

## 1. TITLE PAGE

# INTEGRATED CLINICAL AND STATISTICAL REPORT

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## **Controlled Randomized Multi-Center Study of the Effects of Composite Cultured Skin Containing a Collagen Matrix Seeded with Allograft Cells on the Management of Split Thickness Donor Sites in Burn Patients**

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**Study Device:** Composite Cultured Skin (Ortec International, Inc., New York, New York)

**Control Device:** Biobrane<sup>®</sup> L (Bertek Pharmaceuticals, Inc., Sugarland, Texas)

**Indication:** Wound closure of split thickness skin donor sites in burn patients

**Study Design:** Prospective, randomized, controlled, multi-center

**Protocol Number:** 98-004/OR

**Sponsor:** Ortec International, Incorporated  
Audubon Business and Technology Center  
3960 Broadway  
New York, New York 10032

**Sponsor Contact:** Steven R.Peltier

**Study Report Contact:** Liza Moore

**Date Study Initiated:** May 3, 1999

**Date Study Completed:** October 25, 2000

**Date of Study Report:** February 28, 2001

**2. SYNOPSIS**

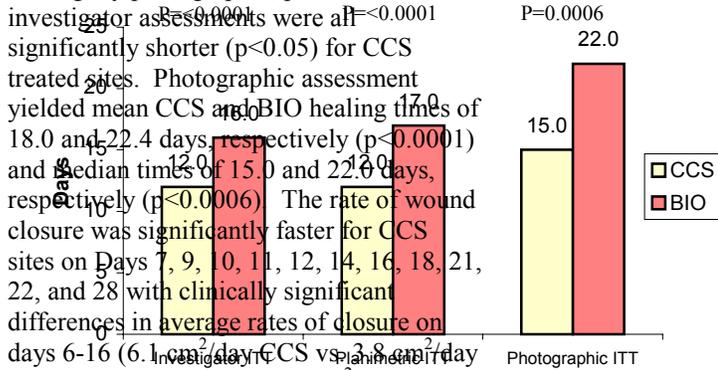
<b>Company:</b> Ortec International, Incorporated	
<b>Title:</b> Controlled Randomized Multi-Center Study of the Effects of Composite Cultured Skin Containing a Collagen Matrix Seeded with Allograft Cells on the Management Of Split Thickness Donor Sites in Burn Patients	<b>Trial Number:</b> 98-004/OR
<b>Investigator:</b> Multicenter	<b>Country:</b> USA
<b>Publications (Reference):</b> None	
<b>Trial Period:</b> Start: May 3, 1999 End : October 25, 2000	<b>No. of study centers:</b> 12
<b>Objectives:</b> To examine the safety and efficacy of Composite Cultured Skin (CCS) in facilitating timely wound closure of split thickness skin donor sites in burn patients, compared to a standard of care dressing (Biobrane L <sup>®</sup> ).	
<b>Trial Design:</b> Prospective, randomized, controlled	
<b>Number of patients entered:</b> 82 <b>Number of patients randomized:</b> 82 <b>Number of patients treated:</b> 82	
<b>Methodology:</b> The study was a prospective, controlled, randomized multi-center study involving patients requiring conventional split thickness skin autografting for the management of burn injuries. The study had a matched pairs design, where each patient had two designated donor sites of equivalent surface area and depth. Each site was randomized to receive a single treatment of either the control dressing (Biobrane-L) or investigational device (CCS).	
<b>Main Criteria for Inclusion:</b> Patients ≥12 months of age with burns involving 10-80% of total body surface area and a life expectancy of ≥6 weeks. Donor sites were to be virgin skin sites on non-articulated surfaces with total donor surface area of 72-360 cm <sup>2</sup> for patients ≥3 years old and 36-180 cm <sup>2</sup> for patients <3 years old. Split thickness autografts from donor sites were to be 0.006-0.014 in with both sites of the same depth.  Exclusion criteria included: sepsis, pregnant or lactating females, severe inhalation injury, history of allergy to collagen material, insulin-dependant diabetics with HbA1c >10%, use of systemic corticosteroids <30 days prior to entry, immunosuppressive/radiation/chemotherapy <3 months prior to entry, injury severity scores of >40 for patients 15-49 yrs, >29 for patients 45-65 yrs, >25 for patients >65 yrs, and for patients <15 yrs a Pediatric Trauma Score ≤5 or Pediatric Glasgow Coma Score <8. Additionally, concurrent use of investigational product on burn sites or previous participation in a skin donor site management trial was exclusionary.	
<b>Investigational Product:</b> CCS Treatment: Up to four allogeneic bilaminar cultured skin substitute devices, each measuring 36 cm <sup>2</sup> to 45 cm <sup>2</sup> . Mode of Administration: Topical application Formulation code: none Duration of Treatment: Single application with a 24-week follow up and bi-annual follow-up visits.	
<b>Comparator Product:</b> Biobrane-L Treatment: One area measuring up to 144 cm <sup>2</sup> . Mode of Administration: Topical application Formulation code: 0514-0096-02 Duration of treatment: Single application with a 24-week follow up and bi-annual follow-up visits.	
<b>Criteria for Evaluation:</b> The primary efficacy outcome was time to 100% wound closure (complete re-epithelialization) as assessed by blinded photographic analysis. Secondary efficacy variables were the time to 100% wound closure as assessed by computerized planimetric analysis and investigator assessment, percentage of wound closure over time and readiness for recropping.  Safety variables were the infection rate of the donor site wound, the durability of wound closure as assessed by blistering/breakdown, the presence of pain, itching at the treatment site, scar outcome and adverse events.	

**Summary Results:**

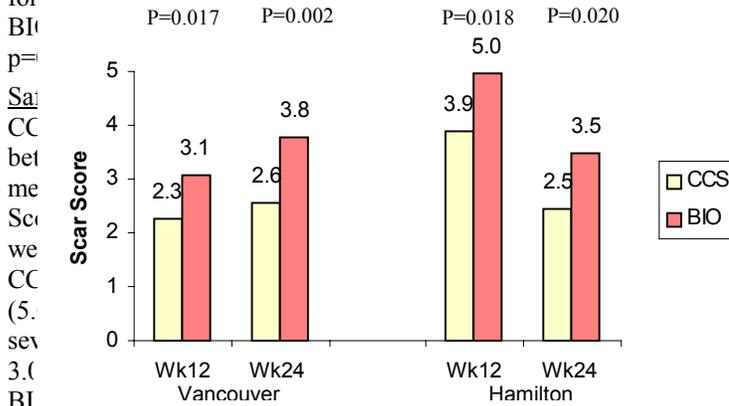
Efficacy

The mean and median times to 100% wound healing, by photographic, planimetric, and investigator assessments were all significantly shorter ( $p < 0.05$ ) for CCS treated sites. Photographic assessment yielded mean CCS and BIO healing times of 18.0 and 22.4 days, respectively ( $p < 0.0001$ ) and median times of 15.0 and 22.0 days, respectively ( $p < 0.0006$ ). The rate of wound closure was significantly faster for CCS sites on Days 7, 9, 10, 11, 12, 14, 16, 18, 21, 22, and 28 with clinically significant differences in average rates of closure on days 6-16 ( $6.1 \text{ cm}^2/\text{day}$  CCS vs.  $3.8 \text{ cm}^2/\text{day}$  BIO) and days 17 to 32 ( $4.0 \text{ cm}^2/\text{day}$  CCS vs.  $2.7 \text{ cm}^2/\text{day}$  BIO).

**Median Time to 100% Wound Closure (ITT Population)**



**Scarring Severity**



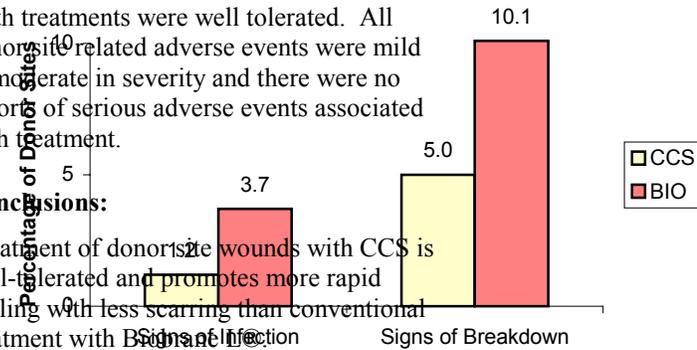
increase in itching for CCS sites (72.2% CCS vs. 68.8% BIO).

**Signs of Infection and Breakdown**

Both treatments were well tolerated. All donor-site related adverse events were mild to moderate in severity and there were no reports of serious adverse events associated with treatment.

**Conclusions:**

Treatment of donor-site wounds with CCS is well-tolerated and promotes more rapid healing with less scarring than conventional treatment with BIO.





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#### 4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ATC	Area Therapy Code per WHO Medication Dictionary
BIO	Biobrane L®
Biobrane-L	Biobrane L®
CCS	Composite Cultured Skin
cm	centimeter
CRF	Case Report Form
D	day(s)
DC	Drug Code per WHO Medication Dictionary
DRF	Data Resolution Form
FIO <sub>2</sub>	Inspired Oxygen Concentration
GCP	Good Clinical Practice
IEP	Independent Ethics Committee
IRB	Institutional Review Board
ISS	Injury Severity Score
ITT	Intent-to-Treat
K-M	Kaplan-Meier
mon	month(s)
PEEP	Positive End Expiratory Pressure
PGCS	Pediatric Glasgow Coma Score
PP	Per Protocol
PTS	Pediatric Trauma Score
SAS	Statistical Analysis System
SD	Standard Deviation
TBSA	Total Body Surface Area
wk	week(s)
yr	year(s)

## **5. ETHICS AND GENERAL STUDY CONDUCT CONSIDERATIONS**

### **5.1 INDEPENDENT ETHICS COMMITTEE (IEC) OR INSTITUTIONAL REVIEW BOARD (IRB)**

The trial protocol and amendments were reviewed and approved by an independent Ethics Committee/ Institutional Review Board. A list of all IEC's or IRB's consulted is provided in Appendix 16.4.

### **5.2 ETHICAL CONDUCT OF THE STUDY**

The trial was conducted in accordance with the ethical principles originating from the Declaration of Helsinki and GCPs and in compliance with United States (federal) and local regulatory requirements.

### **5.3 PATIENT INFORMATION AND CONSENT**

All patients in this study were completely informed, in accordance with GCPs and local regulatory authority requirements concerning the pertinent details and purpose of the study. A written consent form, approved by an IRB, was supplied by the investigators and was to be understood and signed by each patient prior to initiating any study procedures. The investigators were responsible for maintaining each patient's consent form in the study file and providing each patient with a copy of the consent form. See Appendix 16.4 for a sample patient consent form.

## 6. INVESTIGATORS AND TRIAL ADMINISTRATIVE STRUCTURE

### Investigators

- Bruce M. Achauer, M.D., Irvine Medical Center, Orange, CA.
- John Dawson, M.D./ Joseph Still, M.D., Doctors Hospital, Augusta, GA.
- Paul M. Glat, M.D., St. Christopher's Hospital for Children, Philadelphia, PA.
- David Greenhalgh, M.D., Shriners Hospital for Children, Sacramento, CA.
- John A. Griswold, Texas Tech Medical Center, Lubbock, TX.
- Richard Grossman, M.D., FACS, Sherman Oaks Hospital Research Institute, Sherman Oaks, CA.
- David M. Heimbach, M.D., University of Washington Burn Center, Seattle, WA.
- Arnold Luterman, M.D., FRCS, FACS, University of South Alabama Medical Center, Mobile, AL.
- David W. Mazingo, M.D., University of Florida, Gainesville, FL.
- Bruce Shack, M.D., Vanderbilt University Medical Center, Nashville, TN.
- Paul Silverstein, M.D., Integris Baptist Burn Center, Oklahoma City, OK.
- Roger W. Yurt, M.D., New York Presbyterian Hospital, New York, NY.

### Ortec International, Inc.

- Steven R. Peltier, Vice President of Clinical and Regulatory Affairs, responsible for clinical and regulatory management
- Suzanne Schwartz, M.D., Medical Director, responsible for overall clinical management and serious adverse events (SAE)
- Liza Moore, Clinical Project Manager, responsible for overall coordination of clinical trial
- Kathy Tygum, Clinical Project Manager, responsible for overall coordination of clinical trial
- Kenya Edmondson, Quality Assurance Manager, responsible for quality assurance audits of study of processes and data

- Danny Radonjic, Head of Pharmaceutical Unit, responsible for labeling, shipping and destruction of all clinical supplies

#### Contract Research Organization

- The Phoenix-responsible for monitoring at clinical sites for approximately one half of the study period.
- Target Research Associates-responsible for data management for duration of study and Regulatory Affairs for a portion of the study.
- Independent Clinical Research Monitors-responsible for monitoring at the clinical sites for the second half of the study period.

Appendix 16.3 contains a list of the investigators and other persons whose participation materially affected the conduct of the study.

## **7. INTRODUCTION**

Significant morbidity is associated with split thickness skin donor sites, namely infection and delayed healing. The presence of a donor site infection will delay healing and can result in conversion to a full thickness defect<sup>1</sup> that will then warrant excision and autograft coverage. Pain and discomfort at the donor site is a routine complaint of the patient, often worse than at the grafted site. Also, long-term scar outcome of the donor site, particularly with regard to pigmentation and texture is too often ignored but is not of minimal consequence. Although they are often given less consideration than the primary burn wounds undergoing surgical tangential excision, skin graft donor sites warrant efforts directed at improved wound management. The advantages of accelerated skin graft donor site healing are myriad particularly in the burn population.

Minimizing donor-site associated problems is an issue of cost-effectiveness as well. Massive surface area injuries are associated with decreased availability of viable donor sites. One of the options available to the burn surgeon faced with this challenge is

“recropping” or serial use of the same donor site after its complete re-epithelialization. Recropping has its limitations because the waiting period for maturation of donor site healing so as to yield a technically easy to handle conventional split thickness skin graft which functions effectively in facilitating wound closure can take as long as 3-5 weeks. This delay can influence the likelihood for survival. Thus, for patients with extensive body surface area involvement, the ability to recrop or re-harvest donor sites expediently will hasten permanent coverage of injured body surface areas<sup>2</sup>, may result in a shortened intensive care unit stay and even a decrease in length of hospitalization.

Despite the importance of donor site wound management there is no consensus among burn specialists regarding standard dressings for donor site coverage<sup>3</sup>. Cost, convenience, availability, ease of application, conformability and initial adherence are only some of the issues, which are factored into consideration when evaluating the advantages and shortcomings of specific dressings. Other critical factors include the purported rapidity with which healing occurs, the degree of pain or discomfort associated with its use at rest as well as during dressing care and wound intervention, the ease of removal and its capacity for absorption of drainage and its associated incidence of infections.<sup>2</sup>

Options for donor site management range from the open technique to semiopen, occlusive and semioclusive dressing types. The open technique is certainly the least expensive but it is also painful due to adherence and is associated with prolonged healing times.<sup>4,5</sup> Semiopen dressing materials include fine mesh gauze, vaseline gauze and Xeroform gauze. Success of this technique requires adherence of the dressing material to permit healing beneath it. There is, however, significantly less comfort since the adherence of the dressing to the open wound predisposes the patient during dressing care to damage of newly formed epithelium. The occlusive technique includes polyurethane film dressings (i.e., Opsite<sup>®</sup> [Smith & Nephew PLC]). This type of dressing provides a moist environment conducive to wound healing and is indeed non-adherent. Its disadvantages, however, include its relative non-permeability, which results in the

accumulation of exudate and hematoma, requiring earlier and more frequent wound intervention. Other occlusive dressing materials have an additional, separate outer impermeable layer into which exudate can accumulate but the size of the treatment area and dressing cost are the limiting factors in its use. Use of hydrocolloid dressings result in exudate accumulation and these types of materials are characterized by their predisposition to dissolve into a viscous paste that can be difficult to remove and therefore damaging to the underlying epithelializing bed.<sup>2</sup>

During the last ten years, evolution in the field of engineered skin substitutes/equivalents have brought about the recognition that a dermal component is critical to promoting durable wound healing. Leigh et al.<sup>6</sup> has demonstrated that there is a complex interaction between cell-cytokine-receptor-extracellular matrix. This cell signaling occurs because the activated keratinocytes in the biologic substitute produce neurotrophic, angiogenic and other growth factors, which influence the proliferative and migratory activity of dermal fibroblasts. Regulation of the mesenchyma by the epidermal keratinocytes is thought to play an important role in normal development, and regeneration of the epidermis and cutaneous wound repair.

Composite Cultured Skin (CCS) is an allogeneic bilaminar cultured skin substitute containing donor keratinocytes and fibroblasts obtained from neonatal foreskins by an enzymatic release process and then seeded onto a cross-linked bovine collagen sponge matrix. The cell inhabited sponge is then maintained in culture for 10-15 days to encourage proliferation and migration of the allogeneic cells throughout its interstices.

This study was designed to evaluate the safety and efficacy of CCS in facilitating timely wound closure of split thickness donor sites in severely injured burn patients compared to a standard of care commercially available dressing. The focus of this study was to determine a difference, if any, in time to complete healing, as well as in the quality of the healed wound (i.e., its readiness for recropping), the rate of infection at the donor treatment site, the reporting of pain or discomfort at the donor treatment site and scar outcome.

## **8. STUDY OBJECTIVES**

The objective of this multi-center randomized study was to examine the safety and efficacy of Composite Cultured Skin (CCS) in facilitating timely wound closure of split thickness skin donor sites in burn patients, compared to a standard care dressing, Biobrane-L.

## **9. INVESTIGATIONAL PLAN**

### **9.1 OVERALL STUDY DESIGN AND PLAN**

This study was a prospective, active controlled, randomized multi-center study involving patients requiring conventional split thickness skin autografting for the management of burn injuries. The study incorporated a matched pairs design (i.e., each patient had two designated donor sites of equivalent surface area and depth). Each donor site was randomized to receive a single treatment of either the control dressing (Biobrane-L) or investigational device (CCS). The goal was to enroll approximately 100 patients in up to 12 burn centers in order to have 75 patients complete the trial and provide data for analysis.

The two donor sites were evaluated for time to complete wound closure (i.e., 100% re-epithelialization) over the 24 week post surgery period by three different methods: blinded photographic review, blinded computerized planimetric measurements of wound size, and clinical/physical evaluation of the donor sites by the investigator. Additionally, during the 24-week post surgical period, the two donor sites were assessed for pain, itching, signs of breakdown, and readiness for recropping. Scarring severity at each donor site was assessed 12 and 24 weeks after surgery by two different methods: blinded photographic review and clinical assessment by the investigator. Concomitant medications and adverse events were assessed at each visit.

Reharvest of either the investigational treatment site or the control treatment site was performed at the discretion of the investigator after the criteria for 100% wound closure

had been met and a tactile evaluation of the test site by the investigator revealed that the donor site would withstand harvest via dermatome without shearing.

The subset of patients with massive surface area injuries were permitted to undergo recropping of either or both of their treatment sites as well as re-treatment with the appropriate randomly assigned dressing. When recropping of either treatment site was carried out the investigator followed the same study procedures outlined for Treatment Day 0. Original donor sites that were subsequently re-cropped were to be evaluated for functionality and durability as an autograft.

### Screening Visit

The initial screening was performed one to two days prior to surgery. At this visit informed consent, medical history and patient demographics were obtained; physical examination, vital signs assessment (temperature, blood pressure, pulse, and weight) and nutritional evaluation were performed; blood was collected for hematologic<sup>1</sup>, chemistry<sup>2</sup>, and anticollagen antibodies; and urine was collected for glucose, protein, and pregnancy testing. All laboratory testing was performed at a central laboratory.

### Day 0 (Day of Surgery)

On the Day 0 Visit, baseline (i.e., pre-harvest) photographs of both donor sites were obtained using the standardized, protocol-specified photography method (See Appendix 16.1, Protocol, Section III). A body map of the designated test sites as well as an anatomic description of each test location was completed and documented in the Case Report Form (CRF). A partial split thickness autograft was harvested (0.006 inches to 0.014 inches in depth) from each donor site with an air hydraulic dermatome. Post-harvest, baseline microbiologic surveillance swabs for culture were obtained and standardized photography of both test sites was performed. After CCS and Biobrane-L

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<sup>1</sup> CBC: hemoglobin, hematocrit, absolute and differential counts of white blood cells, and platelet count.

<sup>2</sup> Sodium, potassium, chloride, bicarbonate, calcium, alanine aminotransferase, aspartate aminotransferase, total bilirubin, total protein, albumin, alkaline phosphatase, blood urea nitrogen, creatinine, and fasting glucose.

were secured on the appropriate donor surfaces, measurement of the surface area (cm<sup>2</sup>) of both test sites was obtained via wound tracings. Repeat photography of the investigational device (CCS) and control (Biobrane-L) site were obtained after the devices were in place. Each donor site was overlaid with appropriate dressings as outlined in Section 9.4.2 of this report. Outer layer dressings on the CCS site were changed every 48 to 72 hours until day 7. For the Biobrane-L site, dressings were evaluated every 24 hours and changed as needed.

#### Post-Surgical Follow Up

Patients were examined postoperatively on the Day 3 and the Day 7 Study Visits and every 48 hours thereafter until 100% wound closure of both treatment sites was demonstrated. Once the patient had demonstrated 100% wound closure of both treatment sites, assessments were performed on a weekly schedule starting with the next pre-established weekly interval Study Visit (i.e., Day 14 Study Visit, Day 21 Study Visit, or Day 28 Study Visit). After the Day 28 Study Visit, follow-up assessments occurred at the Week 12 Study Visit (3 months) and at the Week 24 Study Visit (6 months). All study visits were to occur as close as possible to the scheduled time point based on Day 0. Patients were considered to have completed the study if the Week 24 Visit assessments were conducted.

Additional biannual follow-up continued beyond the Week 24 Visit until the last enrolled patient completed his/her six-month follow-up.

#### Re-Cropping of Original Donor Site(s)

Patients who underwent recropping of either the CCS site or the control site were permitted to receive additional CCS or Biobrane-L, respectively, during the reharvest procedure.

Original donor sites that were subsequently re-cropped were evaluated for functionality and durability as an autograft. At the time of reharvest of CCS treatment sites, a 1-2mm tissue was separated and submitted for DNA fingerprinting analysis to distinguish re-

epithelialization via autologous means from CCS device “take” (engraftment or incorporation of allogeneic cells onto the patient).

Table 9.1.1 presents the study related sequence of events. Copies of the protocol and protocol amendments are provided in Appendix 16.1. A sample CRF is provided in Appendix 16.2.

**Table 9.1.1: Schedule of Assessments**

Study Procedures	Screening Visit (Prior to Surgery)	Day 0 Visit (Day of Excision and Autograft Procedure)			Day 3 Visit	Day 7 Visit	Day 14 Visit	Day 21 Visit	Day 28 Visit	Week 12 Visit	Week 24 Visit
		Pre-Harvest	Post-Harvest	Post CCS & Biobrane							
Informed Consent	x										
Inclusion/Exclusion Criteria	x										
Medical History, Demography, and Physical Exam	x										
Vital Signs	x					x	x	x	x	x	x
Laboratory Tests	x										X <sup>4</sup>
Nutritional Evaluation	x					x	x	x	x	x	x
Outline of Donor Sites with Sterile Surgical		x									
Surface Area Measurements		x									
Photography		x	x	x		X <sup>2</sup>	x	x	x	x	x
Body Map		x									
Swab Culture			x		X <sup>3</sup>						
Sterile Tracings				x		X <sup>2</sup>	x	x	x	x	x
Outer Layer Dressing Change					X <sup>1</sup>						
Removal of CCS Backing and Biobrane						X <sup>2</sup>	x				
Pain and Itching Assessment					x	X <sup>2</sup>	x	x	x	x	x
Infection Assessment					X <sup>3</sup>						
Assessment of Percentage Unhealed						X <sup>2</sup>	x	x	x	x	x
Readiness for Recropping Assessment (wound)								x	x	x	x
Vancouver Scar Scale										x	x
Concomitant Medications	x				x	x	x	x	x	x	x
Adverse Events					x	x	x	x	x	x	x

<sup>1</sup>Outer dressing layers could be changed on the CCS site every 48-72 hours thereafter until day 7. The Biobrane-L test site was evaluated every 24 hours thereafter, if indicated, outer dressing layers were changed.

<sup>2</sup>If areas of CCS backing or Biobrane-L remained adherent despite soaking, adherent portions of dressing were to be left in place. Assessments were to be performed every 48 hours in which attempts to further remove CCS backing were made. Note: Assessments were to be performed on each test site every 48 hours until complete wound closure had occurred.

<sup>3</sup>If signs of infection were present, swab culture was performed.

<sup>4</sup>Collagen Type I IgG autoantibodies only.

Source: Appendix 16.1, Protocol

## 9.2 DISCUSSION OF STUDY DESIGN AND CHOICE OF CONTROL GROUPS

For this comparative study of donor site wound healing, Biobrane-L<sup>®</sup> was selected as the control dressing. Biobrane-L is a biocomposite dressing made from ultrathin, semipermeable silicone membrane mechanically bonded to a flexible knitted nylon fabric. Biobrane-L utilizes monofilament nylon. A nontoxic mixture of highly purified peptides derived from porcine dermal collagen has been bonded to the nylon/silicone membrane to provide a highly flexible and conformable composite dressing with adherence properties and a hydrophilic, biocompatible surface.

In clinical use, Biobrane-L is similar to semi-open fine mesh gauze. Many of its features are ideal for the severely injured burn patient. For example, its flexibility and stretch allow for treatment of many diverse donor site locations encompassing extensive surface areas. The transparency of the product allows ongoing wound evaluation. The mechanism of attachment for Biobrane-L is unique from traditional fine mesh gauze because Biobrane-L collagen peptide content promotes incorporation into the wound bed allowing the “dressing and tissue to move together.”<sup>3,7</sup> Initial adherence results from the fibrin on the clean wound surface preferentially bonding to the collagen surface of the dressing. Stronger secondary adherence results from physical entrapment of fibrin and tissue ingrowths into the nylon fabric. Less secondary adherence results from use of the lower weight monofilament thread used in Biobrane-L.

As a result of its movement properties, Biobrane-L has been shown to be more comfortable than fine mesh gauze. There are, however, significant drawbacks to its use. Several studies have demonstrated a significantly increased rate of donor site infection, ranging from 20% in some studies to 57% in others.<sup>3,7,8</sup> In addition, healing of donor sites treated with Biobrane-L has required a mean time of 13.78 days in one study.<sup>9</sup> This first allows for reharvesting of donor sites at a minimum of 14-16 days, but more commonly between 18-21 days from the initial surgery since the quality of the healed

wound may not be adequate for reharvesting upon its initial closure. Thus, healing time is not necessarily equated with the time at which recropping can be performed.<sup>2</sup>

The matched pairs design of this study required that, within each patient, the two randomized treatment sites had to be anatomically comparable so they could, therefore, be expected to behave biologically in a similar manner. Attempts were made to identify two similar donor sites on each patient. Non-articulated contiguous or discrete sites were used.

In a recently completed controlled randomized single center exploratory study of eight patients<sup>10</sup> that compared donor site healing of CCS-treated wounds in burn patients with matched wounds treated with Biobrane-L, healing time at the CCS-treated site was consistently accelerated over time to complete wound closure as compared to the Biobrane-L dressing site for each of eight patients enrolled. In that study, there was no incidence of infection at the CCS sites and one infection at a Biobrane-L site. Additionally, patients endured less itching discomfort and less pain at the CCS site compared to the Biobrane-L site.

### 9.3 SELECTION OF STUDY POPULATION

#### 9.3.1 INCLUSION CRITERIA

To be eligible to participate in this study, the following criteria must have been met:

1. Patients could have been male or female, 12 months of age and older.
2. Women of childbearing potential must have been using adequate birth control procedures; all women of childbearing potential must have had a negative pregnancy test prior to receiving the test graft.
3. A patient had to have burns involving at least 10% but not exceeding 80% total body surface area; burns could have been the result of thermal (flame, scald, contact), chemical and friction (road burn) injury.

4. Patients  $\geq 3$  years of age had to have a total donor site surface area of at least 72-90cm<sup>2</sup> (one investigational device of 36-45 cm<sup>2</sup> and one control site of an equivalent area); the maximum body donor site surface area to be treated had to be 288-360 cm<sup>2</sup> (4 investigational devices of 144-180 cm<sup>2</sup> making up one test site and the control site of an equivalent area. Patients under 3 years of age had to have a total donor site surface area minimum of 36-45 cm<sup>2</sup>; this assumed the use of one-half of an investigational device measuring 18-22.5 cm<sup>2</sup> and one control site of equivalent area. The maximum donor site surface area to be treated had to be 144-180 cm<sup>2</sup>; this assumed the use of two investigational devices measuring 72-90 cm<sup>2</sup> as one test site and the control site measuring the equivalent area. Each device measured between a minimum of 36cm<sup>2</sup> (6 cm x 6 cm) and a maximum of 45 cm<sup>2</sup> (6.7 cm x 6.7 cm).
5. A patient's donor sites had to be virgin areas (i.e., never previously harvested for skin nor could they be healed superficial partial thickness burn wounds); selected donor sites had to be on anterior or posterior non-articulated surfaces, including back, buttocks and scalp.
6. In each patient the split thickness autografts harvested from donor sites had to be between 0.006-0.014 inch in depth and donor site 1 and 2 had to be of the same depth. Treatment sites were permitted to be slightly shallower, between 0.004-0.014 inches, if the sites were undergoing recropping.
7. The patient had to be able to provide informed consent or, if the patient was under 18 years of age, parental/guardian informed consent had to be provided.
8. The patient had to be willing to comply with protocol design.
9. The patient had to have a life expectancy of at least six weeks after study entry.

### 9.3.2 EXCLUSION CRITERIA

Patients were excluded from the study if they met any of the following criteria:

1. Unable to provide informed consent
2. Sepsis with hemodynamic instability requiring pressor support or a microbiology report of positive blood cultures drawn within 48 hours prior to surgery
3. Pregnant or lactating
4. Severe inhalation injury requiring PEEP > 20 and FiO<sub>2</sub> > 60% within 12 hours prior to surgery.
5. Injury severity score (ISS) > 40 and was 15-49 years of age; if the patient had an ISS >29 and was 45-65 years of age; if the patient had an ISS >25 and was over 65 years of age; if the patient was < 15 years of age with a pediatric trauma score (PTS) ≤ 5 or a pediatric Glasgow Coma Scale (PGCS) score <8.
6. Treatment with systemic corticosteroids during the 30 days prior to injury.
7. Immunosuppressive, radiation or chemotherapy during the three months prior to injury.
8. Previous participation in a trial for management of donor sites.
9. Concurrent use of any investigational product on the burn sites.
10. History of allergy or sensitivity to collagen material.
11. History of insulin-dependent diabetes accompanied by a glycosylated hemoglobin A1C >10%.

### 9.3.3 SELECTION OF DONOR SITES

Donor sites were selected according to the Principal Investigator's routine surgical practice, guided by donor site availability. Attempts were made to identify two similar donor sites on each patient. Non-articulated contiguous or discrete sites were used.

#### 9.3.4 REMOVAL OF PATIENTS FROM THERAPY OR ASSESSMENT

Patients could be removed from the study at the request of the sponsor, investigator, the patient, or the patient's legal guardian. In the event of premature study termination, a record of the reason for termination was made, however, follow-up for the entire study duration was required for evaluating the safety of device use.

### 9.4 TREATMENTS

#### 9.4.1 DONOR SITE IDENTIFICATION

For patients at least three years of age, the total donor site surface area had to be at least 72cm<sup>2</sup> (one investigational device of 36-45 cm<sup>2</sup> and one control site of equivalent area). The maximum body donor site surface area to be treated was 144-360 cm<sup>2</sup> (four investigational devices of 144-180cm<sup>2</sup> making up one test site and the control site of equivalent area). For patients under the age of three years, total donor site surface area had to be a minimum of 36-45 cm<sup>2</sup>. This assumed the use of one-half of an investigational device measuring 18-22.5 cm<sup>2</sup> and one control site of equivalent area. The maximum donor site surface area to be treated was 144-180 cm<sup>2</sup>, assuming the use of two investigational devices measuring 72-90cm<sup>2</sup> at one test site and the control site measuring the equivalent area. The rationale for splitting the CCS in half was to enable treatment of patients under three years of age who have a small body surface area. For patients with extensive surface area involvement who required recropping, a total of eight CCS devices were allowed for those age three years and older (maximum of four devices at each harvest procedure). A total of four CCS devices were permissible for those patients under three years of age (maximum of two devices at each harvest procedure).

Once the two sites were identified, the following codes were assigned: if the sites were superior/inferior to each other, the site most superior was designated "1" and the other site was designated "2". If the sites were medial/lateral to each other, the site most medial was designated "1" and the most lateral was designated "2". If the sites were mirror images of each other, the test site on the right side of the patient's body was

arbitrarily designated “1” and the test site on the left side of the patient’s body was arbitrarily designated “2”. Through random number tables a randomization code for each site was established to designate the experimental and standard treatment sites.

#### 9.4.2 TREATMENTS ADMINISTERED

The treatments administered in this study were control dressing (i.e., Biobrane-L) and test device (i.e., CCS).

Wounds assigned to the control dressing received Biobrane<sup>®</sup> L coverage. Biobrane-L was applied to the donor site wound with staples then covered with gauze wraps, as is the standard recommended procedure for this dressing. Removal of the outer dressing layers on the Biobrane-L site was generally performed after the initial 24-48 hours following surgery. The timing of Biobrane-L removal was expected to be variable from patient to patient, however attempts to peel Biobrane-L from newly formed epidermis generally began between the 7<sup>th</sup> and 10<sup>th</sup> postoperative days. Those areas where Biobrane-L separated easily from the underlying donor surface were trimmed back. Those areas where Biobrane-L remained adherent to the test site despite soaking were considered non-epithelialized and open.

Wounds assigned to the investigational treatment received CCS for coverage. Staples were used to secure the device at the discretion of the investigator. The overlying dressing layers consisted of non-adherent, moisture retentive synthetic materials followed by gauze wrap and Ace<sup>®</sup> conforming bandage. The outer dressing layers over the CCS test site remained undisturbed during the initial 72-hour postoperative period. On the third post-operative day, the outer layers were taken down to allow inspection of the CCS backing surface overlying the treatment site. The backing material was left in place at this time and gentle normal saline irrigation of the area was permitted to remove any exudate or debris that was adherent to the backing material. Thereafter, removal and replacement of the outer dressing wrap on the CCS was permitted every 48-72 hours until

Day 7, at which time attempts were made to remove the backing to allow the first direct visual assessment of the donor treatment site.

#### 9.4.3 IDENTITY OF INVESTIGATIONAL PRODUCT

The investigational device (CCS) is an allogeneic bilaminar cultured skin substitute containing donor keratinocytes and fibroblasts derived from neonatal foreskins. To prepare the CCS device, keratinocytes and fibroblasts are enzymatically released from the foreskin tissue, cultivated to confluence and then serially passaged until the third expansion of the individual cell line and subsequently cryopreserved. The cells constituting these allogeneic cultured grafts are sequentially seeded into the biomaterial component of the test device, the cross-linked bovine collagen sponge coated with an overlay of pepsinized insoluble collagen, to form a dual layered cell-populated matrix. This cell-inhabited sponge is then maintained in culture for 10-15 days to encourage proliferation and migration of the allogeneic cells throughout its interstices. About 24 hours prior to clinical use, CCS is rinsed from its media and tested for pyrogens and sterility. The CCS is then placed in media without bovine serum and growth factors. Within the 24 hours prior to the procedure, the media is removed. The biologic dressing, upon readiness for clinical use, is now referred to as CCS device.

Each CCS device measures approximately 36-45 cm<sup>2</sup>. The CCS device is supplied to the study site in a sterile cassette within a double layer pouch. Dual sided backing layers are in place (N-Terface<sup>TM</sup>, Winfield Laboratories) to facilitate transport and handling. The blue-tinted backing layer lies directly against the fibroblast side of the device whereas the white backing is placed directly against the epidermal surface to protect the underlying cultured keratinocytes from shearing off with manipulation. Upon opening the sterile cassette, the blue N-Terface backing material is discarded. When placed on the surgically prepared donor wound surface, the white backing should always be oriented superiorly. The white backing remains in place for at least seven (7) days.

#### 9.4.4 IDENTITY OF CONTROL PRODUCT

The comparison material was Biobrane<sup>®</sup> L synthetic wound dressing (Bertek Pharmaceuticals, Inc., Sugarland, Texas). It is a composite of ultrathin semipermeable silicone membrane and a flexible monofilament nylon fabric; both layers covalently bonded to porcine collagen peptides to increase wound adherence and maintain a hydrophilic biocompatible surface. Its flexibility and stretch allow it to conform to surface irregularities for treatment of many diverse donor site locations. Its transparency permits ongoing wound evaluation. The lower weight monofilament thread utilized in Biobrane-L results in less secondary adherence with a reduction in tissue ingrowths into the nylon fabric.

#### 9.4.5 BLINDING

The analyses of photographs for assessment of 100% wound closure/healing were performed by three independent burn experts who were masked to study treatment. The photographs for all enrolled patients were mixed together upon the entire study's completion and then presented for scoring in random order. These experts were blinded to specific patient and the treatment at each of the donor sites.

Quantitative planimetric analysis of wound tracings for 100% wound closure was performed at a central laboratory (Canfield Scientific) whose personnel were masked to the assigned treatment.

#### 9.4.6 PRIOR AND CONCOMITANT THERAPY

Medication history was performed prior to surgery and at follow-up visits. Treatment with corticosteroids during 30 days prior to the date of injury was prohibited. Previous treatment with immunosuppressive agents, radiation or chemotherapy during the three months prior to injury was prohibited. All concomitant medications, including antibiotics, were recorded on the CRF at each study visit.

## 9.5 EFFICACY AND SAFETY MEASUREMENTS ASSESSED

The focus of this study was to determine the difference in time to complete donor site healing between CCS (investigational product) and Biobrane-L (control product). Complete healing was defined as the presence of a dry, opalescent-pink external confluent surface representing the newly formed outer cornified layer of the epidermis (the stratum corneum). Additionally, the quality of the healed donor wound (i.e., its readiness for recropping), signs of infection and breakdown at the donor wound site, the reporting of pain and itching at the treatment site, and the scar outcome were assessed and compared to control dressing.

The primary efficacy variable was the time to complete wound closure, as determined by photography. Secondary efficacy variables were time to complete wound closure as determined by computerized planimetric assessment of unhealed wound, time to complete wound closure as determined by the investigator through clinical assessment, the rate of wound healing as determined by the percent change in wound area from baseline as determined by planimetric data, time to readiness for recropping as assessed by the investigator, and time to actual recropping of an original donor site.

Safety variables that were compared between the two treatments were: incidence of donor site specific adverse events, scar outcome, pain and itching scores, and incidence of donor site infection and breakdown, time to actual recropping, and recrop outcome. Adverse events were tabulated by preferred term, body system and severity (mild, moderate, severe, life-threatening or fatal).

## 9.5.1 EFFICACY MEASUREMENTS

### 9.5.1.1 Primary Efficacy Variable

The primary outcome measure was the time to 100% re-epithelialization (i.e., complete healing) as measured by blinded photographic assessment.

A standardized protocol was developed for procuring photographs of donor sites immediately post-harvest and over the 28-week post-surgery time period (See Appendix 16.1, Protocol). This protocol was strictly adhered to at all study sites. All centers were provided with identical camera equipment as well as on-site training in the proper technique for good clinical trial photography. The film, which was also provided, was processed at a central facility and reviewed from a quality assurance standpoint. After study completion the photographs from all enrolled patients were mixed together and presented, in random order, to three independent burn experts for assessment of 100% re-epithelialization. The photographic reviewers were blinded to specific patient and the treatment at each of the donor sites. Designation of the presence or absence of complete re-epithelialization required agreement of at least two of three blinded reviewers.

The total lapsed time (days) from initiation of treatment to first occurrence of 100% re-epithelialization was compared for the two treatments.

### 9.5.1.2 Secondary Efficacy Variable(s)

#### Computerized Planimetric Analysis of Wound Size

Quantitative planimetric analysis of wound tracings was used to determine and compare the percentage of wound closure over time between the two donor sites. The investigator traced the open, un-epithelialized regions of the donor site post surgery and at each subsequent study visit until complete (100%) healing had occurred. Digital scanning of these tracings provided computerized planimetric calculations of the perimeter and surface area (cm<sup>2</sup>) of the remaining open, unhealed donor sites. The computerized planimetric analysis was performed at a facility (Canfield Scientific) whose personnel

were masked to the assigned treatment. If, at any time during the study duration, there was clinical evidence of wound breakdown at a donor site, tracings were re-instituted until complete wound closure was regained.

Complete wound closure by planimetry was defined as a wound size of 0 cm<sup>2</sup>. The total lapsed time (days) from initiation of treatment to first occurrence of 0 cm<sup>2</sup> wound size was compared for the two treatments.

#### Clinical/Physical Assessment of Wound Closure By Investigator

The investigator clinically evaluated the donor sites post surgery and at each subsequent study visit for (1) the percentage of unhealed treatment site (i.e., numeric value) and (2) assessment of 100% re-epithelialization as an absolute “yes” or “no” response.

Complete wound closure by clinical/physical assessment was defined as the visible presence of a dry, opalescent-pink external confluent surface representing the newly formed outer cornified layer of the epidermis (the stratum corneum). Additionally, in the clinician’s assessment this site was to no longer require an absorbent dressing to collect serous drainage, or a protective dressing acting as a barrier against infection or mechanical trauma. The freshly re-epithelialized wound, which no longer produced transudate and could be visibly characterized as a dry, intact surface, to be left open to the air, qualified as 100% closed (i.e., completely healed).

The total lapsed time (days) from initiation of treatment to first occurrence of 0% unhealed and 100% re-epithelialization was compared for the two treatments.

#### Rate of Wound Healing

Rate of wound healing (cm<sup>2</sup>/day) was determined by quantitative computerized planimetric analysis, in blinded fashion. The daily and average rates over time for the two treatments were compared.

### Readiness for Re-Cropping

Once 100% re-epithelialization had occurred, the investigator assessed the donor site's readiness for re-cropping as an absolute "yes" or "no" response. Assessment was made by a tactile evaluation consisting of a light pinch of the healed donor site and the investigator's subjective determination of whether the site could withstand re-harvest (i.e., could withstand dermatome pressure without shearing) and yield a viable, thin split thickness skin graft.

The total lapsed time (days) from initiation of treatment to first occurrence of readiness for recropping was compared for the two treatments.

### Time to Actual Re-Cropping

The total lapsed time (days) from initiation of treatment to actual re-cropping of the donor site was compared for the two treatments.

## 9.5.2 SAFETY MEASUREMENTS ASSESSED

### Pain at Donor Sites

Pain assessment at the donor sites was conducted prior to dressing change or wound intervention involving donor sites, at each study visit following surgery. Pain rating scales were age group specific as follows:

<b>Pain Rating Scales</b>		
<b>Age Group</b>	<b>Scale</b>	<b>Assessments</b>
8 years of age and older	Numeric Pain Intensity Scale	Subject was asked to indicate the scale point that best represented the pain intensity at the specific donor site on a ruled line containing numbers from 1 to 10, with 0 indicating no pain and 10 indicating the worst pain possible. The subject could indicate a response either verbally and/or by pointing to a position on the ruled line.

<b>Pain Rating Scales</b>		
<b>Age Group</b>	<b>Scale</b>	<b>Assessments</b>
3 to 7 years of age	Wong-Baker Faces Pain Rating Scale	Subject was shown a set of five faces with various expressions. Subject was instructed as follows: Face 0 was very happy because he/she doesn't feel any pain at all; Face 1 hurts just a little bit; Face 2 hurts a little more; Face 3 hurts even more; Face 4 hurts a whole lot; Face 5 hurts as much as possible although subject didn't have to be crying to feel this bad. The subject was asked to choose the face that best described how he/she was feeling with respect to the specific treatment site.
Less than 3 years of age	Objective measurements, (overall and site specific)	Categories Assessed: <ul style="list-style-type: none"> <li>▪ Active use of treatment site, site specific (yes or no)</li> <li>▪ Appropriate activity level (yes or no)</li> <li>▪ Increased heart rate (yes or no)</li> <li>▪ Crying or irritable (yes or no)</li> <li>▪ Evaluation of overall pain (not site specific) per assessor: None (0), Mild (1), Moderate (2), Severe (3)</li> </ul>

Itching Severity at Donor Site

Itching at each donor site was assessed by the subject as none (0), mild (1), moderate (2), or severe (3) at each study visit following surgery.

Blister Formation or Breakdown of the Donor Site

The investigator assessed durability of wound closure by indicating the presence or absence of blister formation/site breakdown at each study visit after 100% wound closure had been achieved at the donor site.

Donor Site Infection

Presence of infection at the donor site was established based on clinical observations and supported by microbiological testing. At each Study Visit, symptoms and signs consistent with infection were evaluated as present or absent. The evaluated symptoms and signs of infection included: purulence, malodor, increased warmth, pain and tenderness, erythema, induration, or swelling.

Any donor site for which infection was suspected was to be treated at the investigator's discretion. If the investigator elected to use antibiotic treatment (systemic or topical) the

site was to be swabbed and cultured for the infecting pathogen. The microbiological culture and susceptibility results were utilized in guiding the clinician in the selection of an appropriate antibiotic. The route of antibiotic administration was selected based on the clinical status of the patient.

### Recrop Functionality and Durability

Recrop outcome (specifically, the functionality and durability of the recropped graft as well as the time to 100% wound closure of the recropped and retreated donor site) were assessed for all patients undergoing recropping of the original donor sites.

### Scar Outcome

Scar outcome was evaluated approximately 12 and 24 weeks following surgery, using two separate methods: clinical assessment (Vancouver Scar Scale) and photographic assessment (Hamilton Scar Scale). Clinical assessment of scarring severity was also assessed at the subject's final visit.

On-site physical or occupational therapists, trained in the use of the Vancouver Scar Scale, conducted the clinical assessment of scarring severity. This scale, a standardized, comprehensive measurement of scarring, utilized visual and tactile evaluations of the donor site's vascularity, pliability, pigmentation, and height, as indicated below, after complete healing had been achieved. For each patient, a total Vancouver score was determined by summing the scores of the four individual parameters. A total Vancouver score could range from 0 to 15. The individual parameter scores as well as the total score were compared for the two treatments.

<b>Vancouver Scar Scale</b>		
<b>Scarring Parameter</b>	<b>Test Description</b>	<b>Rating Scale</b>
Pigmentation	Assessed by applying pressure with a clear piece of plastic (e.g., "UVEX") to blanch the scar, in order to eliminate the influence of vascularity on the assessment of pigmentation. The blanched scar was compared to a nearly blanched area of the subject's unburned skin. A variation from the	0 = Normal 1 = Hypopigmentation 2 = Mixed pigmentation 3 = Hyperpigmentation

<b>Vancouver Scar Scale</b>		
<b>Scarring Parameter</b>	<b>Test Description</b>	<b>Rating Scale</b>
	color of blanched normal skin indicated a pigmentation change	
Vascularity	Assessed by observing the color of the scar at rest. Additionally, the scar was blanched with a piece of clear plastic and the rate and amount of blood return was assessed. Scars which were congested and refilled slowly or could not be completely blanched were rated in the highest category (i.e., 3/purple).	0 = Normal* 1 = Pink 2 = Red 3 = Purple *color and rate of blood return closely resembles that of normal skin)
Pliability	The scar was positioned to minimize tension and then manually palpated between thumb and index finger to assess the ease of distortion under pressure.	0 = Normal (resembles pliability of normal skin) 1= Supple (flexible with minimal resistance) 2 = Yielding (can be distorted under pressure without moving a single unit, but offers normal moderate resistance) 3 = Firm (inflexible; scar moves up as a single unit) 4 = Banding (rope-like tissue that blanches with extension of the scare; full range of movement) 5 = Contracture (permanent shortening of scar, producing limited range of movement)
Height	Visual estimation of the height of the scar as compared to normal surrounding skin.	0 = Normal (flat, flush with normal skin) 1 = Over ¼ of the donor site was elevated more than 0 mm and ≤ 1 mm above the normal skin 2 = Over ¼ of the donor site was elevated more than 1 mm and ≤ 2 mm above normal skin 3 = Over ¼ of the donor site was elevated more than 2 mm and ≤ 4 mm above normal skin 4 = Over ¼ of the donor site was elevated more than 4 mm above normal skin

A specialist in burn wound care and scar management, who was masked to the specific patient and study treatment, conducted a photographic assessment of scarring severity after completion of the trial. The Hamilton Scar Scale, reported to have substantial inter-rater and test-retest reliability, was utilized for this assessment.<sup>11</sup> For each patient, a total Hamilton score was determined by summing the scores of the six individual parameters. A total Hamilton score could range from 0 to 20. The individual parameter scores as well as the total score were compared for the two treatments.

<b>Hamilton Burn-Scar Rating Scale</b>	
<b>Assessments</b>	<b>Rating Scale</b>
Rate the thickness or height of the scar.	0 = No thickness or raising 1 = Thickness is slight 2 = Thickness is moderate 3 = Thickness is severe
Is it all the same height, or are there areas of irregularity?	0 = All smooth; scar area not bumpy or irregular 1 = About ¼ of scar area is bumpy or irregular 2 = About ½ of scar area is bumpy or irregular 3 = About ¾ of scar area is bumpy or irregular 4 = Majority of scar area is bumpy or irregular
Rate the majority of the scar for vascularity, which describes how pink/red the scar is when compared with normal skin	0 = Normal or pigmented; mature 1 = Light to medium pink 2 = Deep pink to light red 3 = Medium to deep red 4 = Purplish
Look at the color/pigmentation of scar and compare it with normal skin (preferably an anatomically homologous area).	0 = Normal or paler than normal 1 = Scar area is slightly darker 2 = Scar area is darker 3 = Scar area is much darker
When the scar is uncovered, how would you rate the overall appearance?	0 = Acceptable 1 = Slightly disfigured 2 = Moderately disfigured 3 = Severely disfigured
When reviewing the projected slides, please equate and score the left slide to the right slide as follows:	0 = Considerably superior 1 = Slightly superior 2 = Slightly inferior 3 = Considerably inferior

Vital Signs

Temperature (°F), blood pressure (mmHg), pulse (bpm), and weight (kg) were assessed at screening, pre-treatment, and each subsequent study visit.

Nutritional Evaluation

A nutritional evaluation was performed by a unit dietician at screening (baseline), at the Day 7 Study Visit and at each subsequent visit. Caloric requirement for each subject was calculated based upon basal energy expenditure plus the calculated needs of a thermally injured patient.

### Laboratory Assessments

Hematology, serum chemistry, and urinalysis were conducted at the screening visit.

Assay for collagen Type I IgG autoantibodies were conducted at the Screening Visit and at the Week 24 Visit.

### Adverse Experiences

Adverse events were tabulated by preferred term, body system and severity (mild, moderate, severe, life-threatening or fatal).

The following definitions were employed in the conduct of this trial:

Adverse Experience:	An adverse experience was defined as any untoward medical occurrence in a patient or clinical investigation administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.
Unexpected Adverse Drug Reaction:	An adverse reaction, the nature or severity of which was not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational medicinal product).
Serious Adverse Experience:	A serious adverse experience was defined as any adverse experience that resulted in any of the following: death, a life-threatening adverse experience, in-patient hospitalization (>23 hours) or prolongation of existing hospitalization, congenital anomaly or birth defect, persistent or significant disability or incapacity. Additionally, important medical events that may not result in the above might be considered a serious adverse experience when, based upon appropriate medical judgment, they could jeopardize the patient and could require medical or surgical intervention to prevent one of the outcomes listed above.

Relationship of adverse experiences and unexpected adverse drug reactions to the investigational product were assessed by the investigator using the following categories:

- Likely: A reaction that followed a reasonable temporal sequence from administration of the device; that followed a known or expected response pattern to the suspected device; that was confirmed by improvement on stopping or reducing the application of the device and reappearance of the reaction on repeated exposure; and that could not be reasonably explained by the known characteristics of the subject's clinical state.
- Unlikely: A reaction that followed a reasonable temporal sequence from administration of the device; that did not follow a known response pattern to the suspected device; but that could be reasonably explained by known characteristics of the subject's clinical state, environment, toxic factors, or other modes of therapy administered to the subject.

Severity of adverse experiences and unexpected adverse drug reactions were assessed by the investigator using the following categories:

- Mild: Caused no limitation of usual activities; the subject might experience slight discomfort; no medical intervention or therapy was required.
- Moderate: Caused some limitation of usual activities; the subject might experience annoying discomfort, no or minimal medical intervention or therapy was required.
- Severe: Caused marked inability to carry out usual activities; the subject might experience intolerable discomfort or pain; medical intervention/therapy required; hospitalization possible.
- Life Threatening: Caused extreme inability to carry out usual activities; the subject might experience intolerable discomfort or pain; significant medical intervention/therapy required; hospitalization definite.
- Fatal: Caused death

For each adverse experience the following were recorded: date of onset, date of remission, severity, causal relationship to study device, action taken, the outcome of the experience, action taken with regards to treatment/intervention, results of any diagnostic procedures or laboratory tests, and all treatments administered due to the experience.

All adverse experiences were recorded on CRF as literal terms and coded to preferred term (medical term) and primary body system utilizing the World Health Organization

(WHO) Adverse Event Dictionary. In cases where an adverse experience could not be coded using WHO terminology, ICD-9 coding was employed.

### Concomitant Medications

Administrations of any medications (including antibiotics) as well as the reason for administration were recorded during the study from Day 0 through the final visit.

All concomitant medications, recorded on CRF, were coded to Drug Code (DC) and Area Therapy Code (ATC) utilizing the WHO Medication Dictionary.

### 9.5.3 APPROPRIATENESS OF MEASUREMENTS

Wound measurement and determination of wound healing are matters of significant controversy in published reports. Three independent methods were used in this study: photography, planimetry, and investigator's clinical assessment. Among these three methods, photography is the most objective while investigator's clinical assessment and planimetry are more sensitive and accurate. Three assessment methods were used in this study so that the primary endpoint (time to wound closure) could be assessed not only by an objective methodology (photography), but also by accurate (planimetry) and sensitive (clinical assessment) methodologies.

Review of wound photographs by three independent expert reviewers, who were totally unconnected to the study and blinded to the specific patient and to the treatment at each donor site, provided the objective measurement of the primary endpoint. The objectivity of this method, however, is not as accurate or sensitive as planimetry or the investigator's real-time clinical assessment due to confounding factors such as the possibility of inconsistent lighting, limitation to viewing the wound in one direction, light reflections, and the inability to touch the wound. Additionally, there is no patient interactivity with this method.

Planimetry depends upon the clinician's judgment of the wound edges, and uses computerized tools to accurately calculate wound area. Because the clinician is able to view the wound from multiple directions, to touch the wound, and to question the patient about pain, irritation, and sensitivity at different locations, the resulting wound tracing is inherently more accurate than photography.

It should be noted that when the wound is completely resurfaced with thin, translucent, epithelium, both photography and planimetry may not be sufficiently sensitive to detect 100% healing; only physical examination can determine if the reepithelialization is truly complete. Detecting continued wound drainage on physical examination, for example, suggests the presence of microscopic skin defects not demonstrable on photography or planimetry.

Photography was selected as the primary assessment method as it is the most objective of the three. Planimetry and investigator's clinical assessment were included to provide the accuracy and sensitivity that the photographic method lacks.

## 9.6 DATA QUALITY ASSURANCE

### 9.6.1 STANDARDIZATION OF ASSESSMENTS

A standardized protocol was developed for photography of the treatment sites (Canfield Scientific). This protocol was strictly adhered to at all study visits. All centers were provided with identical camera equipment as well as on-site training in the proper technique for good clinical trial photography. The film, which was also provided, was processed by a central facility and reviewed from a quality assurance standpoint (Canfield Scientific).

For uniformity of analyses, blood and urine samples for hematology, serum chemistry, and urinalysis were analyzed at a central laboratory (Covance Central Laboratories, Indianapolis, Indiana) according to the laboratory's standard operating procedures.

Assay for collagen Type I IgG autoantibodies and tissue samples from patients who underwent recropping of the CCS treatment site were shipped to and analyzed at a central laboratory (Cellmark Diagnostics, Germantown, Maryland) according to the laboratory's standard operating procedures.

Scar outcome assessment for the safety analysis was standardized and performed by an on-site burn physical or occupational therapist trained in the use of the Vancouver Scar Scale. Photographic evaluation, utilizing the Hamilton Burn Scar Rating Scale was performed by an independent specialist in burn wound care and scar management, who were unaware of the specific patient, study treatment or visit date of assessment when scoring the photographs for scar appearance. The scale that was used for photographic analysis had been previously tested and reported to have substantial inter-rater and test-retest reliability.<sup>11</sup>

#### 9.6.2 CLINICAL INVESTIGATOR QUALITY CONTROL

The clinical investigators were responsible for the quality of the clinical conduct at their respective centers. They were also responsible for complying with the protocol, standard assessments, data collection procedures, and adherence to Good Clinical Practice (GCP).

#### 9.6.3 ORTEC INTERNATIONAL AND CONTRACT RESEARCH ORGANIZATION QUALITY CONTROL

A study monitor, qualified by training and experience, visited each investigator prior to the study and at regular intervals during the course of the study. Monitoring visits included review of CRF against source documents to assure the validity and accuracy of recorded results, audit of investigational and control products, storage facilities, and adherence to GCP.

#### 9.6.4 DATA QUALITY CONTROL

Data were collected from investigational centers while respecting the anonymity of all subjects. Two independent data entry clerks entered all data, collected on CRF, into a SAS database utilizing a double data entry procedure. Discrepancies between the two entries were resolved by on-line comparison. After completion of this verification procedure, any subsequent changes to the data were recorded in an audit trail journal file.

Computerized and manual edit checks were performed on all entered data to ensure logic and consistency of the captured data. Data discrepancies uncovered during manual and computerized checks were recorded on Data Resolution Forms (DRF), resolved by study center personnel, with appropriate changes made to the database. All resolved DRF were filed with the appropriate subject's CRF.

Laboratory and other external data received electronically (i.e., planimetry, photographic assessment for wound healing, and photographic assessment for scarring severity) were converted to SAS data sets and verification of accurate data conversion were performed on 100% of the transmitted and converted variables.

Prior to database lock, a sample of 10% of the subjects in the database was randomly drawn and 100% of SAS data for these subjects were compared to CRF. Acceptable error rate for this study was less than or equal to 0.1%.

#### 9.7 STATISTICAL METHODS AND SAMPLE SIZE

A separate statistical analysis plan was written prior to unblinding and is included in Appendix 16.8.

## 9.7.1 STATISTICAL AND ANALYTICAL PLANS

### 9.7.1.1 Analysis Populations

The primary population for the efficacy analyses was the intent-to-treat (ITT) population. This population included all patients who underwent randomization of donor sites and received treatment, regardless of completion of study.

The population for the safety analyses was the subset of the ITT population who received treatment with a study device, regardless of study completion.

The per-protocol (PP) population was a subset of the safety population who had no major protocol violations and sufficient planimetry data to determine time to wound healing. The per-protocol population was identified at a validity meeting held on 13 November 2000, prior to unblinding the study. A decision was made at this meeting to allow patients who used systemic corticosteroids before and during the study. The rationale for this decision was that the patient received control as well as test device and any healing effect of the steroid would affect both arms of the study equally.

### 9.7.1.2 Methods of Analysis

#### Primary and Secondary Efficacy Variables

The primary measure of clinical effectiveness was time to complete healing as determined by photographic assessment. To demonstrate that the results were consistent and independent of the analysis method (i.e., robust), we used means, medians and Kaplan-Meier statistics. Due to the fact that the mean would be affected by only a few outliers, we believe that the most robust statistic for time to complete healing is the median. However, we present means and standard deviations for completeness of the report. The statistical significance level to test the primary endpoint was set at  $p < 0.05$ , *a priori*.

Time to healing for all assessment methods was censored at 32 days post surgery for time-to-event analyses due to the fact that donor site wounds are expected to heal within 28 days. Thus, censoring was implemented for those patients where an extended duration between visits (60 to 180 days) resulted in the inability to capture an accurate time to wound closure. Day 32 was chosen in order to include as many patients as possible in all assessment methods.

Photography produced the largest number of healing times beyond 32 days for both CCS and Biobrane-L. This was due to the fact that patients who had been evaluated as 100% healed by the investigator often did not return for follow-up visits until two to six months later. Therefore, if the photographic evaluation of 100% healed did not agree with the physician’s assessment of 100% healed, then the next photograph available for the reviewers was taken two to six months later, thereby resulting in an artificially extended healing time. Also, in other instances, patients were discharged before their donor sites were 100% healed and did not return until two to six months later for follow-up visits, thereby also creating artificially extended healing times for all assessment methods. For those patients who died or were withdrawn from the study before day 32, the death or withdrawal dates were used.

Table 9.7.1 presents a summary of the numbers of donor sites by treatment group that were censored at day 32.

**Table 9.7.1: Donor Sites with >32 Days to Healing**

	Photographic Assessment	Planimetric Assessment	Clinical Assessment
Total CCS >32 days	7	3	1
Total Biobrane >32 days	22	11	11

*Source: Section 6.10, Listings 19, 20, and 16*

There were more Biobrane-L sites recorded requiring >32 days to heal than CCS sites with all assessment methods. By censoring all unhealed sites after 32 days, we prevented the influence of outliers on the mean days to healing. This influence would have affected

the Biobrane-L treated sites more than the CCS-treated sites (i.e., mean days to healing for Biobrane-L would have been much larger than that of CCS).

No covariate adjustments were made and therefore no adjustments were made to the reported p-values. Formal statistical comparisons of the two treatments for the primary endpoint in terms of mean and median days to complete wound closure are presented utilizing the ITT and the Per Protocol populations. The p-values reported for treatment group differences in Kaplan-Meier survival curves and estimates are from Log-Rank statistics. The p-values reported for the means are based on t-tests and paired t-tests.

Statistical comparisons for the following secondary and safety endpoints are also presented:

- Time to 100% wound closure as assessed by planimetry
- Time to 100% wound closure as assessed by investigator
- Time to readiness for re-cropping
- Time to 50% wound closure by Day 14 as assessed by planimetry
- Number and percentage of donor sites achieving 50% wound closure by Day 14 as assessed by planimetry
- Scarring severity as assessed by investigator\*
- Scarring severity as assessed by photography\*
- Number and percentage of infections\*
- Number and percentage of wound blistering/breakdown\*
- Itching severity\*
- Pain severity for ages  $\geq 8$  years of age\*
- Pain severity for ages 3 to 7 years of age\*

\*These endpoints were specified as safety outcome variables. In this report we present these results within the efficacy section. It should be noted that p-values reported for these parameters are simply indicators and should be viewed with caution due to the fact

that when multiple statistical tests are conducted, some p-values will be significant (i.e.,  $p < 0.05$ ) due to chance alone.

Kappa statistics are presented for:

- Analysis of Agreement between investigator's clinical assessment of 100% wound closure and photographic review.
- Analysis of Agreement between investigator's clinical assessment of 100% wound closure and planimetry.
- Analysis of Agreement between photographic assessment of 100% wound closure and planimetry.

Descriptive statistics are presented for all other measurements of efficacy. Categorical data are summarized and presented using frequency tables of counts, histograms, and percentages. Continuous variables are summarized and presented as means, standard deviation, medians, and ranges.

### Safety Variables

Descriptive statistics, as described above, are presented for adverse experiences, vital signs, nutritional assessments, concomitant medications, and collagen Type I IgG autoantibodies.

### Missing, Unused, and Spurious Data

An intent-to-treat approach was followed for all data summaries. All available data for safety and efficacy are presented.

All analyses presented in the main body of this report are based on treatment assigned according to the randomization schedule. There were, however, two patients whose donor sites were treated contrary to the randomization schedule (Patient 05-002 and Patient 15-009). Two additional analyses were conducted to assess the impact of this

mis-randomization on photographic, planimetric, and clinical assessments of time to complete healing: (1) exclusion of the mis-randomized patients from analyses, and (2) analysis of the entire population, utilizing treatment actually received. These analyses are presented in Section 14 of this report (i.e., Tables P13.1 through P18.2). The results indicate that the mis-randomization of these two patients had no impact on the overall results of the study (i.e., there was no difference in the direction of the results or in the p-values from these additional analyses as compared to those based on treatment assigned). For this reason, we chose to report, in the main body of this report, all results according to treatment assigned.

#### Subpopulation Analyses

Customary subgroup analyses (i.e., age, race, and gender) were performed for the ITT and PP populations on time to wound closure by photography, planimetry, and clinical/physical assessment. Additionally, subgroup analyses for the ITT population are presented for time to wound closure by photography and planimetry based on percentage of total body surface area (TBSA) burned, and surface area of the donor site.

#### 9.7.1.3 Calculation of Sample Size

Sample size calculations were performed using the sample size methodology for the total number of events required for a test based on the proportional hazards assumption. Using this method, estimates of the mean (or median) time to 100% re-epithelialization were used to estimate the ratio of the hazard rates.

Sample size was estimated based on (1) the photographic assessment results of time to 100% re-epithelialization obtained from a pilot study in donor sites,<sup>10</sup> (2) a type I error rate (alpha level) of 0.05, two-tailed, (3) power of 80%, and (4) the proportion of wounds randomized to each treatment would be the same and equal to 0.5 or ½.

### 9.7.2 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSIS

Three amendments to the original protocol, dated 10 March 1999, were made:

- Amendment 1  
05 May 1999
- In response to FDA comments, the following were instituted:
- Enrollment increased to 100 patients in 12 burn centers to obtain 75 completed patients
  - Primary efficacy variable changed from time to complete wound closure by planimetric analysis to blinded photographic assessment
  - Addition of genetic testing of tissue samples from re-cropped CCS sites
  - Addition of a 6-month minimum follow-up period for patients undergoing re-cropping
  - Addition of continued 6-month follow up visits until the last patient enrolled had completed one 6-month follow up visit
  - Clarification that administration of any antibiotic and the reason for administration would be recorded
  - Addition of scar outcome analyses on re-cropped and re-treated donor sites
  - Addition of safety parameters assessments (itching, pain, and signs of infections) at the Study Day 3 Visit
  - Allowed treatment sites undergoing re-cropping to be harvested at a depth of 0.004-0.014 inches, as clinically indicated
  - Addition of exclusion criteria: sepsis, severe inhalation injury, steroid and immunosuppressive treatment, unstable diabetic history, age-specific parameters for baseline injury/trauma/coma scores
  - Allowed either or both of the donor sites to be re-cropped
  - Addition of definition of infection
  - Addition of patient instruction sheet providing instructions in the event of wound breakdown
- Amendment 2  
29 June 1999
- In response to FDA comments, the following were instituted:
- Clarification that photographs are to be taken of each wound at each visit
  - Clarification that durability is time to wound breakdown and will be evaluated by Kaplan Meier
- Amendment 3  
03 Dec 1999
- The following revisions were instituted:
- Entrance criterion for percentage of total surface area burned set at  $\geq 10\%$
  - Increased the required number of completing patients from 75 to 85 (Note: enrollment was stopped after 82 patients completed the trial)

The following analytical changes were made:

- Some time-to-event analyses were originally planned to be based on the name of the Study Visit (e.g., the Day 14 Study Visit) not the actual elapsed days to 100% wound closure. All time to event analyses were modified to reflect the actual elapsed days since surgery.

- Censoring at Day 32 was implemented for time-to-event analyses due to the long duration between visits after this point and the resulting inability to accurately capture wound closure time. Furthermore, all wounds were expected to heal by day 32.
- The calculation of elapsed days was modified to date of wound closure minus date of Day 0, based on current literature.
- No analyses were conducted on time to re-cropping or on re-cropping outcome events due to the small number of patients undergoing re-cropping.

## 10. STUDY PATIENTS

### 10.1 DISPOSITION OF PATIENTS

The study enrolled 82 patients among the 12 study sites. There were 22 patients (27%) who discontinued study before the week 24 visit. However, 20 of 22 discontinued patients had complete healing at both sites by at least one of the assessment methods prior to discontinuation. Both donor sites for patients 01-008 and 15-009 were unhealed as of the discontinuation date.

Reasons for study discontinuation are summarized in Section 14 Table C2 and detailed in Appendix 16.10, Listing 4. The majority of subjects were discontinued due to lost to follow up (16/82, 19.5%). Three of 82 subjects (3.7%) discontinued due to an adverse event (death, unrelated to investigational or comparator product) and three subjects (3.7%) were discontinued due to other reasons, including incarceration (n=1) and patient non-compliance with follow up visits (n=2).

Mean and median times on study for the 82 patients were 235 days and 186.5 days, respectively (range: 9 to 549 days), as indicated in Table 10.1.1.

**Table 10.1.1: Mean and Median Time On Study (days)**

N	82
Mean (SD) Days on Study	235.04 (212.71)
Median Days on Study	186.5
Min, Max	9, 549

*Source: Section 14, Table C5*

## 10.2 PROTOCOL DEVIATIONS

In total, eight patients were recorded as having major protocol violations. Listing 5 in Appendix 16.10 provides details concerning these violations. Planimetry data was incomplete in four patients. An additional four patients violated inclusion/exclusion criteria. The specifics for each of the protocol violations are as follows:

- Site 1 Subject 13 had an Injury Severity Score (ISS) of 50, which violated exclusion criterion #5.
- Site 3 Subject 1 had burns >80% of Total Body Surface Area (TBSA), which violated inclusion criterion #3.
- Site 3 Subject 7 was missing planimetry and photos for treatment days 9, 11, 16, 21 and 28.
- Site 4 Subject 5 was 9 months old, which violated inclusion criterion #1.
- Site 4 Subject 12 was 11 months old, which violated inclusion criterion #1.
- Site 8 Subject 9 was missing planimetry data for treatment days 16, 18, 21, 23/25 and 28.
- Site 12 Subject 2 was missing photos and planimetry data for days 18, 28, week 12 and week 24.
- Site 15 Subject 6 missed treatment days 16, 18, 21, 23/25.