myOwn skin™

CELLULAR CULTURE OF AUTOLOGOUS SKIN

Information Packet

Produced at the laboratories of

BIOLAB SCIENCES

Version 10 Effective: 08/22/19

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MyOwn Skin™: A Wound Care Therapy

When injured soft tissue is exposed, the greater the amount of time of this exposure is in direct correlation to the greater risk and percentage of complications. The primary goal in a wound care therapy is to decrease this exposure time.

The ideal wound care therapy is one that is definitive, leaves minimal scarring, and does not require extensive donor areas which create secondary wounds on the patient. This ideal wound care therapy is also available at the acute stage of the injury.

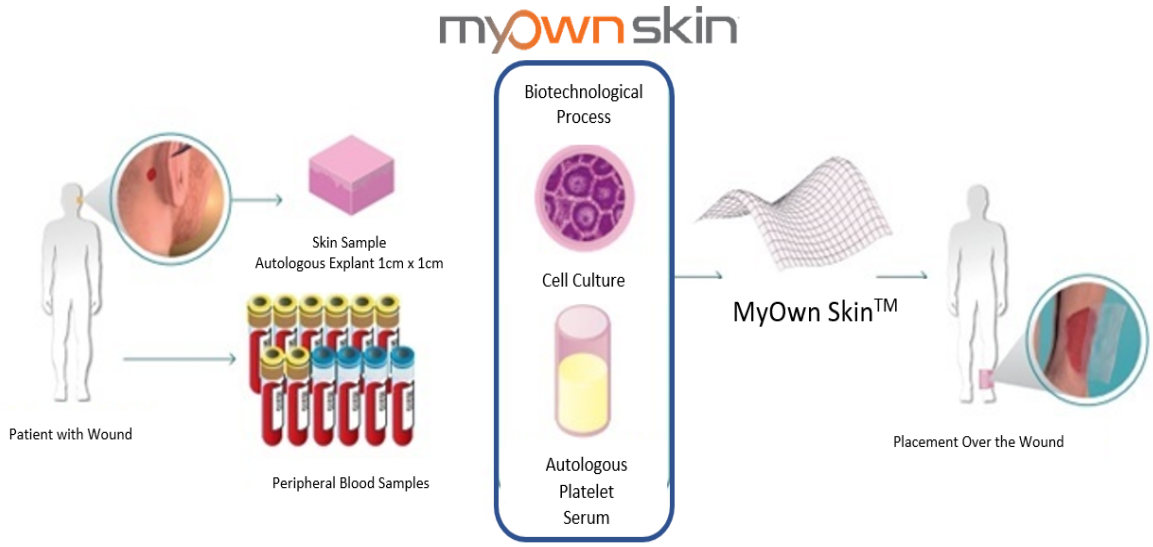

Figure 1. Homograft / Autograft with autologous skin culture from a human source

MyOwn Skin™, an autologous skin cell culture, is an advanced wound care therapy developed by a group of renowned physicians and patented around the world. These autologous skin sheets are cultured, using the patient's own skin cells, blood, and plasma. When placed over this patient's wound will allow the regeneration of all layers of a patient's skin in wounds caused by burns, trauma, non-healing diabetic ulcers, plastic surgery reconstructions, and any other wound where a typical therapy might have been to harvest a wound-equivalent sized skin graft.

These inherent characteristics allow MyOwn Skin™ to be classified as an autologous skin cell culture and used in the most advanced wound care therapies. A typical skin graft harvested from a patient's back, abdomen or thigh, will cover damaged tissue and eventually become integrated. MyOwn Skin™ not only will cover the defect but

will create the niche environment necessary for tissue regeneration within the wound. With a perfect biocompatibility due to its autologous nature, MyOwn Skin™ significantly reduces the risk of complications and rejections, while shortening the healing time.

This procedure is more cost-effective since it does not require multiple specialties or complex equipment for the application. The MyOwn Skin™ autologous skin sheets can be applied in a treatment room, an outpatient clinic or any other sterile environment; an operating room is not necessary.

The Mechanisms of Wound Care Therapy

The dermo epidermal tissue and its physiological processes are altered in different types of injuries, as in the case of trauma (avulsions), extensive burns, ulcers, and other injuries that cause loss of cells in the various skin layers.[1]
The physiological process of wound areas repair is a complex mechanism that requires the interaction among different elements, such as fibroblasts, myofibroblasts, smooth muscle cells, endothelial cells and immune cells. These interactions are mediated by growth factors, hormones, blood components and second messengers.[2]

Wound repair is a mechanism that depends on hemostasis and an initial inflammatory state, caused by the injury. This stage is known as acute phase. Subsequently, it enters a proliferative phase of epidermal, endothelial and fibroblast cells, which will generate an initial granulation tissue.[3]

Afterwards, a late inflammatory phase results, characterized by neovascularization, dependent on regulatory factors such as the factor of vascular endothelial growth (VEGF), and different neurotrophies that stimulate proliferation, chemotactic activity and survival of different cellular populations in the skin, responsible for generating a new collagen matrix. Generally, an eschar is formed, and remodeling of the granulation tissue is produced with the generation of new collagen fibers and the differentiation of fibroblasts in myofibroblasts, which increase tensile strength and allow the approximation of the edges of the lesion.[4]

From these cell populations, human keratinocytes are skin cells resulting from the embryonic ectoderm, responsible for the production of keratin (a high molecular weight polypeptide) and pro-inflammatory cytokines, in addition of the expression of intercellular adhesion molecules (ICAM1) and immune reactive surface molecules such as HLA-DR.[5]

The fibroblasts in turn appear to be the most specialized cells that make up the connective tissue being dispersed throughout the body, where they secrete a non-rigid extracellular matrix, rich in type I and / or type III collagen. When a tissue is injured, the closest fibroblasts proliferate, migrate to the wound area and produce large amounts of

collagen, which help to isolate and repair the damaged tissue. The ability to survive in the context of a wound, as well as their style of solitary life, can explain why fibroblasts are the easiest cells to cultivate.[6]
In regard to the extracellular matrix of the connective tissue, the most common in the body, it consists of a fundamental hydrated substance similar to a gel, with fibers included in it. The fundamental substance resists compression forces and the fibers support tension forces. The present water allows the rapid exchange of nutrients and waste products transported by extracellular fluid as which is filtered through the fundamental substance.[7]

Collagen molecules are produced by cells, such as fibroblasts, and they self-assemble into hierarchical structures, such as fibrils and then fibers. These newly formed collagen fibrils and fibers then form the tissue architecture and provide such qualities as the resistance, elasticity and capacity of elongation, in greater or lesser degree, depending on whether it is tissue such as skin, tendon or bone.[8] The growth factors essential for tissue repair are the epidermal growth factor (EGF), the fibroblast growth factor (FGF), the Insulin-like growth factor (IGF), keratinocyte growth factor (KGF), the platelet derived growth factor (PDGF), the transforming growth factor (TGF) and vascular endothelial growth factor (VEGF).[9]
When tissue loss occurs in a wound, MyOwn Skin™ is effective by providing a niche environment for the wound below MyOwn Skin™ to regenerate up to the new skin.

The MyOwn Skin™ Difference

Unlike a traditional skin graft harvested from another site on the patient's body, MyOwn Skin™ does not require a donor area of similar size to the coverage area. MyOwn Skin™ requires a skin sample of approximately 1cm², thus alleviating additional scarring from the secondary wound traditionally created when a skin harvest for grafting is performed. MyOwn Skin™ effectively decreases wound healing time and lowers the risk of rejection due to its autologous nature.

From the conceptual point of view multiple studies have historically addressed this therapy. There are many reports and case studies in the medical literature and journals regarding the use of keratinocytes to grow regenerative skin patches. However, most of these studies have been conducted using cells taken from other humans or animals. In these cases, multiple tests needed to be performed on both the donor and the receiver. These tests (multiple pathogen detection tests and/or infectious or communicable disease presence testing) proved to make the resulting products not only cost-prohibitive but not viable in developing countries.

In the aforementioned literature there are also reports of using autologous keratinocytes but seeded them on heterologous fibroblasts, sometimes irradiated or on fibrin glue.[10],[11] Other publications speak about how autologous keratinocytes have even been applied directly in suspension over the wound areas.

It has also been reported that in these types of bioengineering, autologous cultures can take at least 4 weeks to manufacture. Thus, in 2009, Doctors Gaona, Zambrano, and Nieto standardized the autologous keratinocyte culture method with autologous serum for the covering of a wound. The culture and production time of *these* skin patches was reduced to approximately seven (7) days. This revolutionary MyOwn Skin™ showed adequate growth and cellular proliferation in four (4) days, avoiding the senescence of the keratinocytes.

MyOwn Skin™ Clinical Data

A multicenter cohort study was conducted to follow up 44 patients. This patient group was composed of 27 men and 17 women, between 15 and 75 years of age, and all with wounds and indications treated with MyOwn Skin™. These patient treatments were studied at the initial time of treatment and then at 5, 7, 15, and 30 days post-application. Particular attention was paid to assessing with cultured keratinocytes. It was found a non-significant inverse correlation between age and the percentage of epithelialization (R_2 = -0.194 p = 0.089, spearman) which was decreased when adjusting for depth of the bloody area (R_2 = -0.115 p = 0.321, spearman).[12]

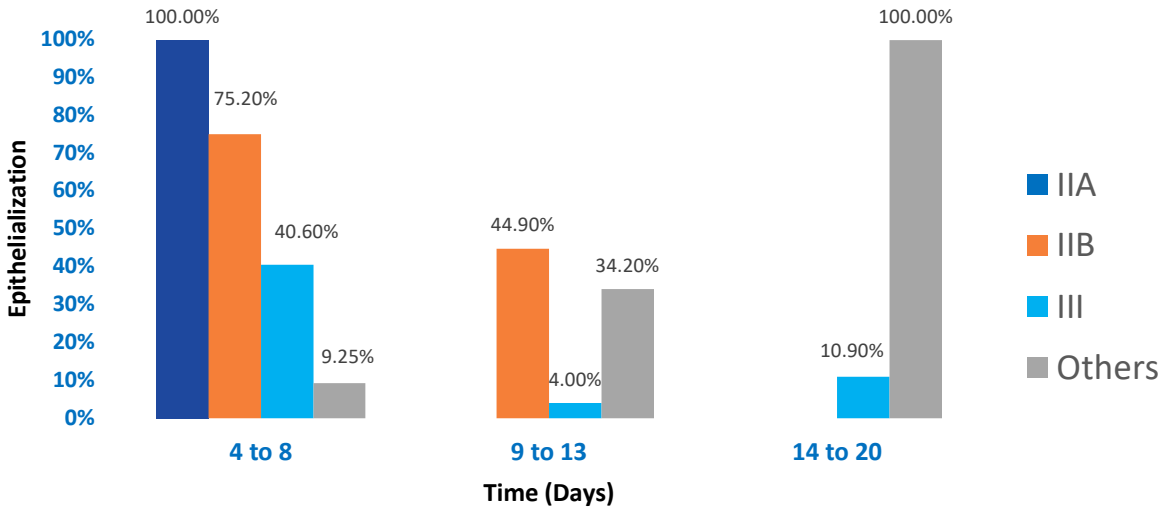

Figure 2. Percentage of burns with epithelialization regarding time elapsed for MyOwn Skin application

The epithelialization was on average 53%, finding significant differences in the percentage of epithelialization and the depth of the wound (p <0.001) being greater to a

lesser degree of depth. The size was very variable with an average of the total size of the areas of 41 cm² for which it was grouped by quartiles.[13]
Significant differences were found in the percentage of epithelialization in relation to the size of the wound area (burns) and its depth (p = 0.001). Being mainly grade IIA burns with a size of 0 - 9.75 cm²; n = 8 with a percentage of epithelialization of 100% of cases.[14]

The average time between the injury onset and the harvesting of the skin sample was 28.4 days. The average time between the harvesting of the skin sample and the application of the new MyOwn Skin² sheets was an average of 6 days. The stratification of the average time was performed and the differences in the medians of the percentage of epithelialization of the interaction between the days elapsed between the skin sampling (autologous explant) and the application of the graft with the depth of the wound (p <0.001), being greater for burns IIB (between 4 - 8 days), which is compatible with what is reported in the literature in which it suggested that viability cell decreases above day 8.[15] See Figure 2.

Healing was satisfactory in 93% of the patients; the cases that presented hypertrophy were patients with grade III burns. Finally, the coverage was evaluated, finding scarce, serous and clean secretion in 35% of the cases that required drainage during the controls at days 5-7, but were not related with signs of local infection (edema, erythema, fever, or local heat). 18% of patients received oral antibiotic treatment, as prescribed by the attending physician.[16]

MyOwn Skin™ provides adequate cutaneous viability and complete epithelialization in short time, with zero cases of rejection, allergic reactions, or adverse side effects, during the study. Early coverage of the wound with MyOwn Skin™ is an important advantage for the decreased risk of infection, and the increased possibility of a short-term integration and epithelialization.

MyOwn Skin™ Candidates

MyOwn Skin™ is best for wound care treatment of patients suffering from most open tissue wounds such as burns, trauma, non-healing diabetic ulcers, plastic surgery reconstructions, and any other wound where a typical therapy might be to harvest a wound-equivalent- sized skin graft.

It is important that the bed of the wound be prepared in advance to optimize metabolic, physiological and vascular conditions, as well as controlling any sign of infection or necrosis.

Other necessary conditions are the absence of cavitation, perforation, fistulas, tunneling, bone exposure, tendon exposure and pocket wounds. An appropriate control of pathologies and base contributors, especially associated with injuries, such as autoimmune conditions, diabetes and/or vascular insufficiency should be addressed prior to the application of MyOwn Skin™. Particularly, it is not recommended to use MyOwn Skin™ in pressure ulcers in which it is not possible to guarantee that the pressure does not persist. MyOwn Skin™ should not be applied in wounds with signs of superinfection, necrotic tissue with exposure of fascia, bone, tendon and or vital structures.

1. Clinical selection of the patient.

The process begins with the clinical selection of the patient and medical criteria:

1.1. Each treating physician should use their own clinical criteria to specify which patients are candidates for this procedure. They must address the time frame in which to perform this procedure as well as identify the amount and number of membranes that will be needed.

1.2. Patient with a diagnosis of a wound, acute or chronic ulcer, which requires a definitive coverage through a stimulating procedure of tissue regeneration. Example: burns, vascular ulcerations, traumatic wounds with significant tangential avulsion of medium depth skin, etc.

1.3. Patient candidates for partial skin grafting to improve tissue regeneration.

1.4. It is recommended not to select patients with pressure ulcers where it is not possible to guarantee that the mechanical effect of pressure does not persist.

1.5. In patients with diabetic foot ulcers (DFUs), it is important to take into account the degree of classification.

1.6. In cases of patients with injuries subsequent to a cancerous tumor removal, it is necessary to confirm free margins in the autologous skin – sample or in the surgical specimen, to verify the absence of residual malignant cells.

1.7. It is necessary that there is no evidence of fascia exposure in the lesions, tendons, muscle and / or bone.

1.8. The patient's injuries should not have excessive exudate, hemorrhage, signs of superinfection, necrotic tissue, maceration or cicatricial fibrosis of the perilesional edges, or other signs of tissue infeasibility.

1.9. Adequate tissue perfusion signs should be verified on the affected tissue as in the perilesional zone.

1.10. A patient with injuries with an appropriate granulation process and without evidence of over granulation.

1.11. Patients with systemic comorbidities to the wound areas which have been properly controlled medically and / or surgically, are candidates for this procedure, however the treatment outcomes have not been tested.

Procedure Guide for Collection

1. Microcentrifuge tube w/medium for skin explant
2. Local anesthetic
3. Alcohol swab
4. Sterile gloves
5. Sterile non-woven gauze

6. Blue blood tubes (4)
7. Yellow blood tubes (8)
8. Vacutainer
9. Sterile Tweezers
10. Syringe
11. Size 20 sterile scalpel

Figure 4. Collection Kit Contents

The procedure can be prescribed following the usual procedure regulated by *the Food and Drug Administr*ation (FDA). Any Patient information forwarded to BioLab Sciences, intentionally or accidentally, will be handled under the legal guidelines of HIPAA data and under the ethical parameters of confidentiality.

Blood and Skin sample collection for cell culture.

When the patient has been chosen and cleared for the procedure, the physician proceeds to the initial step which consists of the collections of both the skin sample ("explant"), and the blood sample using the following detailed steps: See steps 3.5.a-g for blood sample. See steps 3.6 a-e for skin explant.

3.1 BioLab Sciences will send the Biologic Sample Collection Kit (Collection Kit) to be received by the Physician on or before the predetermined date. See picture below for detailed list of kit components. Physician, or hers/his authorized personnel, will perform the procedures outlined in steps below. The integrity of the external packaging, and expiration date of materials, must be verified.

3.2 The patient's ID must be confirmed and must be placed on all tubes containing blood and skin samples prior to shipping back to BioLab Sciences.

3.3 Prior to engaging in the sample and blood collection, the health provider must collect the patient's signed consent, which is then archived into the patient's medical records. NOTE: This consent is not shared or stored with BioLab Sciences, however, in the cases of an audit must be readily available.

3.4 Because the blood sample is taken under a vacuum closed system, to avoid the loss of the vacuum the tubes **must not** be opened at any time.

3.5 Instructions for Blood Draw

a. Wash hands and put on sterile gloves.
b. Place the tourniquet between 7.5 cm or 10 cm above the point of puncture.
c. Clean the arm area with an alcohol pad.
d. Perform the venipuncture with the bevel upwards with a smooth and fast movement and secure the needle once the vein is reached.
e. Take blood samples at the maximum capacity of each tube; yellow tubes (without anti-coagulant) and blue (with anticoagulant). The blue cap tubes should be gently shaken to homogenize the samples and avoid clot formation.
f. Once all the tubes have been filled, first remove the tourniquet and then the tube. Next remove the needle with a quick and gentle movement backwards.
g. In cases where the patient is less than 5 years old, the laboratory will confirm the number of blood tubes needed for the autologous skin cell culture (MyOwn Skin™).

3.6 The sample of skin ("explant") is taken in this way:

a. Wearing gloves and using sterile gauze, thoroughly wash the area from which the explant will be taken with antibacterial soap, and then wipe the area with an alcohol pad.

b. A minimum of 0.5 ml of local anesthetic shall be used to generate a blister. With the syringe, infiltrate the area where the sample will be obtained.

c. With the scalpel blade and the sterile tweezers, cut parallel or slightly oblique, a partial skin sample (superficial, but guaranteeing that it has dermis) with an area of 1.0 cm² and a thickness between 0.30 mm - 0.65 mm approximately. See figure to the right for size reference. The silver top of the lidocaine bottle provided in the kit is approximately 1.0 cm^2.

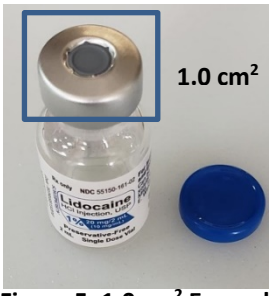

Figure 5. 1.0 cm^2 Example

d. Using the microcentrifuge tube supplied in the kit and taking care to not spill the medium contained within it, place the skin sample into the tube with attention to not touch the inner sides of the tube. Cover it immediately. If for any reason this vial arrives in the kit without the medium, immediately call BioLab Sciences (480) 207-1884 for a replacement vial. Do not use formaldehyde or any other liquid medium.

e. Cover the area where the sample is taken with a healing cream and the gauze dressing contained in the kit.

f. Place this microcentrifuge tube containing the skin explant in the return container (that came with the kit) along with the blood sample vials. The return container must be shipped back to BioLab Sciences.

Biotechnology Processing

From the aforementioned skin explant and blood samples, BioLab Sciences will grow up to three (3) 10cm² x 10cm² autologous skin sheets and return to the provider for application. If the patient requires more than 300 cm², the provider will submit another skin explant and blood samples per 300 cm² required.

Procedure Guide for Placement of MyOwn SkinTM

The wound area must be prepared prior to receiving the MyOwn Skin™ sheet(s). The area needs to be washed and received the respective debridement (if necessary), in order to have the best conditions.

Membrane placement

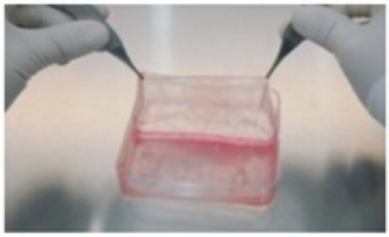

Figure 6A

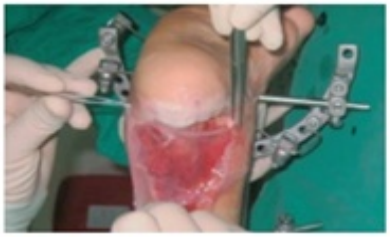

Figure 6B

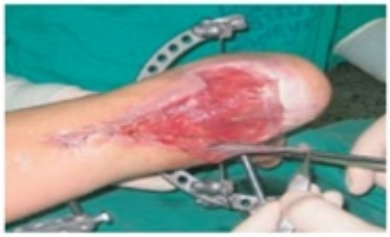

Figure 6C

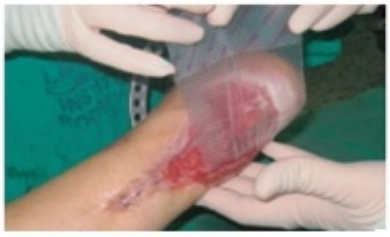

Figure 6D

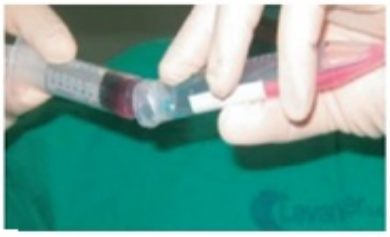

Figure 6E

Figure 6F

The following steps must be considered **before** placing the MyOwn Skin™ membrane:
a. Verify the patient ID is the same in the external packaging on all petri-dishes and conical tubes.

b. The patient to whom MyOwn Skin™ will be applied must have been previously controlled for excessive exudate and hemorrhage, and there should not be signs of infection or necrotic tissue, at the application site.

c. Verify that the sheet(s) packaging is completely sealed and in perfect condition.

d. Open the packaging in a horizontal position. The packaging and the tube with the patient's growth factors and cytokines should be opened aseptically.
The following steps must be considered **during** the placement of the MyOwn Skin™ membrane:
1. With the sterile tweezers take the membrane at two corners. See figure 6a

2. Apply on the area to be treated, **always with the upper face in contact with the wound**. NO sweeping should be done on the membrane and it must be completely adhered to the wound bed (without bubbles). See figure 6b.

3. The excess MyOwn Skin™ can now be folded onto itself or be trimmed. See figure 6c.

4. Cover the MyOwn Skin™ membrane with the porous silicone cover. See figure 6d.

5. After applying MyOwn Skin™ on the area to be treated, remove the cap from the tube that contains the patient's growth factors and cytokines. Use these growth factors and cytokines to hydrate sterile gauzes and place them onto the porous silicone cover with slight pressure to adhere them to the silicone. See figure 6e.

6. Cover the treated area with a bandage as appropriate. See figure 6f.

Continuous Wound Monitoring

7.1 Qualified personnel should check the treated area every 5-7 days, at least until day 21.

7.2 During each checkup, secondary healing dressings should be removed with care so as to not damage the MyOwn Skin™ membrane. It is also important to not remove the porous silicone cover during these checks.

7.3 Excess secretion from the wound should be cleaned with a dry dressing.

7.4 No other substance should be applied over the treated area.

7.5 Only after day 21, can the porous silicone cover be removed. On day 21, the attending physician will assess the level of tissue regeneration to determine if it is necessary to perform a new MyOwn Skin™ procedure. It is possible to experience some stinging in the area of application. If it is evident any sign of anomaly, unusual symptom or any other alteration, the treating doctor should be informed immediately.

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