



The Search for an Ideal Temporary Skin Substitute: *AWBAT* Plus, a Combination Product Wound Dressing Medical Device

E. Aubrey Woodroof, PhD, MBA,^a Richard P. Phipps, PhD,^b John E. Greenwood, MD,^c William Hickerson, MD,^d and David Herndon, MD^e

^aAubrey Inc, 5930 Sea Lion Pl, Carlsbad, CA; ^bLung Biology and Disease Program, University of Rochester School of Medicine and Dentistry, Rochester, NY; ^cBurns Unit, Royal Adelaide Hospital, South Australia; ^dFirefighters/Regional Burn Center, Memphis, TN; and ^eShriners Burn Hospital, Galveston, TX

Correspondence: awoodroof@aubreyinc.com

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Objective: To create an ideal temporary skin substitute. **Methods:** Temporary skin substitute (Biobrane, *AWBAT* and *AWBAT* Plus) porosity, 3D matrix, and biochemical composition were evaluated for impact on healing wounds (pain, fluid accumulation, infection, and time to heal). Twenty-four Sprague-Dawley [H1a: (SD) CVF] rats were used to measure the histology of healed full-thickness wounds. Tissue culture methods were used to measure the influence of Immuno-10 on human dermal fibroblasts: proliferation, collagen, and alpha-smooth muscle actin. **Results:** In full-thickness wounds (rats), histological evidence suggests better reepithelialization of wounds covered with *AWBAT* Plus than those with *AWBAT*. Tissue culture techniques revealed that key biological additives to *AWBAT* Plus stimulated human dermal fibroblast growth, collagen synthesis, and alpha-smooth muscle actin production. Human keratinocyte growth was also promoted by a key biological element in *AWBAT* Plus. Interestingly, human mesenchymal stem cells grew well on the *AWBAT* Plus membrane. Clinically, *AWBAT* Plus protected widely meshed autograft, which healed more uniformly and faster than cadaver allograft. **Conclusion:** *AWBAT* Plus shows a great promise as a major advancement in wound care.

The search continues for a safe and cost-effective ideal temporary skin substitute that functions as well as, or better than, the gold standard (cadaver allograft). Minimal functional requirements are as follows: (a) protect excised deep wound surfaces until autografting takes place, resulting in equal or greater autograft and equal or fewer complications (infection or dressing changes due to rejection, nonadherence, etc); (b) protect excised wounds covered with widely meshed autograft until the interstices heal; and (c) limit conversion of mid-dermal burns to full-thickness wounds

Table 1. *Types of AWBAT Plus and intended use*

AWBAT Plus (6 biologicals)	Intended Use
S	Partial-thickness burn
D	Donor site
M	Widely meshed autograf
Gloves	Hand burns

Novel and meaningful terminology¹ was proposed for wound-dressing products referred to as *bioengineered skin*, *tissue-engineered skin*, and *bioengineered skin equivalents*. “Bioengineered Alternative Tissue” applies to products like Biobrane, Integra, TransCyte, Alloderm, Apligraf, Orcell, Oasis, Epicel, Gamma Graft, Laser skin, AWBAT, and AWBAT Plus. Composition may include biologicals, such as collagen (various types and forms), chondroitin 4 and/or 6 sulfate, vitamin C or E, growth factors and cytokines, and living cells. AWBAT Plus was cleared by the Food and Drug Administration (FDA) in January 2010 and declared to be a “Combination Product” wound dressing device containing properties of both a drug and a device.

Desirable properties of an ideal temporary skin substitute or bioengineered alternative tissue^{2,3} include adherence, moisture permeability control, safety, pain management, flexibility, transparency, stability, cost-effectiveness, and ease of application and removal. Greenwood et al⁴ have shown Biobrane to possess these properties. The desirable properties are expanded for AWBAT Plus: stimulate fibroblast proliferation, increase collagen synthesis, provide better drapability, and can be preformed into anatomically shaped gloves, which enable efficient and precise fit to the hand wound surface, reducing time for wound closure and anesthesia.

Six biological components are added to the 3D matrix of AWBAT Plus to increase its hydrophilic and hygroscopic properties enabling a moist, but not wet environment, to heal more rapidly. Vitamin C and E are natural antioxidants. Hypoallergenic type I collagen peptide and chondroitin 4 and 6 sulfate contribute hydrophilic properties.³ Immuno-10 is a purified carbohydrate fraction of Aloe vera, which increases the hydrophilic and hygroscopic nature of the 3D matrix. Different porosities (5.5% or 7.5%) accommodate wounds with varying exudative characteristics.

AWBAT product configurations are described later. The distinction between AWBAT and AWBAT Plus is that the former contains collagen peptide as the sole biological component, same as Biobrane; AWBAT Plus contains 6 biological components. The S, D, and M configurations distinguish the level of porosity and adherence needed for the type of wound managed.

METHODS AND RESULTS

Preliminary evaluation of AWBAT-D on donor sites and AWBAT-S on superficial burns⁵ showed that donor sites treated with AWBAT-D healed on average in 11.2 days. Pain rating after 24 hours was 1.2. Average number of days to healing of superficial burns treated with AWBAT-S was 8.1. Pain rating after 24 hours was 1.5. There was no infection and no hematoma/seroma.

Table 2. Summary of Epidermal Healing

Grade of Reepithelialization*	<i>AWBAT-S Plus (Test Article)</i> (N = 12 animals)					<i>AWBAT-S (Control Article)</i> (N = 12 animals)				
	None	Minimal	Partial	Most	Complete	None	Minimal	Partial	Most	Complete
Incidence	0	0	5	4	3	0	2	3	7	0

Sood et al⁶ compared *AWBAT-D* with Acticoat and BGC Matrix. In 13 donor sites, there was no fluid accumulation under *AWBAT-D* and no infections. There were 4 infections with Acticoat and 7 infections with BGC Matrix (19 wounds each). Average healing time (days) was: *AWBAT-D* 10.8, BGC Matrix 12.4, Acticoat 14.4.

REEPITHELIALIZATION OF FULL-THICKNESS WOUNDS IN RATS

A study⁷ was conducted to evaluate systemic toxicity and wound healing of the *AWBAT-S Plus* wound dressing following application to full-thickness dermal wounds. Twenty-four Sprague-Dawley [H1a: (SD) CVF] rats were randomly assigned to either a comparator control (*AWBAT* wound dressing) or test article (*AWBAT-S Plus*) group, composed of 6 animals per sex per group. On day 0, animals were anesthetized and 2 cm by 2 cm full-thickness dermal wounds were aseptically created on each side of the back (2 wounds per animal). Both wounds were covered with either the test or the control article (one article section per wound site). Body weights and detailed examinations for clinical signs were conducted at randomization, and at days 8 and 15. On day 15, blood was collected for hematology and serum chemistry analysis and the animals were euthanized. A gross necropsy was conducted; wound sites and selected organs were collected and weighed. Microscopic evaluation of the wound sites and selected major organs was performed by a veterinary pathologist.

There were no clinical signs, changes in body or organ weight, clinical laboratory tests, or microscopic findings indicative of systemic toxicity to *AWBAT-S Plus*. The inflammatory cellular response consisted of mild-to-moderate infiltrates of polymorphonuclear cells, lymphocytes, and macrophages. Rare multinucleated giant cells and mineralized tissue debris were also present, and there was no evidence of necrosis. Crusts composed of degenerating inflammatory cells and keratin were frequently present on the wound surface. Granulation tissue formation and fibrosis in the dermis were generally minimal to mild. Reepithelialization of the wound sites occurred in the control and test animals (Figs 1-4), but tended to be more complete in the test animals of both sexes (Table 2). For the control and test articles, males tended to have more complete reepithelialization than did the females.

INFLUENCE OF IMMUNO-10 ON HUMAN DERMAL FIBROBLASTS: PROLIFERATION, COLLAGEN, AND ALPHA-SMOOTH MUSCLE ACTIN

Fibroblasts are a key structural cell in skin and are crucial for wound repair. Studies were performed to test whether or not Immuno-10, a key component of *AWBAT Plus*, possessed beneficial properties in terms of stimulating fibroblast proliferation.⁸ Our initial studies

looked at whether various amounts of Immuno-10 had any obvious deleterious effects on the human dermal fibroblasts. Fibroblasts were cultured in 96 well plates with Immuno-10 (1 mg/mL) for up to 7 days. No signs of cell toxicity such as rounding or loss of adherence were observed. Next, the 3H-thymidine assay was used to evaluate fibroblast proliferation (see Fig 5). 3H-thymidine is taken up into newly synthesized DNA in proliferating cells. Cells were cultured in low serum (0.1%) to render them moderately quiescent. Addition of Immuno-10 at 1.0 or 0.1 mg/mL stimulated cell proliferation at least as well as the positive controls (10% serum or transforming growth factor beta [TGF β]).

Immuno-10 was also tested for ability to stimulate collagen production.^{9,10} Dermal fibroblasts were cultured for 48 hours with Immuno-10 (0.1 mg/mL) and collagen production was detected using immunofluorescence (see Fig 6). Transforming growth factor beta, a known potent inducer of collagen synthesis, was used as a positive control. Interestingly, Immuno-10 did stimulate collagen synthesis although not as strongly as TGF β . This ability of Immuno-10 to increase collagen could be beneficial and could result in faster wound healing. Another aspect of wound healing involves the synthesis of alpha-smooth muscle actin (α -SMA) by fibroblasts that convert to contractile cells called *myofibroblasts*. These cells are important for wound contraction. We detected α -SMA in fibroblasts treated with the positive control cytokine TGF β and also when the cells were exposed to Immuno-10 (albeit less strongly than TGF β). Fibroblasts that ramp up their production of collagen and α -SMA may result in faster wound healing.

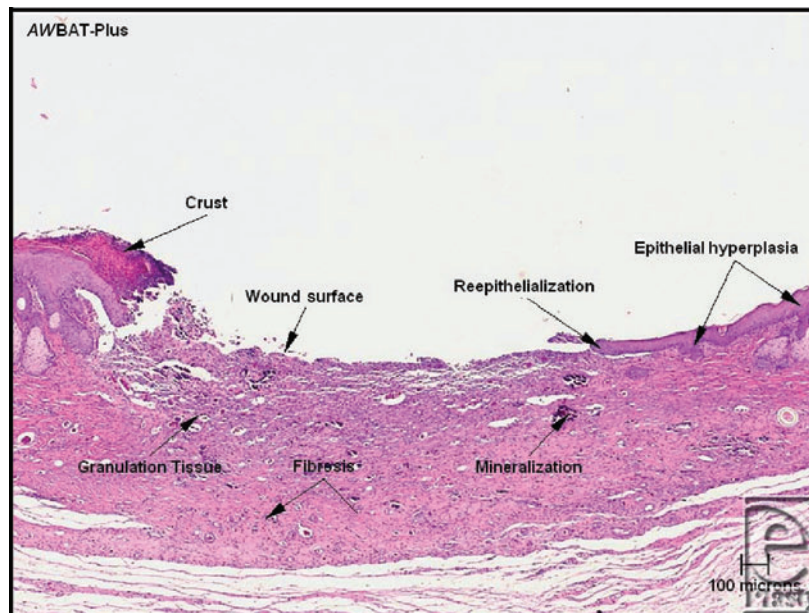


Figure 1. Most of the wound surface is reepithelialized and healing of the underlying dermis is nearly complete. Small foci of mineralized debris are present in the dermis. A small crust containing inflammatory cells is adhered to the epithelium at the edge of the unhealed wound surface (H & E stain, 40 \times magnification).

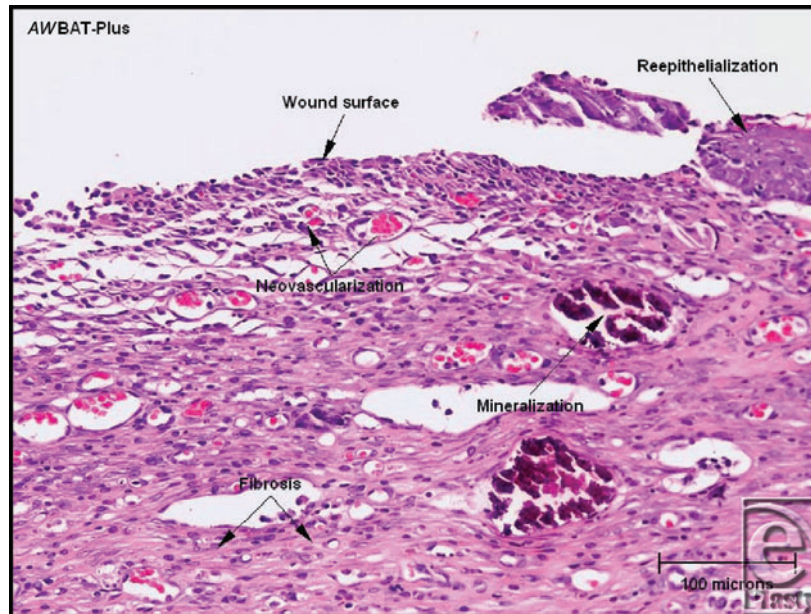


Figure 2. Granulation tissue with interspersed inflammatory cells is present in the superficial dermis underneath the wound surface, and neovascularization is prominent (H & E stain, 200× magnification).

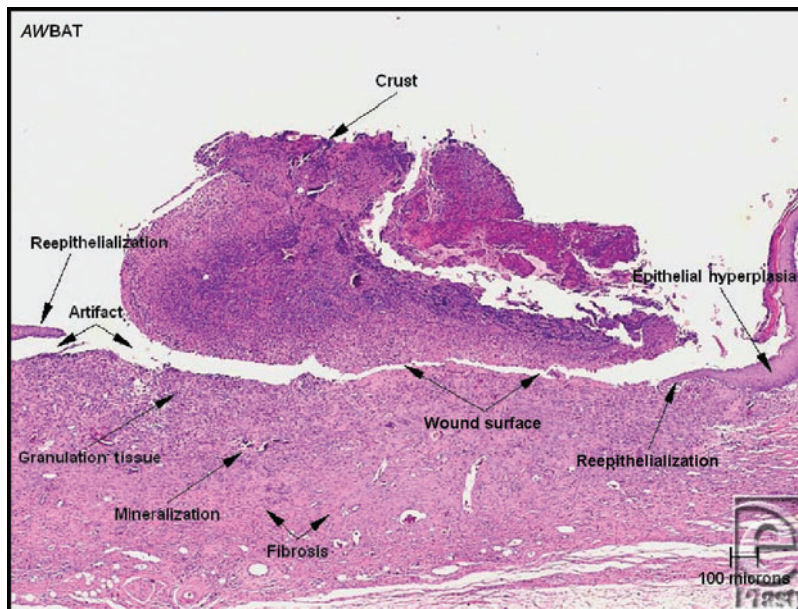


Figure 3. The wound surface is partially reepithelialized, and healing of the underlying dermis is nearly complete. Small foci of mineralized debris are present in the dermis, and a large crust containing inflammatory cells covers the unhealed wound surface (artifactual space present) (H & E stain, 40× magnification).

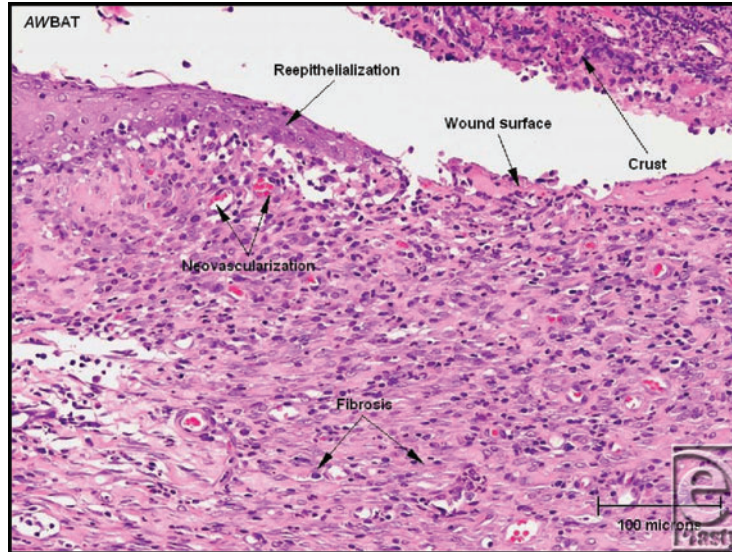


Figure 4. Granulation tissue with prominent neovascularization and interspersed inflammatory cells is present in the superficial dermis underneath the wound surface and adjacent epithelium. Many degenerating inflammatory cells are present in the cellular crust (H & E stain, 200× magnification).

Human dermal fibroblast proliferation (72 hr ³H Thymidine incorporation)

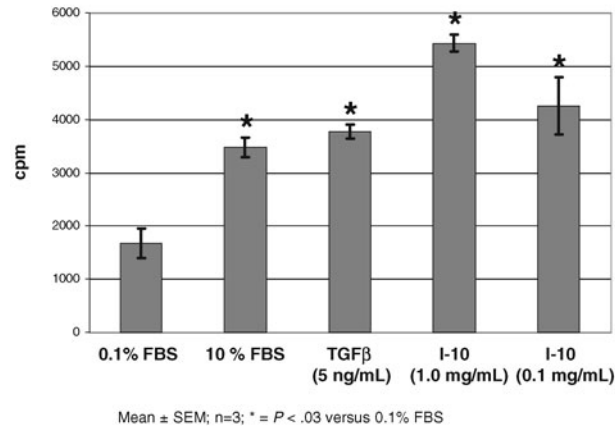


Figure 5. Immuno-10 stimulates human dermal fibroblast proliferation. Human dermal fibroblasts were maintained in complete DMEM 10% FBS or starved for 48 hrs in DMEM 0.1% FBS. Starved cultures were stimulated with TGFβ (5 ng/mL) or Immuno-10 (I-10) (1.0 or 0.1 mg/mL) for 72 hours are compared to nonstimulated control. Tritiated thymidine labeling for all cultures was during the final 24 hours. Statistics were evaluated using the Student *t* test. DMEM indicates Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum.

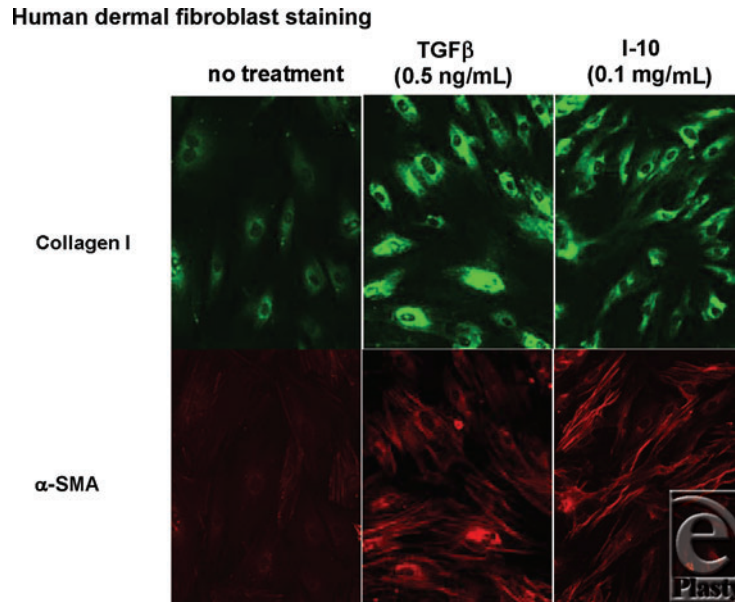


Figure 6. Immuno-10 stimulates fibroblast collagen and α -SMA production. Human dermal fibroblasts were maintained in complete DMEM 10% FBS or starved for 48 hours in DMEM 0.5% FBS. Starved cultures stimulated with TGF β (0.5 ng/mL) or Immuno-10 (I-10) (0.1 mg/mL) for 48 hours. Cells were fixed and labeled with fluorescent antibodies for collagen I and α -SMA. Cells were observed with a digital fluorescence microscope and images acquired by using the same intensity and photodetector gain to allow quantitative comparisons of relative levels of immunoreactivity between samples. DMEM indicates Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum.

INFLUENCE OF IMMUNO-10 ON HUMAN KERATINOCYTE PROLIFERATION

Human keratinocytes were also tested for their ability to be influenced by Immuno-10 in terms of their rate of proliferation by using the ³H-thymidine incorporation assay (Fig 7). Here, the keratinocytes were cultured with a low, suboptimal amount of a special keratinocyte growth medium (medium 154 at 0.1 \times concentration) with or without Immuno-10. Interestingly, keratinocytes cultured in the suboptimal media supplemented with Immuno-10 grew much better than those in 0.1 \times medium 154. In fact, the cells proliferated as well as those grown in full strength medium 154.

HUMAN MESENCHYMAL STEM CELLS GROW ON *AW*BAT-S-PLUS

Human mesenchymal stem cells (MSC), derived from human cord blood, were purchased as a viable culture (Vitro Biopharma, Golden, CO) and were maintained in low serum mesenchymal stem cell culture medium without antibiotics or antimycotic. To test the growth on the *AW*BAT-S Plus (nylon mesh side up) 5.0×10^5 MSC were plated onto the

material in the bottom of a 24 well plate. A small amount of sterile vacuum grease was applied to the bottom of the dish to keep the *AWBAT* Plus adhered to the plate bottom. Cell growth was monitored for up to 10 days at which point the *AWBAT* Plus-Cell complex was transferred, nylon mesh side up, to a glass bottom, 24 well plate and images captured (Fig 8, panels A and B).

Human epidermal keratinocyte proliferation is stimulated by Immuno-10

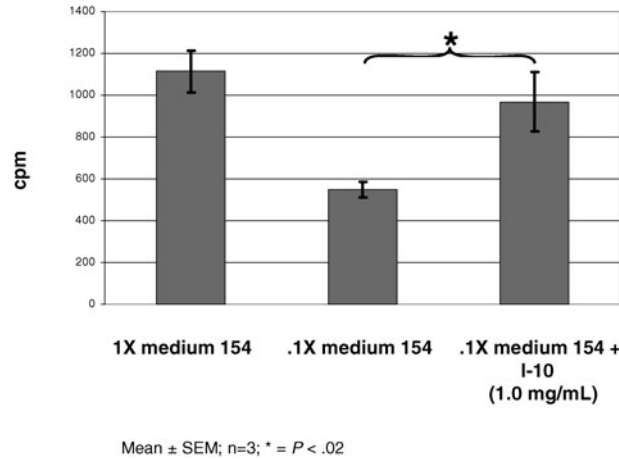


Figure 7. Human keratinocyte proliferation is enhanced by Immuno-10. Human epidermal keratinocytes were maintained in complete (1×) medium 154 or starved for 48 hours in 0.1× medium 154. Starved cultures stimulated with Immuno-10 (I-10) (1.0 mg/mL) for 72 hours are compared to nonstimulated control. Tritiated thymidine labeling for all cultures was during the final 24 hours. Statistics were evaluated by using the Student *t* test.

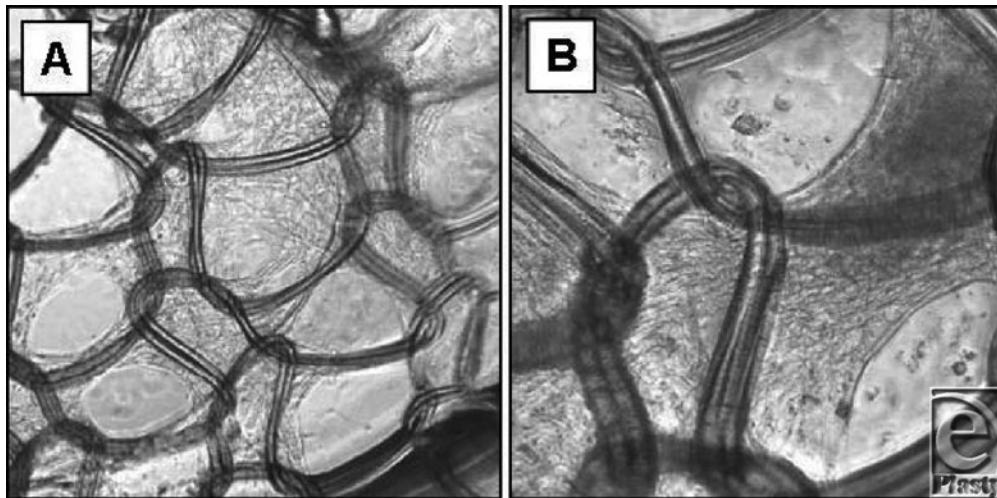


Figure 8. Human mesenchymal stem cells growing on *AWBAT*-S Plus. Panel A shows cells growing well on the backing material and in some cases growing over the mesh (original magnification 100×). Panel B shows a higher magnification (200×) of a different area.



Figure 9. Sixty percent total body surface area (TBSA) third degree postoperative day 10.



Figure 10. Sixty percent total body surface area (TBSA) third degree postoperative day 42.

CLINICAL EXPERIENCE WITH *AWBAT PLUS*

AWBAT Plus has the potential to take wound coverage to the next level. It provides not only an optimal milieu but also a stimulus to promote active wound healing. With this in mind *AWBAT-M Plus* was used to cover 4:1 meshed autografts to the upper extremities of a 26-year-old man. Because of the lack of donor sites and no finding for cultured epithelial autografts, the lower extremities were grafted with 5:1 meshed autografts that were covered with allograft meshed 1:1.

Two weeks later, the upper extremities were reexcised and covered with 4:1 meshed autograft. Since *AWBAT Plus* was now available, it was used as a protective barrier with factors to accelerate reepithelialization. The *AWBAT Plus*-treated sites were reepithelialized by 14 days; yet, the allograft-covered sites on the lower extremities still had open areas at 4 weeks. Furthermore, there was excellent adhesion of the *AWBAT Plus*, there was no fluid accumulation, and it was easily removed between the third and fourth weeks postoperatively. Figures 9 and 10 show the results at 6 weeks. He continues to have a very

satisfactory aesthetic appearance. The lower extremities have more hemosiderin deposits within the dermis and are more heavily pigmented. In addition, there is an excellent range of motion of the upper extremities and the grafts are very durable.

The 2 differences were the 4:1 mesh and 5:1 mesh, and *AWBAT*-M Plus used to protect the grafts on the upper extremities and 1:1 meshed allograft on the lower extremities. A single-case study seldom indicates a true solution, but the impressive results shown in the photographs warrant further investigation.

DISCUSSION

Biobrane has been clinically available for 3 decades and found to be an effective temporary skin substitute.^{2,4,12,14} El-Khatib et al¹¹ showed that Biobrane has better long-term adherence on an excised wound than pigskin (EZ-Derm, Genetic Laboratories, St Paul, Minnesota); 10 days versus 3 to 4 days. Purdue et al¹² showed Biobrane to be equally effective as frozen allograft (gold standard) on excised wounds with respect to autograft takes and infection. Barret et al¹³ showed Biobrane to be superior to 1% silver sulfadiazine with respect to reduction of (a) pain and pain medications, (b) wound healing time, and (c) length of hospital stay. Whitaker et al¹⁴ have shown Biobrane to be effective for a variety of clean wounds. Hansbrough¹⁵ showed dodecylamine responsible for hypersensitivity reactions to Biobrane.

Frequent fluid accumulation⁴ beneath Biobrane on donor sites presents an opportunity to improve the performance by making the membrane more porous and free of cross-linking agents (cyanuric chloride, dodecylamine). Methods were developed for making *AWBAT* with a uniform 3D nylon matrix physically bonded to a precision porous silicone membrane coated with hypoallergenic type I porcine collagen peptide.

Aloe vera has been used for centuries for burn wound care. Maenthaisong et al¹⁶ showed that Aloe vera-treated burn wounds resulted in significantly faster burn wound healing. A purified fraction of Aloe vera (Immuno-10) is a key component of *AWBAT* Plus and is shown herein to stimulate the proliferation of human skin fibroblasts and keratinocytes. In addition, stimulation of human fibroblast production of collagen and smooth muscle actin may all be desirable properties for healing of burned skin. Of interest is the finding shown here that human mesenchymal stem cells grow well on *AWBAT* Plus. This finding shows that in the future, stem cells themselves or stem cells differentiated to different kinds of skin cells could readily be used with *AWBAT* Plus or be already attached to *AWBAT* Plus to serve as a living “skin” replacement.

CONCLUSION

AWBAT and *AWBAT* Plus represent a new generation of Temporary Skin Substitutes intended to better satisfy the desired attributes of an ideal temporary skin substitute.¹⁷⁻²² *AWBAT* was cleared by the FDA (February 2009) for use on superficial wounds, donor sites, and meshed autograft and was initially evaluated on a total of 69 wounds. Low pain was noted when used to protect superficial burns and donor sites. Importantly, there was no fluid accumulation and no infection.

AWBAT Plus was cleared by the FDA in January 2010 as a “Combination Product Medical Device.” Immuno-10 has the ability to stimulate fibroblast and keratinocyte

proliferation and fibroblast collagen synthesis. Human mesenchymal stem cells grow well on *AWBAT* Plus. Reepithelialization of rat dermal wounds tended to be more complete in animals treated with *AWBAT*-S Plus than in those treated with *AWBAT*-S.

Preliminary observation shows *AWBAT* Plus functions well in humans to protect widely meshed autograft.

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