Revita®

Scientific and Clinical Compendium

stimlabs

Introduction

Since the early 1900s a substantial number of clinical benefits using amniotic membranes have been demonstrated in a range of wound and surgical applications[1]. The first reported use of amniotic membrane (AM) as a surgical material was for a skin grafting surgery in 1910. Notably, superior results were reported compared to xenograft or cadaveric wound coverings[2]. Clinical use of AM to treat wounds continued to proliferate, and by the 1940s, clinical usage had expanded to ophthalmology and reconstructive surgery. In more recent years the placental tissue market has advanced from single-layer amnion allografts to dual-layer allografts being introduced in the late 2000s. However, despite this progress, the characteristics of commercially available grafts still posed difficulties for clinicians. These problems included the inability to visualize the graft once rehydrated and applied to the wound and the difficult handling characteristics of thin, single and dual-layer grafts particularly when rehydrated.

StimLabs, a Roswell, Georgia based bioimplant manufacturer, developed a new product, Revita®, the first placental tissue graft that retains all layers found in the native placental membrane to address these clinical unmet needs. Revita is produced using the Clearify™ process, a patent-pending process that is capable of cleaning all the layers of the placental membrane through minimal manipulation of the tissue without the addition of toxic cleaning agents. The Clearify process is an advancement in tissue processing techniques allowing the retention of all three (3) main layers, including the hyaluronic acid-rich intermediate layer. This product satisfies the unmet clinical needs, as described below, by retaining a substantial amount of the native placental tissue matrix.

Native Placental Membrane

Native placental membranes act as a natural barrier and provide support and protection for the fetus during pregnancy. This function is dependent upon the morphology and defining structural components of the native placental membrane that can be seen in the cross-sectional image in Figure 1. The innermost layer of the placental sack is the amnion, which is adjacent to the fetus in-utero. The intermediate layer, or the "spongy layer", is found between the fibroblast layer of the amnion and the condensed matrix of the reticular layer of the chorion (the maternal facing side of the membrane). This layer is the interface between the amnion and chorion and consists of a spongy, acellular network. The outermost layer, or the chorion layer, is the maternal-facing side of the membrane. In utero, these three (3) layers work in unison to provide the structural functions that support fetal development. Each one of these placental membrane layers contributes to overall membrane structure, thickness, and protein content, including many of the cytokines, growth factors, extracellular components, and cell communication signals the body uses to heal, protect and grow tissues[1-7]. Notably in unprocessed native placental membranes, the intermediate layer can account for nearly 42% of the overall membrane thickness[1]. Each layer is made-up of a unique combination of core matrix components, that make every layer vital to the overall physiology of the native placental membrane.

Native Placental Membrane Layers

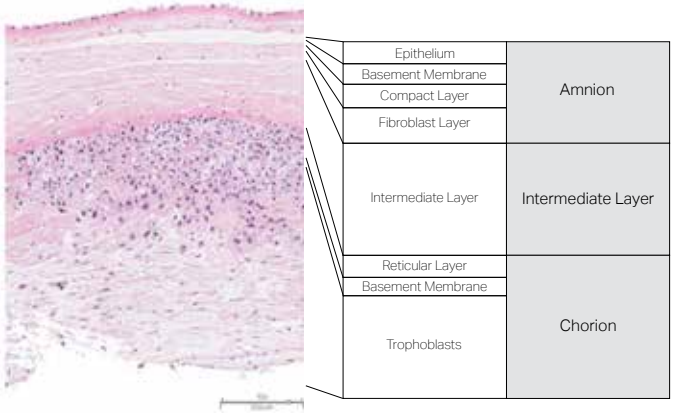


Figure 1. Histological staining on fresh, unprocessed placental membrane performed by Premier Laboratory, LLC.

The Clearify™ process

StimLabs developed the patent-pending Clearify process as a method of removing unwanted constituents and blood remnants from the placental membrane, while preserving the key components naturally found in the tissue. The differentiation between Clearify and traditional processing methods is that Clearify maintains the intermediate layer by design. This process allows the placental tissues to be cleaned, dehydrated, and terminally sterilized while maintaining all three (3) layers of the native barrier membrane, resulting in a user-friendly graft with improved handling characteristics. Tissues processed using the Clearify process have a distinct opaque appearance at the point-of-use. Clearify was engineered using 10 iterative core processes that were evaluated by qualitative and quantitative means across a series of over 70 process runs. The process design was accepted using quantitative metrics to evaluate both the final product composition and along the various process iterations, to ensure retention of native membrane components whilst the inherent variability in donor tissues.

Cleaning

During Clearify process development, the removal of contaminants and retention of protein content was assessed for different process iterations by qualitative and quantitative metrics before finalizing the manufacturing process[3]. Each cleaning step has been designed to maximize contaminant removal before proceeding to the preservation stage. StimLabs' tissue products are processed aseptically in a state of the art, purpose-built cleanroom facility, certified to ISO standards for cleanroom cleanliness. All tissue processing is performed under certified ISO Class 5 conditions. The placental membrane tissue layers are never separated during processing, and the intermediate layer is preserved. Through a series of six cleaning stages, using sterile reagents suitable for tissue processing per 21CFR§1271.10(3), the tissue is cleaned under conditions designed to maintain the native membrane protein content content and to reduce the risk of contamination.

Drying and Preservation

The pharmaceutical and biotechnology industries have used freeze drying (lyophilization) techniques for decades to

maintain the stability and shelf-life of proteins and cytokines, including growth factors[8]. The Clearify process includes the preservation of Revita by lyophilization to yield a dehydrated graft that can be stored at ambient temperature for up to five (5) years. The resulting off-white, opaque appearance of Revita is a result of the preservation process, and aids in visualization of the graft for placement at the wound or surgical site. This preservation process mechanically removes water from each placental layer whilst maintaining membrane thickness. On average, Revita grafts are 400µm in thickness[3]. The enhanced thickness of Revita grafts also translates to ease of handling at the point of use.

Sterilization

StimLabs' uses a validated method of terminal sterilization for all tissue products, proven to provide a Sterility Assurance Level (SAL) of 10-6 for an added assurance of safety. This level is the industry standard for medical products labeled as "sterile". Validation of sterilization methods ensures that the manufacturer has performed the required studies to show that the sterilization process is reliable and repeatable, and that it meets the requirements for standards set for the industry (ISO 11137 Sterilization of Health Care Products – Radiation). Each batch of StimLabs' tissue products are sterilized and released based on dosimetric release. StimLabs confirms the effectiveness of the validated sterilization dose (as required by ISO 11137) by performing routine testing of bioburden levels and sterility of finished products[14].

The Science of Revita

Through the processing and preservation techniques of Clearify, all three (3) native placental layers of the barrier membrane are maintained (Table 1). Revita has enhanced membrane thickness, dry weight, protein content and retains many of the cytokines and growth factors the body uses to protect and grow membranes when compared with the previous generation of AM products[1,3].

Placental Membrane Structure: Amnion, Intermediate Layer and Chorion

The amnion layer is the thinnest layer of Revita. It is composed of a surface epithelial layer on the fetal side, a basement membrane, a compact layer and then a fibroblast layer containing collagens, laminins and fibronectin. The intermediate layer is another vital layer of Revita as it contains an acellular network of multiple extracellular-matrix and regulatory proteins[3,4,5]. It is also concentrated with proteoglycans and glycosaminoglycans, including hyaluronic acid[3,5,6-10]. As reported in the literature, hyaluronic acid is well known for its cushioning and lubricating properties[11]. Hyaluronic acid can be found in the amnion and in smaller quantities in the chorion; however, the most concentrated amounts of HA are found between the amniotic and chorion layers within the intermediate layer[6]. The densest regions of HA in Revita have been found in the intermediate layer[3]. Proteoglycans, like the ones found in the intermediate layer, are also known to sequester growth factors[3,12]. The final layer of Revita is the chorion layer which is composed of a reticular layer, basement membrane and trophoblast layer. This layer contains extracellular matrix proteins including collagens, proteoglycans, laminin, elastin and fibronectin, as well as a large range of regulatory proteins[7,13].

Table 1. Individual layers in native membrane and Revita grafts

Placental Membrane Layer	Amnion	Intermediate Layer	Chorion
Native Membrane	√	√	√
Revita	√	√	√

Regulatory Proteins

Each layer of the placental membrane contains regulatory proteins, including but not limited to cytokines, growth factors, interleukins and enzyme inhibitors that contribute to the overall protein content[2,13]. Revita regulatory proteins are most accurately measured by enzyme-linked immunosorbent assay (ELISA), a standard immunoassay to estimate a protein's concentration in a sample. An analysis was performed on multiple Revita products randomly selected from a range of donor tissues available in commercially available inventory. Some of the highest concentrations of cytokines and enzyme inhibitors detected in Revita are PDGF, bFGF, TIMP-1 and TIMP-2. Additional regulatory proteins relevant to healthy wound management were also assayed and measurable within the limits of detection, including TGFα, IL-5, IL-10, TGFβ-1, PDGF-AA, PDGF-BB, EGF, VEGF and TIMP-4[1]. During the development stages of the Clearify process, a panel of twelve relevant regulatory proteins were used to quantitatively evaluate and guide the selection of process settings and limits. A host of regulatory proteins, including those assayed in this panel, have also been identified in the intermediate layer[2]. Therefore, Revita, through retention of the intermediate layer, offers a differentiated solution that retains desirable regulatory protein content from each placental membrane layer.

Table 2. Regulatory protein content in Revita grafts

Regulatory Protein	Revita
PDGF-AA	√
PDGF-BB	√
TGFα	√
TGFβ-1	√
bFGF	√
EGF	√
IL-5	√
IL-10	√
TIMP-1	√
TIMP-2	√
TIMP-4	√
Lactoferrin	√
VEGF	√

In conclusion, the retention of the clinically relevant extracellular matrix, regulatory protein and hyaluronic acid content of the full placental membrane yields an enhanced wound covering product. The following case series demonstrate the unique capabilities and performance of Revita in a range of clinical applications including diabetic foot and venous leg ulcer wound management.

Case 1: Venous Leg Ulcer

Initial Patient Evaluation and Product Application: Day 0

An 87-year-old female presented with a severe Venous Leg Ulcer (VLU) on her lower right leg measuring 11 cm in length and 4 cm in width with exposed tendon and heavy exudate of purulent consistency. Conservative therapies were previously attempted for months without success. Patient had a history of diabetes and smoking, and all toes had previously been amputated. Patient reported high pain of 8 on a Visual Analog Scale (VAS) of 1 to 10 at site of VLU. At the time of initial evaluation, the wound was cleaned and five Revita allografts were used to cover the wound. Standard facility protocol for tissue application was followed, including placement of a non-adherent hydrogel for protection. Clinicians rated the ease of Revita application as excellent.

Follow Up: Day 27

Healthy granulation tissue began to fill the wound bed. The wound was cleaned, debrided, and clinicians re-applied five Revita allografts. Clinicians rated the ease of Revita application as excellent.

Follow Up: Day 41

Clinicians noted "good results" with healthy tissue developing around the previously exposed tendon. The wound was cleaned, debrided, and clinicians re-applied four Revita allografts.

Final Treatment: Day 62

Clinicians noted a "considerable improvement" with low exudate levels and healthy tissue development and re-applied three Revita allografts to continue treatment.

Wound Resolution

Wound continued to progress through to resolution with "incredible results" noted by the clinicians.

Day 0 Wound

Day 0 Application

Day 41

95% Closure

Case 2: Diabetic Foot Ulcer

Initial Patient Evaluation and Product Application: Day 0

A 69-year-old male presented with a Diabetic Foot Ulcer (DFU) on the plantar aspect of his right heel measuring 1.8 cm in length,1.7 cm in width and 0.3 cm in depth, with scant exudate of serous consistency. Previous therapies, including other amniotic tissue grafts failed for at least 12 weeks prior to application of Revita. Patient had a history of type II diabetes, hypertension, charcot foot, gout, and diabetic neuropathy. At the time of initial evaluation, the wound was cleaned and one 2x2 cm Revita allograft was used to cover the wound. Standard facility protocol for tissue application was followed, including placement of a non-adherent dressing and sterile gauze for protection. Clinicians rated the ease of Revita application as excellent.

Follow Up: Day 14

After good initial progress, the wound decreased in size to 1.7 cm long, 1.4 cm wide, and 0.3 cm deep. The wound was cleaned, debrided, and clinicians re-applied one 2x2 cm Revita allograft. Clinicians rated the ease of Revita application as excellent.

Follow Up: Day 33

After good initial progress, the wound decreased in size to 1.5 cm long, 1.1 cm wide, and 0.1 cm deep. The wound was cleaned, debrided, and clinicians re-applied one 2x2 cm Revita allograft. Clinicians rated the ease of Revita application as excellent.

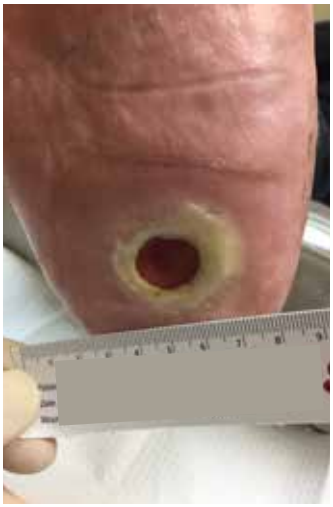
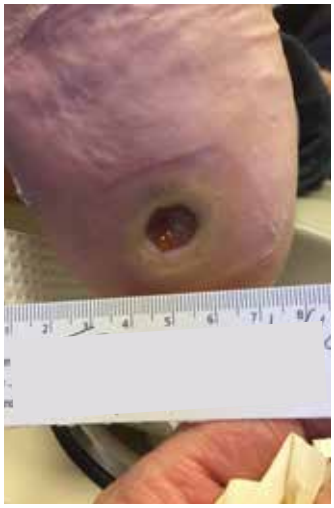
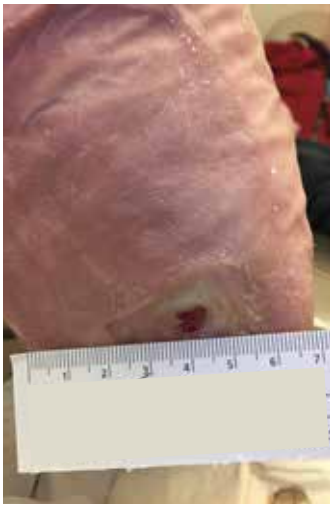
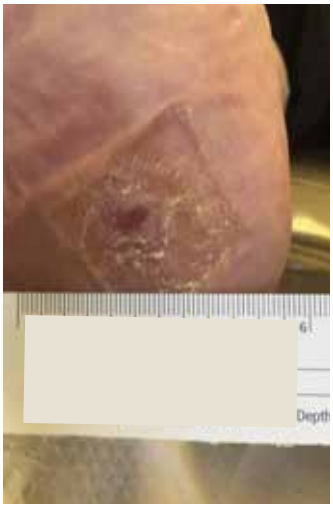
Final Treatment: Day 69

Additional Revita allografts were applied at day 48 and 62. The wound continued progressing and decreased in size to 0.7 cm long, 0.4 cm wide, and 0.1 cm deep.

Wound Resolution: Day 83

The wound accelerated quickly to resolution by day 83 of treatment.

Day 0 | Day 22 | Day 69 | Day 83

Case 3: Diabetic Foot Ulcer

Initial Patient Evaluation and Product Application: Day 0

A 58-year-old female presented with Diabetic Foot Ulcer (DFU) on the plantar aspect of her right heel measuring 4 cm in length and 5 cm in width with minimal exudate of serous consistency. Previous therapies failed for approximately two years. Patient had a history of diabetes, osteomyelitis, and peripheral vascular disease. At the time of initial evaluation, the wound was cleaned, debrided and three 2x3 cm Revita allografts were used to cover the wound. Standard facility protocol for tissue application was followed, including placement of a non-adherent dressing, sterile gauze and hydrogel for protection, and the patient was instructed not to bear weight on her foot. Clinicians rated the ease of Revita application as excellent.

Follow Up: Day 20

After the initial Revita application, the wound decreased in size to 2 cm long and 3 cm wide. No additional allografts were applied, and the wound was redressed.

Follow Up: Day 27

The wound continued to progress, measuring 1 cm long and 2 cm wide. The wound was cleaned, mechanically debrided, and clinicians re-applied one 2x3 cm Revita allograft. To ensure continued offloading, the patient was provided a wheelchair. Clinicians rated the ease of Revita application as excellent.

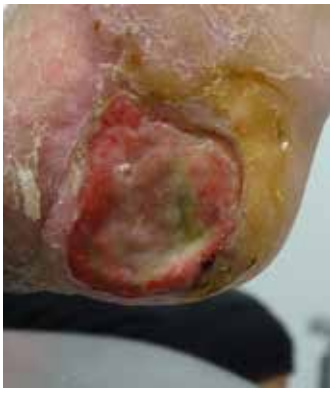
Follow Up: Day 32

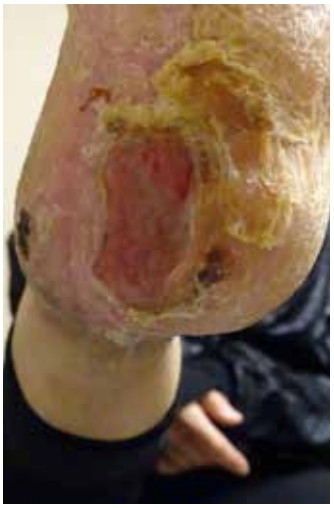
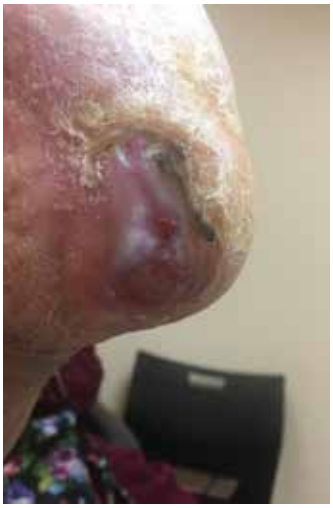
The wound completely filled in with healthy tissue and clinicians expected full resolution within the next 2 weeks.

Wound Resolution: Day 97

By day 97, the treated wound completely resolved.

Day 0 | Day 20 | Day 39 | Day 91



Case 4: Diabetic Foot Ulcer

Initial Patient Evaluation and Product Application: Day 0

A 55-year-old male presented with a Diabetic Foot Ulcer (DFU) on the bottom of his right foot measuring 1.6 cm in length, 1.5 cm in width and 0.2 cm in depth with scant exudate of serous consistency. Previous therapies including wound care, offloading, and oral antibiotics failed for at least 10 months prior to application of Revita. At the time of initial evaluation, the wound was cleaned, mechanically debrided, and one 2x2 cm Revita allograft was used to cover the wound. Standard facility protocol for tissue application was followed, including placement of a non-adherent dressing and sterile gauze for protection. Clinicians rated the ease of Revita application as excellent.

Follow Up: Day 33

After the initial Revita application, the wound decreased in size to 1.0 cm long, 0.7 wide and 0.2 cm deep. The wound was cleaned and debrided, and a single 2x2 cm Revita allograft was applied. The patient was also provided a cast boot for offloading.

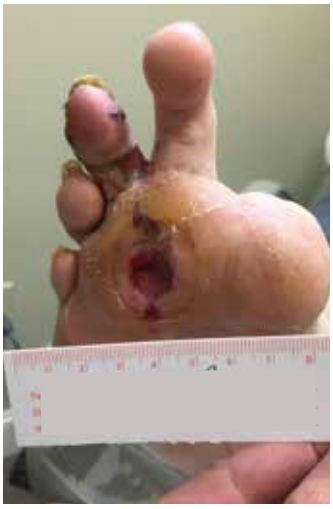
Follow Up: Day 69

An additional Revita graft was applied at days 41, 48, and 62. The wound continued to progress, measuring 0.8 cm long, 0.3 cm wide and 0.2 cm deep on day 69. The patient was provided a pneumatic walking boot to continue offloading.

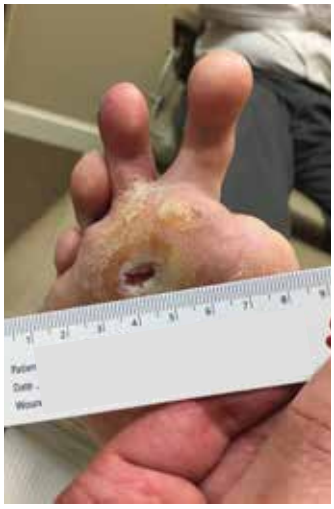
Wound Resolution: Day 104

By day 104, the treated wound completely resolved.

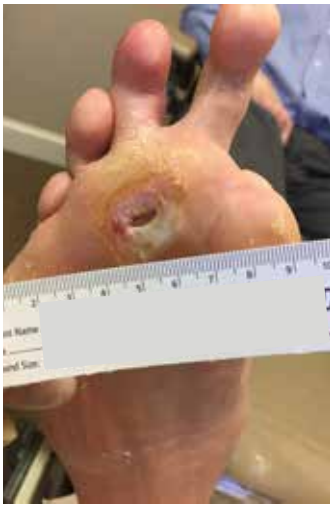
Day 0



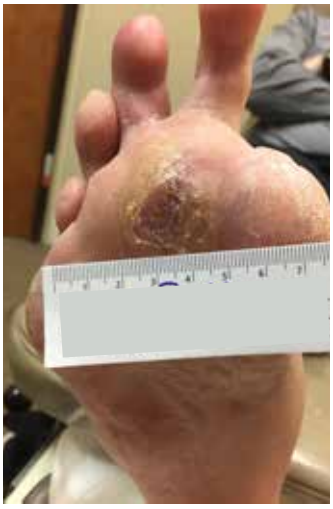
Day 55



Day 69



Day 104



Case 5: Diabetic Foot Ulcer

Initial Patient Evaluation and Product Application: Day 0

A 75-year-old male presented with a Diabetic Foot Ulcer (DFU) on the toe of his left foot measuring 1.5 cm in length, 1.0 cm in width and 0.2 cm in depth with no significant exudate. Previous therapies failed for at least 5 months prior to application of Revita. At the time of initial evaluation, the wound was cleaned, mechanically debrided and one 2x2 cm Revita allograft was used to cover the wound. Standard facility protocol for tissue application was followed, including placement of a non-adherent dressing and sterile gauze for protection. Clinicians rated the ease of Revita application as excellent.

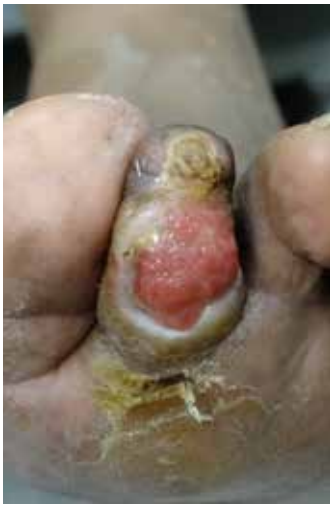
Follow Up: Day 42

After the initial Revita application, the wound decreased in size to 0.25 cm long and 0.25 cm wide. The wound was cleaned and the patient was asked to return in 7 days.

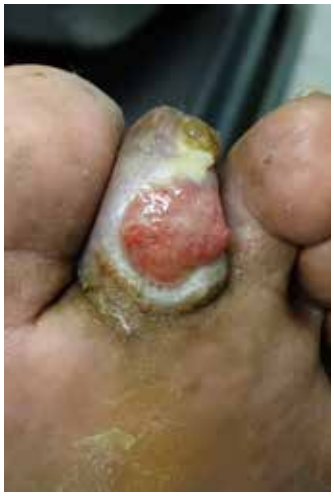
Wound Resolution: Day 49

By day 49, the treated wound completely resolved.

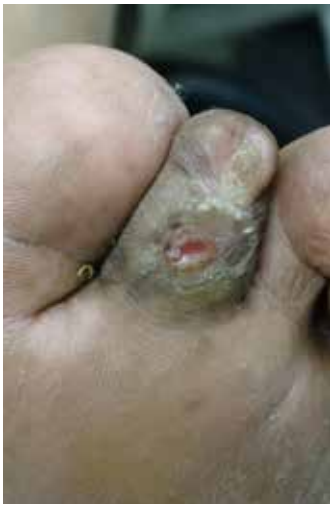
Day 0



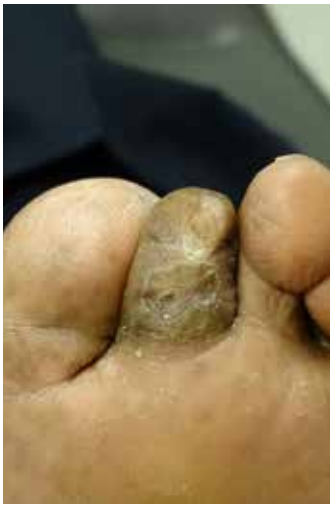
Day 12



Day 42



Day 49



Case 6: Traumatic Wound

Initial Patient Evaluation and Product Application: Day 0

A 39-year-old female presented with an injury resulting from complications of bariatric surgery. The surgical complications resulted in septic shock and gangrene in her lower extremities. The patient had a trans-metatarsal amputation with a split thickness skin graft that had not healed in 7 months. At the time of initial evaluation, exposed bone was present. The wound was cleaned, surgically debrided and six square centimeters of Revita were used to cover the wound. Standard facility protocol for tissue application was followed, including placement of a non-adherent dressing for protection. Clinicians rated the ease of Revita application as excellent.

Follow Up: Day 6

Clinicians debrided the wound and changed the dressing.

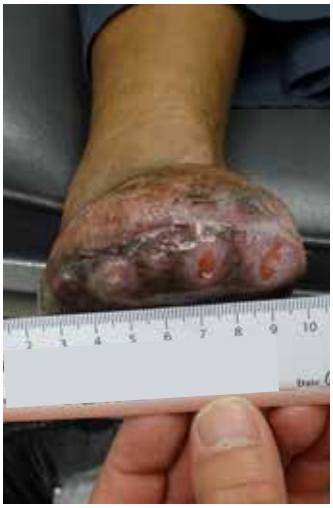
Follow Up: Day 13

Clinicians changed the wound dressing and noted evidence of bone coverage.

Wound Resolution: Day 28

By day 28, the treated wound completely resolved.

Day 0



Day 6

Day 13

Day 28

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