PRECLINICAL REVIEW MEMO

Composite Cultured Skin (CCS) was first used in Australia on RDEB (*Recessive Dystrophic Epidemolysis Bullosa*) patients.

Ortec International Inc. introduced this product in the USA for the first time in their clinical studies with burn patients via G920046. Later the product was approved for several additional clinical studies: G960200 (indicated for the treatment of Epidermolysis Bullosa patients), G980037 (indicated for the treatment venous ulcer patients), G990063 (indicated for the treatment of donor sites in burn patients), G990204 (indicated for diabetic foot ulcers). Recently, the same device (Orcel™ Composite Cultured Skin has been approved for marketing under a humanitarian device exemption (HDE) for EB patients (H990013).

In addition to the clinical studies referenced above, the subject device, CCS was also approved for use in clinical studies for the indication of donor sites in burn patients. For details regarding the pilot study protocol, see G920046/S28, G920046/S29, G920046/30.

The pivotal study was conducted under the IDE, G990063.

The firm (Ortec International, Inc.) submitted this PMA seeking approval of the subject device for the indication of donor sites in burn patients. This PMA is being reviewed only for the safety and efficacy of CCS for the following indication: For the management of split thickness donor sites in burn patients. The data submitted in this PMA from other studies (burn study, venous ulcer study, EB study, and diabetic foot ulcer study) provide additional data supporting the safety of the product.

DEVICE DESCRIPTION AND PRECLINICAL STUDIES

The device under consideration is Composite Cultured Skin (CCS) indicated for the management of split thickness donor sites in burn patients.

CCS consists of a collagen matrix in which allogeneic human skin (neonatal male foreskin) cells (i.e., epidermal keratinocytes and dermal fibroblasts) are cultured in two distinct layers.

The collagen cross-linked sponge consists primarily of Type I bovine collagen laminated on one side with a thin gel layer of acid soluble bovine collagen. The collagen sponge is a component of the device and such is not a stand-alone product and is not FDA-approved.

The fibroblast cells and keratinocyte cells are tested for human and animal viruses, retroviruses, bacteria, fungi, yeast, mycoplasma, karyology, isoenzymes and tumorigenicity. The final product is tested for morphology, cell density and viability, sterility, mycoplasma, and physical container integrity. The animal-derived reagents used in the manufacture of the device are tested and found to be negative for viruses,

retroviruses, bacteria, fungi, yeast, and mycoplasma. The bovine material used in the device is obtained from countries free of BSE.

The device measures approximately 6 cm x 6 cm. A non-adherent mesh (N-Terface) is placed on both aspects of the device to protect the cells. The device is packaged in a plastic tray with protein-free medium containing DMEM.

The human fibroblast cells and epidermal keratinocyte cells are obtained from neonatal foreskin. The donor's mother's blood is tested and found to be negative for the following infectious agents: HIV I, HIV II, HTLV I/II, CMV, HBVsag, HBVab, Herpes 1 and 2, ALT and RPR.

The cell lines are tested (separately) for the following:

Growth and morphology evaluation, cytogenetic testing, isoenzyme analysis, in-vivo tumorigenicity.

The cell lines (pooled) were tested for the following:

Sterility, mycoplasma, EBV (Epstein Barr Virus), HSV (Herpes Simplex Virus), HBV, HIV I, HIV II, HTLV I/II and HHV-VI.

All the test results meet the acceptance criteria established for the product.

The following table gives the final release criteria of the device:

Table 1
In-Process and Final Release Tests are:

	Test	**Method**
1	Appearance (excess growth/proliferation)	100% Visual inspection
2	Dimensions	Linear Measurement
3	Viable Cell Density	Ortec TM 07-0004
4	Cell viability	Ortec TM 07-0004
5	NHF Morphology (in-process)	Ortec WI 09-0062
6	NHK Morphology in-process	Ortec WI 09-0062
7	Endotoxin	Kinetic Chromogenic LAL
8	Sterility, in-process	USP Sterility
9	Sterility Final product	USP Sterility

Shelf Life of CCS Device:
The sponsor submitted data supporting the shelf life of CCS (see G990063). The data demonstrated that the subject device, CCS is stable for at least 72 hours inside a temperature-maintained shipping container.

Mycoplasma Testing:
Each lot manufactured is tested for mycoplasma.

Cell Lines:
In HDE H990013, the sponsor provided data in support of two cell lines that will be used in product manufacture. They are **FS-143** and **FS-145.**

In this PMA (P010016), the sponsor provides data for cell strain **FS-148.**

Donor Mother's Blood Test results were also provided.

The following table gives presents type of tests performed on FS-148 NHK/NHF strain. Note: NHK stands for normal human keratinocytes; NHF stands for normal human fibroblasts.

Table 2
Cell Testing on FS – 148 NHK/NHF Cells

Test	**FS-148 NHK/ NHF**
Keratinocytes & Fibroblasts (not pooled)	
Growth & morphology evaluation	
Cytogenetic Testing (Karyotype analysis)	Pass (NHF)
Isoenyzme analysis	Pass (NHF)
In-vitro tumorigenicity (soft agar)	Not tumorigenic
In-vivo tumorigenicity (nude mice)	Not tumorigenic
Keratinocytes & Fibroblasts pooled	
Sterility	Sterile
Mycoplasma cultivable & non-cultivable	Negative
In vitro virus - MRC-5, Vero & MDBK	Pass
EBV PCR	Negative
HHV-6- PCR	Negative
HBV PCR	Negative
HIV-I PCR	Negative
HIV II PCR	Negative
HTLV- I/II	Negative
HIV-1 co-cultivation & antigen capture	Negative

Table 3
The cytokine profiles

Cytokine	CCS Production
bFGF	+
EGF	+
GM-CSF	+
IL-1a	+
IL1b	+
IL-6	+
IFN-g	-
KGF-1	+
M-CSF	+
PDGF	+
TGF-a	+
TGF-b1	+
TGF-b2	+
TNF-a	+
VEGF	+

+ Present
- Undetectable

The sponsor has developed a cryo-preserved version of the product called Cryo-CCS. It should be noted that the fresh form of CCS is the only product that was used in the treatment of donor sites patients.

Literature data Vogt, et al *Platic and Reconstructive Surgery,* 102, 117 (1998) on cytokine concentrations in human wound fluids are available. The following are the data obtained from 16 patients:

IGF-1 (21-41 ng/ml)
EGF (3-63 pg/ml)
b-FGF (3- 60 pg/ml)
PDGF – AB (40- 200 pg/ml)
IL-1a – (10-200 pg/ml)
TGF-b – (60 - pg/ml)
TGF-b2 (10 – 30 pg/ml)
IGF-1 binding protein –1 (1-10 ng/ml)
IGF-1 binding protein –3 (1 - 300 ng/ml)

The growth factors IL-1a, TGF-b2, b-FGF and EGF are present in higher concentrations in the subject device than in the patients' wound fluid (literature values).

Biocompatibility of Collagen Sponge:
The following biocompatibility studies were conducted on the collagen sponge: Cytotoxicity, hemolysis, sensitization, acute systemic toxicity, subchronic toxicity, mutagenicity, rabbit pyrogen test. All tests were negative.