CASE STUDY

The Use of a Pure Native Collagen Dressing for Wound Bed Preparation Prior to Use of a Living Bi-layered Skin Substitute

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Abstract Management of chronic wounds in the outpatient setting is quite challenging. The extensive co-morbid medical problems of the chronically ill patient along with the complexities of the wound bed and its biochemical environment has led to a plethora of patients with poor wound healing. This ever increasing population is a challenge for the wound care practitioner and cost to the health care system and patient. Increased wound chronicity has promulgated the use of advanced wound care products, including Living Skin Substitutes (LSS), in an attempt to obtain wound closure, and ultimately both physiological and functional healing.^{1–3} In the outpatient setting, it is evident that the efficacy of the LSS varies widely depending on the patient type with some patients responding quite favorably while others who do not achieve healing despite repeated applications of LSS. This case series demonstrates that a systematic method of wound bed preparation prior to the application of LSS improved healing outcomes. The entire wound bed preparation protocol included autolytic, non-selective, and sharp-selective debridement, if deemed appropriate, followed by the weekly application of a pure native collagen. The wound bed preparation protocol was completed prior to LSS application. This case series presents evidence supporting the application of a 100% native collagen dressing to wound bed prior to the final step of LSS utilization.

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Introduction

The treatment and healing of chronic wounds frequently presents a formidable challenge posing a significant burden to our health care system both here in the United States and internationally. The personal and financial impact of 6.5 million Americans suffering from chronic wounds is a

staggering and growing concern. As of 2008, the United States, unfortunately is not typically a component in the medical school curricula.^{1,2} Physicians are expected to treat and appropriately resolve complex wounds in patients with multiple co-morbidities,³ without a clearly determined path for wound care education and clinical rotations.²

The understanding of wound healing at a cellular level, (including the multifactorial roles of chemokines, cytokines, growth factors, cell signaling and galvanotaxis) has increased exponentially.^{4–8} This has encouraged the development of advanced wound care and the use of biologics to expedite wound healing in patients with multiple co-morbid

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conditions. For example, a patient who has a diabetic foot ulcer superimposed on systemic peripheral vascular disease, a disease largely based in a pro-inflammatory bias, is at increased risk for non-healing secondary to the local and systemic pro-inflammatory bias.^{9–12}

Collagen is an important component of the extracellular matrix (ECM),^{4,13–15} a component of the dermis. Proteins, including collagen, is a natural polymer composed of amino acids. It is the most abundant protein found in mammals and a major component of the ECM. Until recently, it was believed that collagen (primarily Types I, II and III) were responsible for structural support and had little to do with cellular interactions. It is now evident that Type I collagen, the most abundant type in the ECM; interacts with many cellular functions; including migration, change in cell shape and recruitment of keratinocytes and endothelial cells.^{16–20} Activated pro-inflammatory macrophages secrete TNF- α , IL-1 β ; both key pro-inflammatory cytokines that directly influence the deposition of collagen via fibroblasts. Further, the cleavage products of collagen stimulate endothelial cell proliferation.^{21,22} Keratinocytes will adhere to denatured collagen. Collagen demonstrates an absorptive capacity for metalloproteases (MMPs) in-vitro. A decrease in pro-inflammatory MMPs is associated with resolution of the inflammatory state and progression to proliferation.^{23–25} Native mammalian collagen, attracts matrix metalloprotease I (MMP I). When a migrating keratinocyte encounters Type I collagen, the keratinocyte converts the

Type I collagen to gelatin. This conversion to gelatin makes many active sites accessible to cells important in the formation of granulation tissue.²⁶ Natural collagen is relatively inexpensive as compared to advanced biologics. Therefore, its incorporation into a wound bed preparation protocol prior to LSS warranted study.

Bioengineered skin substitutes have evolved over the last 20 years. The particular LSS (Apligraf[®]) used in this study, is a composite bi-layered-cultured-skin substitute. Human foreskin fibroblasts are grown in a bovine collagen gel with a second overlying layer of stratified and differentiated human keratinocyte. After 4–6 days of culturing, a well-formed stratum corneum with keratinocytes and fibroblasts contract the gel matrix to form a dermal equivalent. The final LSS mimics some of the biochemical and histological properties of the skin.²⁷

The ECM provides dynamic mechanical support to the dermis and epidermis through the presence of elastin and collagen fibers. It is a reservoir for growth factors, which enhance thermal and moisture regulation and must be restored for optimal healing of wounds, both in terms of re-epithelialization and function.^{28,29}

This clinical case series sought to evaluate the use of a pure bovine native collagen (Puracol[®]) dressing for wound bed preparation, and a biological living skin substitutes (LSS) (Apligraf[®]) through wound closure. Preparation of the wound bed^{30,31} with either conservative selective sharp debridement or non-selective autolytic debridement in

Table 1 Patient Demographics

Patient	Age	Sex	Wound Etiology	Co-morbidities	Medications/Procedures	Initial % of Necrotic Tissue
Case 1>	72	Male	Venous ulcer on the LLE which had been present for approximately 25 years as a result of a traumatic military event.	None listed	<ul style="list-style-type: none"> • IV antibiotics • Previous autologous skin grafts • Venous ablation procedures • Cardiac evaluation 	80%
Case 2	67	Female	Past medical history included punch biopsy for suspected malignancy which evolved into a large open wound with exposed bone. Positive for pyoderma gangrenosum.	Rheumatoid arthritis	<ul style="list-style-type: none"> • IV antibiotics, decreasing her systemic immunosuppressant medications • Collagen with minimal selective sharp debridement 	95%
Case 3	75	Male	Chronic myelogenous leukemia and LE edema. LE ulcer-medial malleolus		<ul style="list-style-type: none"> • Collagen with silver 	30%
Case 4	72	Male	LE diabetic wound for 3 months	Type II diabetic	<ul style="list-style-type: none"> • Arterial/vascular evaluation • Aggressive control of blood glucose levels. • Oral antibiotics 	50%
Case 5	58	Male	Non healing diabetic wound with resulting BKA on the RLE. Post-operatively the wound dehisces.	Long history of non-healing wound	<ul style="list-style-type: none"> • HBO • Collagen with silver 	40%

combination with the application of a pure native collagen dressing with or without antimicrobial silver, demonstrated facilitation of wound closure without complication or reoccurrence. The case studies are representative of various diagnoses common to wound care centers. This diverse case series presents patients with diagnoses of chronic venous insufficiency wounds, diabetic ulcers, myelogenous leukemia, gangrenous pyoderma, and a non-healing below knee amputation in a diabetic patient with diabetes.

Materials and Methods

The inclusion criteria required that candidates have halted wounds. The general hypothesis was that there would be a cumulative benefit from the combination of a pure native collagen (Puracol[®]) prior to the application of an LSS (Apligraf[®]). There were no exclusions based on wound etiology, longevity or severity. Five patients were identified as suitable candidates for LSSs. Their demographics are listed in [Table 1](#) below. Co-morbidities for

each patient were addressed per standard of care. Following either selective sharp debridement or non-selective autolytic debridement; collagen dressing (Puracol[®]) was placed weekly for a period of up to three weeks, per standard of care. In the final phase of wound healing, LSS (Apligraf[®]) was applied using appropriate aseptic or sterile technique. When appropriate, a collagen dressing was applied between LSS applications to further prepare the wound bed. The outcomes of interest were: 1) a visible impact on granulation tissue, 2) a reduction in wound surface area and 3) a decrease in wound depth. All parameters were measured over time.

The before and after photos for patient 2 and patient 5 are presented below as well as the patient specifics in [Table 1](#).

Results

This small diverse case series, demonstrated that early and repeated collagen introduction into a well-prepared, actively-granulating wound bed, irrespective of wound

Figure 1 Patient 2, wound with pyoderma gangrenosum A) Initial presentation B) Final Presentation at 35 weeks; Patient 5, diabetic wound C) Initial presentation D) After 2 months, the wound achieved closure.

etiology or patient co-morbidities, should be considered prior to LSS application. In this case series the combination of pure native collagen resulted in a tissue bed that was more suitable for LSS application, as evidenced by the incorporation of the LSS into the wound bed. As depicted in Figs. 1–4, substantial changes were observed within the wound bed which appeared to promote wound closure. The solid markers represent time points where either an appropriate debriding agent or the collagen dressing was used, and the black markers indicate the time points when the LSS was applied. The decrease in depth (Fig. 4), increase in granulation tissue (Fig. 2) and decrease in wound size overall (Fig. 3), are all indications that wound conversion from a chronic to a proliferative state has occurred. By day 50, four of the five patients demonstrated 100% granulation. By day 75, four of the five patients had 100% granulation tissue and wound depth approached zero. In each case, the wounds re-epithelialized and remain closed to date.

There was one outlier in each of the graphs (Patient 2). Patient 2 presented with one of the most difficult to heal diagnosis, pyoderma gangrenosum. Although the length of time to closure was approximately twice that of the other four patients in the series, wound healing was indeed achieved in one of the most challenging diagnosis.

Discussion

This is the first published study exploring the use of a pure native collagen prior to the application of LSS. A small case series of patients with varying etiologies is presented. The wound bed was properly prepared for application of an LSS.

The first hypothesis was that regardless of the wound etiology a wound bed preparation protocol was implemented utilizing a pure native collagen weekly. It is hypothesized that the addition of 100% pure native collagen (Puracol®) to the wound bed weekly assisted in the reconstruction of the ECM and decrease of the proteolytic environment, priming the wound bed for application of the LSS. The second hypothesis is that the combination of ECM reconstruction with the decreased proteolytic environment would accelerate the trajectory of wound closure and improve successful integration of the LSS.

Chronic ulcers (pressure, arterio/venous, diabetic or post-operative) defined as ulcerations which have failed to close within a period of 3 months or greater despite optimal wound care and therapy now affect more than 1% of the adult western population^{32–35} Further, the reoccurrence rate in the first year approaches 57%.^{35,36} As identified by Phillip et al in 1994, the quality of the patient's life is profoundly affected in terms of depression, mobility and socialization.³⁷ Yet, 30 years later we remain in search of a set of paradigms and solutions for an ever growing problem affecting increasing numbers of adults in the world each year. Lazarus defined wound healing as both “the closure of the wound and the restoration of the tissue.”³⁸ Re-epithelialization without adequate reconstruction of the ECM likely sets the stage for wound reoccurrence.

Traditional dressings, such as natural/synthetic bandages, cotton wool, lint and gauzes³⁹ are still used to manage wounds. The mechanism of action of these dressings is to keep the wound dry by allowing evaporation of wound exudates and preventing the entry of harmful bacterial. Moist wound healing is now the current standard of care as it facilitates the optimal healing environment.^{40–42}

Ideal selection of advanced and conservative wound care procedures and dressings takes into account complete

Figure 2 Collagen/LSS impact on granulation tissue.

Figure 3 Collagen/LSS impact on wound surface area.

healing which is fiscally responsible,⁴³ evidenced based and is patient centered. Patient centered care requires education, and dedication of both the patient and the multidisciplinary team of health care providers to ensure that the wound is healed and function restored.^{44,45}

Fig. 2 illustrates the impact of collagen application as part of the wound bed preparation protocol which results in an increase of granulation tissue. A visual analog scale approximating the increase of granulation tissue and the corresponding decrease in non-viable tissue was recorded. In this case series, the gradual application of pure native collagen improved granulation tissue and ultimately created a more viable environment for LSS application.

Fig. 3 demonstrates the positive impact of the collagen wound bed preparation on the reduction of wound surface area. Variation by patient was observed both in the time required to prime the wound bed with 100% native collagen and ensure removal of necrotic, non-viable tissue. This corresponded to the point in time at which minimal granulation and proliferation had begun in each patient. Controlling and treating the patient's co-morbid problems and treating any reversible causes of non-healing, such as edema, were done throughout the healing process. It is apparent from Fig. 3 that with appropriate wound bed preparation, which included weekly collagen application for up to 3 weeks prior to the application of the LSS, there was a steep increase in the wound closure trajectory.

Figure 4 Collagen/LSS impact on wound depth.

Decrease in wound depth is apparent, as shown in Figs. 3 and 4. Fig. 4 demonstrates a significant plateau effect in all the patients with minimal to no depth decrease during the wound bed preparation phase. However, a steep decline in depth is noted upon the application of the LSS.

Conclusion

Fiscally responsible care would dictate the necessity of careful wound bed preparation⁴⁶ in order to optimize the LSS integration. This small case series demonstrates that appropriate use of the wound bed preparation protocol, which includes the use of a 100% native collagen dressing, prior to the application of the LSS may facilitate terminal wound re-epithelialization. This case series lends credibility to both the need for physician and advanced health care practitioner education regarding the potential benefit of using the combination of two advanced wound dressings (LSS and native collagen) for positive clinical outcomes. The use of pure native collagen, after debridement, resulted in optimally granulated wound beds; the LSS was used to facilitate wound closure. Future comparative trials are warranted both to investigate the effect of different types of collagen and their impact on LSS take.

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