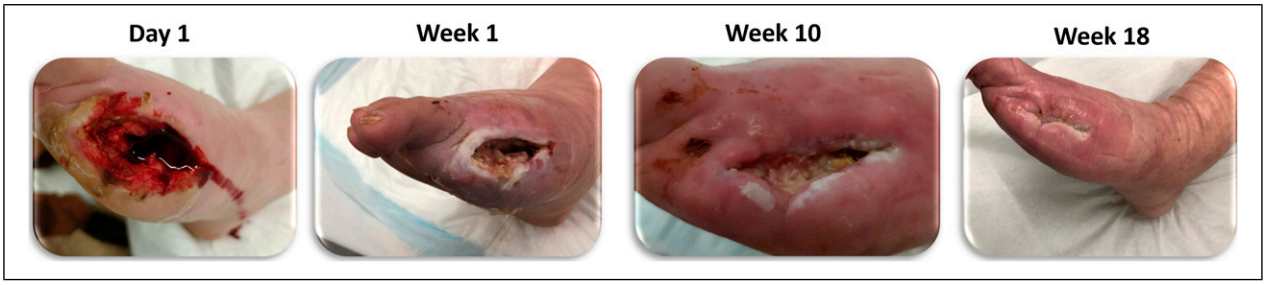

Figure 2. Post-amputation wound with signs of fibrotic tissue, minimal granulation tissue, and findings of necrosis. The wound underwent surgical debridement before ACWB application (left). At week 1 to ACWB application, reduction in wound size with increased tissue granulation (center left). On week 10, no fibrotic tissue present and continuing reduction in wound size (center right). Complete wound closure was achieved with ACWB on week 18 (right).

subsequently underwent Girdlestone procedure (Revision of Total Hip Arthroplasty) due to involvement of the original prosthesis. This procedure was also complicated by a surgical site infection due to *Proteus* sp. She was treated with ceftriaxone, without satisfactory response. Due to the severity of the infection, metronidazole and ceftazidime were added to the treatment regimen for a total of 6 weeks along with NPWT treatment to try and achieve progression in the wound condition. The post-surgical non-healing dehiscence wound remained unhealed for a total of 3 years.

Active coagulation whole blood was introduced to the wound following a surgical debridement. The wound dimensions on the day of the initial application of ACWB were 45 cm^2, and the wound was sutured reducing the open area to 4.2 cm^2 and .4 cm in depth. For the preparation of ACWB, a total 3 ACWB kits were used, and 54 mL of blood was utilized to fill out the entire wound cavity. The blood was allowed to clot inside the surgical site and was dressed with a non-adherent dressing. Active coagulation whole blood was reapplied weekly, and the loose absorbent gauze and PVC dressing were changed bi-weekly. By the third week of ACWB application, the wound exhibited the development of granulation tissue and wound contraction leading to a reduction in size to 3.6 cm^2 and a decrease in depth to .3 cm. There were no overt signs of invasive wound infection. The wound continued to progress in the following weeks, showing more granulation tissue and a reduction in wound depth. The wound was treated with ACWB for a total of 6 weeks. Although the percentage of wound reduction on the last visit was not determined by formal measurements, a visible improvement was noted. The wound was free of visible signs of infection and was considered by the treating physician as closed.

Discussion

Chronic wounds are a huge burden on the health care system and have a major effect on the patient's quality of life, subsequently, increasing morbidity and mortality.[15] Chronic wounds fail to proceed through the inflammatory phase, disrupting the normal balance between deposition and degradation of the ECM components, and exhibit high levels of pro-inflammatory cytokines, proteases, reactive oxygen species, and senescent cells.[15,16] The ECM is a therapeutic target in chronic wounds and a crucial factor in the entire wound-healing process by enabling cellular adhesion, chemotaxis, and migration.[17,18] It provides the wound with a physical infrastructure but most importantly, it regulates the activity of other cells in the wound area, such as fibroblasts and endothelial cells.[19] The degradation of the ECM in chronic wounds is attributed to an increase in inflammatory cytokines and senescent fibroblasts, which are unable to effectively reorganize the ECM and are unresponsive to growth factors and other signals that are essential for the wound healing process.[20] Those properties of the ECM make the reconstruction of the ECM a crucial target for wound healing of chronic wounds.

Active coagulation whole blood clot is a topical treatment and plays a major role in reconstructing and reorganizing the ECM. Active coagulation whole blood clot contains and attracts growth factors, such as platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), and transforming growth factor (TGF). These facilitate and help progress the wound toward healing by promoting cell granulation, coalescence of wound edges, and reduction of nonviable tissue.[10] In deep wounds with cavities and exposed structures, there is a need to have direct contact with all the wound edges and surfaces to fully maximize the treatment effect.

Amongst its many advantages, ACWB clot treatment has a two-way form of application. The flowable form enables the clinician to administer the biological autologous dressing in its liquidated form during the activation phase and before completing the full clotting phase, thus filling the odd shape cavity of the wound where 100% of the wound surface is in direct contact with the ACWB

clot. This ability to completely fill the cavity is superior to any known other dressing technique currently utilized in managing hard-to-heal wounds. The fluid administered traverses the most complex wound cavities, resulting in clot adherence to the entirety of the wound surface. This is virtually impossible for most current advanced wound therapies. The formed fibrin matrix mimics the role of the ECM, recruiting fibroblasts to the wound and promoting granulation and remodeling. The scaffold assists in the reconstruction of the ECM, thus providing a well-organized and regulated healing process for wound contraction and re-epithelialization.

The clinical examples provided demonstrated significant improvements in wound healing without the need for aggressive surgical wound management or complex dressing changes with the application of the ACWB clot. This benefit was most evident for the complex ventral hernia wound with exposed mesh and bowel and the post-amputation wound which avoided a subsequent higher-level amputation with a pre-existing contralateral above-knee amputation. Flowable ACWB wound therapy offers a safe and effective adjunct for treatment of otherwise non-controlled hard-to-heal wounds, allowing for better patient functional status, decreased hospitalization, reduced total cost of medical care, and improved quality of life, simply by facilitating wound healing by weekly, bedside application.

The use of the ACWB kit is extremely easy and does not necessitate any special knowledge or training by the clinical team. Furthermore, the kit does not require the use of any special equipment to facilitate usage in any of the mentioned forms of the ACWB clot, other than altering the timing and method of administration before or after the complete formation of the clot, depending on the discretion of the clinician.

In the five case studies presented herein, all five patients have undergone multiple previous treatments with either no effect on the wound healing process or halting in the progression of wound healing cascade after several months of treatment (case study 2). In all five cases, the ACWB was able to drastically reduce the size of these wounds, and in 4 out of the 5 cases, the wounds were classified as healed and completely closed by the end of the treatment period. To note, no adverse events were encountered during multiple applications of the active coagulation whole blood clot treatment.

Limitation

This study describes the efficacy of the ACWB clot treatment. However, given the small group size and different types of wounds, further investigation will be needed to determine the overall efficacy of ACWB clot therapy.

Conclusions

The ACWB clot treatment was found to be safe and effective in complex wounds that failed to progress with other advanced treatments. The direct contact of the ACWB clot with the entirety of the wound surface area holds a great benefit and a more efficient healing treatment approach to the chronic wound, especially when the wound contains deep areas, crevices, and tunneling. The ACWB clot therapy is a bedside treatment using the patient's own blood to minimize treatment rejection. The ACWB clot unique treatment method holds great promise in treating hard-to-heal and chronic wounds.

Declaration of Conflicting Interests

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