CMC REVIEW

BLA 125400/0

Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen (Gintuit)

Organogenesis, Inc.

Division of Cell and Gene Therapies, Office of Cellular, Tissue and Gene Therapies

Reviewers: **Mark H. Lee, Ph.D.**
Cellular Therapies Branch, DCGT/OCTGT/CBER

Eric Dollins, Ph.D.
Cellular Therapies Branch, DCGT/OCTGT/CBER

Concurred by:

Keith Wonnacott, Ph.D.
Branch Chief, Cellular Therapies Branch

Kimberly Benton, Ph.D.
Deputy Director, Division of Cellular and Gene Therapies

Raj Puri, M.D., Ph.D.,
Director, Division of Cellular and Gene Therapies

EXECUTIVE SUMMARY

Recommendation: This biologic licensing application (BLA) is approvable from a product quality perspective.

Product Overview:
Gintuit consists of cultured allogeneic cells derived from neonatal foreskin, including keratinocytes and fibroblasts, and bovine-derived collagen. It is comprised of two layers. The upper layer is made of human keratinocytes and the supporting lower layer is constructed of bovine-derived collagen, human extracellular matrix proteins, and human neonatal dermal fibroblasts. The manufacturing process involves (1) generation of the two-tiered cell banks of dermal fibroblasts and keratinocytes from donor neonatal foreskin tissue, (2) generation of --b(4)---------- and (3) a custom multi-step culture process in which these components are combined and further processed to generate the uniquely structured tissue-mimetic construct. The final clinical product that is Gintuit is supplied as one circular cellular sheet, approximately 75 mm in diameter and 0.75 mm in thickness. The product is stored on a semi-permeable synthetic membrane which separates it from a semi-solid agarose nutrient medium below and 10% CO2 atmosphere above in a transparent plastic container. Once packaged, Gintuit has a 15 day shelf-life under the validated conditions.

BLA/Review Organizational Note:

- *This review has been organized in a consistent manner as the BLA submission from the applicant. Drug substance is the mature, bi-layered –b(4)- unit through the ----b(4)---------of manufacture. This notably includes all information regarding the cell banks and bovine collagen, which are raw materials for drug substance manufacture. The drug product is the packaged Gintuit product.*
- *Collaborative reviewers who provided input to this review include Dr. Charles Durfor (CDRH/ODE/DSORD/PRSB) regarding bovine type I collagen and critical human/animal-derived reagents used in Gintuit manufacture; and Dr. Qiao Bobo (CBER/OCBQ/DMPQ/PII) regarding facilities and inspection-related issues.*

Review Findings:
The review of Gintuit is supported, in part, by the existing safety database and manufacturing experience gained by the applicant with the related commercial product, Apligraf®, since its approval in 1998 by the Center for Devices and Radiological Health (CDRH) of FDA. However, as a biologic licensing application (BLA) at the Center for Biologics Evaluation and Research (CBER), there are different regulatory requirements that the product has to meet to gain license approval. These included new key issues such as biologically relevant potency assays used for product lot release. After extensive discussion with the applicant, as well as valuable input from the Advisory Committee for this BLA held on November 17, 2011, the applicant and the FDA have been able to reach an agreement by incorporation of relevant bioassays with the existing histology assay as elements of a potency assay matrix for Gintuit. These bioassays included -----b(4)---------- For ---------b(4)----------

- ----------b(4)----------

Another pertinent review consideration was the safety and comparability of the different allogeneic cell banks used for product manufacture. As periodic addition of new banks of dermal fibroblasts and keratinocytes are required for Gintuit manufacture, key cell bank data generated from 13 years of manufacturing experience of the equivalent data to date was reviewed. This included parameters such as microbiological safety, cytogenetic stability and comparability of cellular characteristics among donors. The Advisory Committee meeting of November 17, 2011 also addressed these issues. The safety and comparability of the different cell banks used for Gintuit manufacture were determined to be adequate. The applicant will submit for FDA review, as a PMC, ----b(4)----------

Lastly, the manufacturing process that is used for Gintuit has remained largely unchanged since approval of the related product, Apligraf®, in 1998. Therefore, it was necessary to review whether the chemistry, manufacturing and controls for Gintuit were consistent with the most current regulations and FDA policies for a BLA. This involved both direct observation of the aseptic process during the pre-licensing inspection of the manufacturing facility in Canton, MA and information gained through the interactive review process. Many aspects of the manufacturing process were updated and re-validated to be consistent with current good manufacturing practice (cGMP). The necessary safety information supporting the use of critical human- and animal-derived raw materials in Gintuit manufacture was evaluated as a part of the BLA review. All animal-derived raw materials are tested for viruses, retroviruses, bacteria, fungi, yeast, and mycoplasma before use. While bovine materials are sourced to minimize bovine spongiform encephalopathy (BSE), the use of bovine pituitary extract (BPE) in Gintuit manufacture poses a theoretical safety risk for the transmission of variant Creutzfeldt-Jakob disease (vCJD). -----b(4)---

Agreed-upon Post-Marketing Commitments (PMCs):

2. ----b(4)-------

    a. ----b(4)---

    b. ---b(4)---

c. ---b(4)--- ----------

d. ----b(4)--- ----------

3. ---b(4)--- ----------

4. ---b(4)---- ----------

5. ----b(4)--- ----------

6. ----b(4)--- ----------

Reviewer Note: #1 has been omitted from this review as it refers to the clinical post-marketing requirement. Please refer to the memo of the clinical reviewer for details. For clarification on PMC #6, please refer to the review memo of the facilities reviewer.

Table of Contents for CMC Review

3.2.S DRUG SUBSTANCE

3.2.S.1 General Information

3.2.S.1.1 Nomenclature

Trade Name	Gintuit
United States Adopted Name (USAN)	N/A, combination product
Proper Name	Allogeneic cultured keratinocytes and fibroblasts in bovine collagen
NDC labeler code	42606-044-01
UNII codes	ZJO8CP3Q2A, Foreskin Keratinocyte (Neonatal) T34C307W5N, Foreskin Fibroblast (Neonatal) FHJ3ATL51C, Bovine Type I Collagen
Pharmacologic Class	allogeneic cellularized scaffold product
Dosage Form	cellular sheet
Company Name, Trivial Name, or codes used to identify the product in the application	-b(4)-, CelTx, Apligraf (Oral)

3.2.S.1.2 Structure

Gintuit is a cellular sheet that contains human foreskin-derived allogeneic cells and bovine collagen. It is comprised of two main layers: An upper layer formed by keratinocytes with a cornified layer, and a lower layer constructed of dermal fibroblasts, bovine Type I collagen and human extracellular matrix proteins. Through a custom, multi-step culture process, these components interact and produce the final bi-layered structure that possesses important barrier function and mechanical properties. The cells that are present in the sheet can produce extracellular matrix proteins such as collagen and cytokines such as Vascular Endothelial Growth Factor (VEGF). While the tissue-like structure resembles that of human skin, Gintuit does not contain Langerhans cells, melanocytes, macrophages, lymphocytes, blood vessels or hair follicles.

Each Gintuit unit is an off-white, circular disk with a diameter of approximately 75 mm and a thickness of approximately 0.75 mm. The composition of a Gintuit final unit is provided below.

COMPONENTS	QUANTITY PER UNIT
Gintuit Unit composition:	**(Starting Amounts)**
Human epidermal keratinocytes	---b(4)-----
Human dermal fibroblasts	--b(4)-----
Bovine Type I collagen	--b(4)-------

For Gintuit, the biological substance and biological final product are one and the same.

Reviewer Note: As a part of the discussion with the FDA regarding the product labeling, the applicant has noted that that they do not currently have reliable estimates of the number of keratinocytes and fibroblasts that are present in the final product. ----b(4)----------. This is acceptable.

3.2.S.1.3 General Properties

A Gintuit unit is comprised of two distinct layers:

- The lower layer, also referred to as the dermal equivalent (DE), consists primarily of Type I collagen, extracellular matrix (ECM) proteins and ----b(4)--- ----------, and human allogeneic neonatal dermal fibroblasts. This layer provides biomechanical strength and biochemical support for the formation of the upper layer. ---b(4)--- ----------.

- The upper layer forms by an –b(4)----------process during manufacturing which results in the formation of a stratum corneum. This structure is important for providing the barrier properties of Gintuit. The viable keratinocytes in the upper layer are believed to work in conjunction with the fibroblasts to secrete cytokines that are also important for wound healing.

3.2.S.2 Drug Substance Manufacture

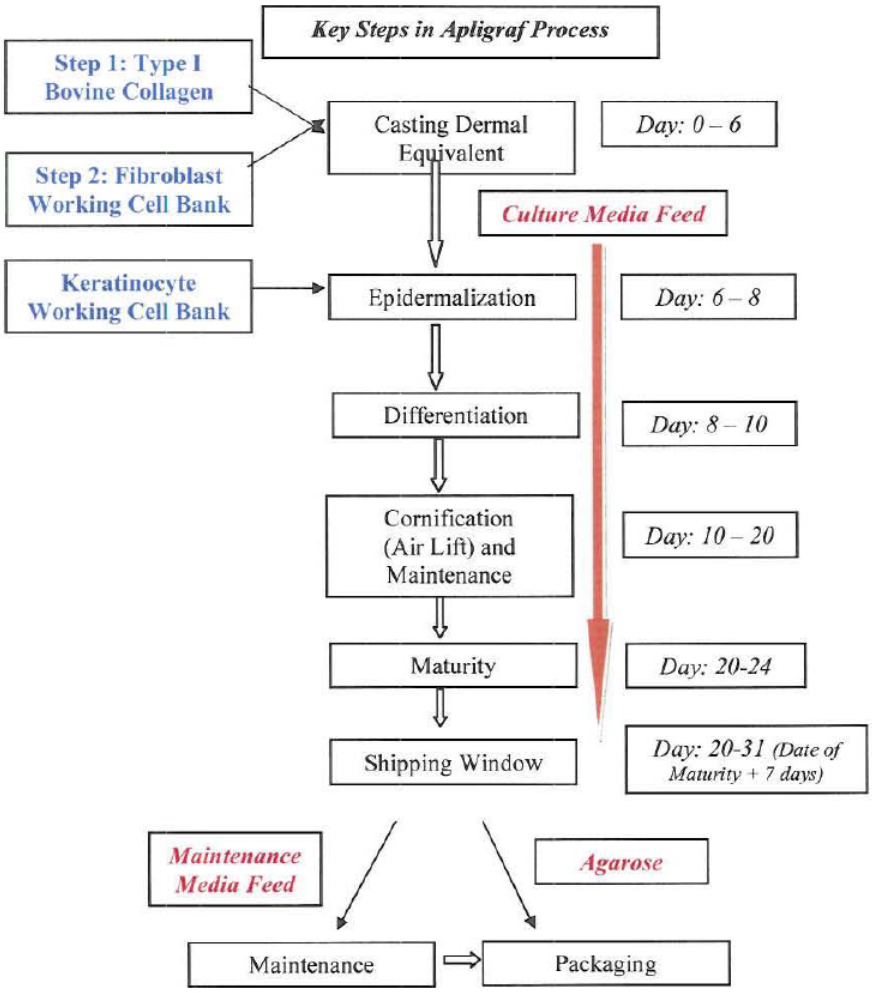
3.2.S.2.1 Manufacturer

The manufacture of Gintuit occurs at:

Organogenesis, Inc.
150 Dan Road
Canton, MA 02021 USA
Establishment Registration Number: 1221816

3.2.S.2.2 Description of Manufacturing Process and Process Controls

An overview of the manufacturing process is presented in the figure below (with more detailed descriptions following):



I. Generation of Fibroblast and Keratinocyte Cell Banks

----------b(4)--- ---------- The allogeneic cells used for the production of Gintuit are derived from neonatal foreskin tissue. Donor testing and screening meet the requirements for Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/P) outlined in 21 CFR 1271. Mechanical and enzymatic dissociation of the excised foreskin tissue releases keratinocytes and fibroblasts. The heterogeneous population of primary cells resulting from tissue dissociation is directed to produce a more homogeneous culture of keratinocytes and/or fibroblasts. This is achieved by means of cell selective and growth promoting media. For cell production, the applicant utilizes a two-tiered banking system

consisting of master cell banks (MCBs) and working cell banks (WCBs) for both cell types. The keratinocytes and fibroblasts are sub-cultured from the dissociated tissue using defined culture conditions to create each respective MCB. The WCBs are derived via serial passage from one or more vials of a single MCB. The keratinocytes and fibroblasts are cryopreserved at the MCB and WCB stages. WCBs are further passaged to generate the keratinocyte and fibroblast seed pools which form the starting material for the constructs. There are defined, limited numbers of passages for each stage of the cell banking procedure. Please refer to Section 3.2.S.2.3.2-3.2.S.2.3.5 below for details.

Reviewer Note: Given the limited yield of primary cells from the dissociated foreskin tissue and the limited expansion potential of these diploid cells, there is an ongoing need for the applicant to generate and qualify new cell banks (strains). Cell bank qualification data will be submitted to the FDA as supplements for approval before release of the cell banks and includes: adventitious agent testing, neoplastic safety testing, in vitro and in vivo comparability testing, identity testing, and cell performance characterization. The applicant anticipates submitting qualification data for several banks annually to the FDA for approval. The approach to qualify and demonstrate comparability for new cell banks used for Gintuit manufacture was discussed during the CTGTAC meeting on Nov. 17, 2011.

II. Bovine Collagen Processing

The production of bovine type I collagen is illustrated in Figure 2.3.S-5 of the BLA submission. ----b(4)--- ----------
---

[b(4)]

2 Pages determined to be not releasable:

b(4)

Reviewer Note: It is noted that the design parameters and formulations have remained largely unchanged since the original approval of the related product, Apligraf®, by FDA in 1998. There have been incremental process improvements (e.g. material changes) to enhance the safety profile and quality of the product. All process improvements implemented prior to and including 2007 were validated under a prospectively designed retrospective process validation protocol (Section 3.2.S.2.5). This validation concluded that the current process continues to operate in the original validated state of control. In STN 125400/027 (Received February 17, 2012), the applicant provided upon FDA request a summary of implemented Gintuit manufacturing changes after the retrospective process validation of December 2007. These changes were determined to be minor in nature and therefore the process validation remains valid for the current state of Gintuit manufacture.

3.2.S.2.3 Control of Materials

3.2.S.2.3.1 Raw Materials

An overview of the raw materials used for Gintuit manufacture are listed in Table 2.3.S-5 of the BLA and are further discussed in detail in Section 3.2.P.2.1 and Section 3.2.P.4. The table below summarizes this information.

[b(4)]

7 Pages determined to be not releasable:

b(4)

-----*b(4)*---------- ----------

---*b(4)*-------- ----------
---------- ----------
--------- ----------
---------- ----------

--b(4----------

All necessary documentation has been provided for the remaining human- and animal-derived reagents from the table above.

3.2.S.2.3.2 Source, History, and Generation of Cell Substrate

Components and Material used for Generation of Cell Banks
Control of starting materials and components used for the generation of Gintuit, including the establishment of cell banks, has been reviewed separately above in Section 3.2.S.2.3.1 of the BLA.

Cell source and donor screening / testing
The tissue donation program is designed to ensure that the necessary documentation and testing are performed in order to determine eligibility and suitability of the donor. Neonatal foreskin tissue is obtained in accordance with established tissue donation guidelines and informed consent procedures. The donor program complies with current regulations for human cells, tissues, and cellular and tissue-based products (HCT/P's) outlined in 21 CFR Part 1270 and Part 1271. All donor sites are qualified in accordance with pre-established internal procedures. The tissue donation protocol and informed consent procedures are approved by the site IRB.

Both the mother and father of the potential donor are screened using a set of comprehensive inclusion and exclusion criteria defined the applicant's NFTDP Donor Screening Form. The maternal donor is required to submit a blood sample for infectious disease testing as on the same

day as tissue collection. The tests mandated by 21 CFR Part 1271 are conducted using FDA approved test kits. The testing laboratory, ----b(4)----------, is registered as a donor testing facility with the FDA as a Human, Tissue, and Cellular and Tissue-Based Products establishment (--b(4)----------).

Within the Organogenesis Quality System, the donor eligibility and suitability is determined by the Tissue Bank Medical Director. The Tissue Bank Medical Director reviews: 1) the donor parent's medical and risk behavior history and medications, 2) maternal physical exam, 3) delivery and circumcision records, and 4) maternal donor testing results.

Maternal Donor Testing Panel (based on BLA Tables 3.2.S.2.3-1 and 3.2.S.2.3-2)

TEST	TEST METHOD (SUPPLIER)	SPECIFICATION
Blood Group and Rh type	-----b(4)----------	--b(4)--
HIV-1/2 antibodies[1]	----b(4)---------- ----------	--b(4)--
NAT HIV-1/HCV/HBV	----b(4)---------- ----------	--b(4)--
HTLV-I/II antibodies	-----b(4)----------	--b(4)--
Hepatitis B surface antigen (HBsAg) antibodies	-----b(4)----------	--b(4)--
Hepatitis B core (HBc) (Total) antibodies	-----b(4)----------	--b(4)--
Hepatitis C (Anti-HCV) antibodies	-----b(4)----------	--b(4)--
Cytomegalovirus (CMV) Total Antibodies (includes IGM)	-----b(4)----------	--b(4)--
----b(4)----------	-----b(4)---------- ----------	--b(4)--
West Nile Virus (WNV)	-----b(4)----------	--b(4)--
Urine Neisseria Gonorrhea[3]	-----b(4)---------- ----------	--b(4)--
Urine Chlamydia Trachomatis[3]	-----b(4)---------- ----------	--b(4)--
HLA (if feasible)		--b(4)--
Blood Group and Rh type (if feasible)	-----b(4)----------	--b(4)--
Alanine Aminotransferase (ALT)[4]	-----b(4)----------	--b(4)--
Hepatitis B Surface Antibody -b(4)---	-----b(4)----------	--b(4)---------- ----------
---------- --b(4)----------	-----b(4)----------	--b(4)--
Epstein-Barr Virus (EBV) ---b(4)---- ----------	-----b(4)----------	--b(4)--
Epstein Barr Virus –b(4)----	-----b(4)----------	--b(4)--

TEST	TEST METHOD (SUPPLIER)	SPECIFICATION
-----b(4)----------	---b(4)----------	--b(4)--
----------b(4)----------	---b(4)----------	--b(4)--

[1]HIV-1 is repeated at 6 months post circumcision if maternal donor is available.
[2]indicative of previous or active Syphilis.
[3]Added to the Maternal Testing Panel effective January 1, 2011.
[4]Indicative of hepatitis.
[5]Donor suitability is determined by IgM results.

----b(4)----------

----b(4)---------- ----------

---b(4)----------

----b(4)--- ----------

-----b(4)---- ----------

---

1 Page determined to be not releasable:

b(4)

----b(4)---- --

<u>Reviewer Note</u>: The cell banking system (procedures and testing) is well established- FDA approved (under PMA#950032) and effective since 1998. The use of a two-tiered system consisting of MCBs and WCBs is appropriate for manufacture of the product. The number of passages of keratinocytes b(4) and fibroblasts b(4) for use in manufacture of the final product has been found to be safe, both clinically (under PMA#950032 and IDE studies for Gintuit) and from in vitro and in vivo cell bank safety testing (described below in Section 3.2.S.2.3.3).

Cell selective and growth promoting media

-----b(4)---- ---

<u>Reviewer Note</u>: The above section summarizes the purpose of various media components. In section 3.2.P.2.2- Formulation Development, the applicant provides the history, rationale, and data (both primary data and literature reference) to support the current media formulation, including base media and supplements.

Nomenclature and traceability

The lot numbering system for the keratinocyte and fibroblast cell banks is described in BLA Table 3.2.S.2.3-13. Of note, traceability of cells from donor to final product is ensured through unique codes. Furthermore, information regarding the exact batches of keratinocyte and fibroblasts strains that are used for each Gintuit unit is captured in the batch records of each manufactured lot. Both aspects of traceability were verified by examining executed batch records during the pre-license inspection of the Canton, MA manufacturing facility.

Cell Bank Storage

---b(4)--- ----------

Reviewers note: During the pre-license inspection, it was noted as a discussion item that the current cell banking archive system, while not in violation of regulation, is not performed according to FDA guidance for the following reasons: 1) there is no MCB archive; 2) there is --b(4)---------- --- and 3) MCB and WCB samples should be stored in two or more separate locations within the facility or at a distant site in order to avoid loss of the cell substrate due to local disaster or equipment malfunction. The applicant noted the discussion item and responded they would take it under advisement. Additional details are described in the EIR.

3.2.S.2.3.3 General Testing Strategy

Overview of Qualification of Cell Banks

Qualification of cell banks includes several broad test categories: adventitious agent testing, neoplastic safety testing, *in vitro* and *in vivo* comparability testing, identity testing, and cell performance characterization. The number of cells obtained from the primary tissue is limited, resulting in a relatively small MCB. There is also a low number of passages between the MCB and creation of the WCB. Therefore, the testing scheme for qualification of the MCB and WCB is designed with overlap. To obtain sufficient cells for MCB testing, a portion of the MCB is passaged using the same conditions used to generate the WCB. The overall testing scheme includes tests that are performed on cells from this MCB passage (referred to as Master Cell Bank Test Cells (MCBTC), cells from the WCB, and on final cell-scaffold constructs made from MCB and WCB cells.

A subset of cell bank testing data will be submitted to the FDA as a BLA supplement for approval before release of the cell bank. Data to be submitted to the FDA include all MCB testing and microbiological safety testing from a minimum of –b(4)----------. Other WCB testing results are used internally for WCB cell bank release but will not be submitted to the agency for review. This testing scheme is outlined below. Unless otherwise noted, the testing performed applies to both keratinocytes and fibroblasts:

MCB Qualification Testing

Testing performed on cells:

- *Adventitious agents*:
  - Microbiological and viral safety testing: Used to show the cells are free from contamination due to bacteria, fungi, mycoplasma, and viruses.
- *Neoplastic safety testing*:
  - Isoenzyme analysis: Used to positively identify the species of the cell line as human.

- Karyology (cytogenetic analysis): Used to determine whether chromosomal aberrations have occurred over time during cell culture. The upper limits of acceptability for abnormalities are calculated using expected frequencies for diploid cell lines.
- Senescence determination: Used to demonstrate that the diploid cells have a finite life span and will not indefinitely replicate. A negative growth index is used to determine when cells no longer have the ability to divide.
- Tumorigenicity testing: Used to evaluate tumorigenic potential of the cells. Groups of nu/nu female mice are subcutaneously injected with one of the following: the cell bank to be tested, positive control –b(4)---- cells or serum free medium. Mice are observed everyday and followed for 3 months. At the end of three months the animals are euthanized and necropsied.

- *In vitro comparability*:
  - Cell purity: Used to detect the level of cellular impurities. A flow cytometer is used to acquire and analyze the percent expression of cell surface markers present on control and test samples.

Testing performed on mature –b(4)- units made from cells:

- *In vitro comparability*:
  - Percutaneous absorption: Used to measure to the barrier function of the --------b(4)-- unit. Percutaneous absorption is determined as a percentage of applied tritiated water –b(4)----------. Test units are compared with control units produced from qualified banks and must not exceed pre-determined absorbance rates.
  - Cytokine profile assay: Used to determine presence of cytokines from keratinocytes and/or fibroblasts in the mature –b(4)- unit. The RT-PCR assay includes three markers for the keratinocyte and/or fibroblast cells (IL-1α, PDGF-α, and TGF-β1), a positive control marker (-b(4)----), and a negative control marker (IL-4).
  - Mitochondrial tetrazolium test (MTT): Used to evaluate metabolic activity and viability of cells in the mature –b(4)- unit. This colorimetric assay is dependent on intact mitochondrial function. Test units are compared with control units produced from qualified banks and must meet pre-determined tetrazolium conversion rates.
  - Morphological analysis of final product: Used to evaluate if the cells of MCB can produce a mature –b(4)- unit which passes pre-determined specifications.
  - ---b(4)--------

- *In vivo comparability in athymic mice*:
  - *In vivo* comparability: Used to examine histological assessment, immunohistochemical staining for human involucrin, graft take and integration as well as % contraction of a graft made from the mature –b(4)- unit. The final

assessment of *in vivo* comparability is determined by a trained pathologist based on comparability of test articles to control articles produced from qualified banks for each test parameter.

WCB Qualification Testing

Testing performed on cells:

- *Adventitious agents:*
  - Microbiological and viral safety testing: Used to show the cells are free from contamination due to bacteria, fungi, mycoplasma, and viruses.
- *Identity and cell performance characterization*:
  - Cell growth: Used to evaluate if cells have a kinetic profile typical of normal doubling mammalian cells.
  - Cell viability: Used to determine if cells are alive. Cells must be –b(4)- viable post-thaw.
  - Isoenzyme analysis: Used to positively identify the species of the cell line as human.
  - Collagen biosynthesis: Used as an identity test for fibroblasts. A key function of fibroblasts is the ability to synthesize collagen. Spent culture media from test fibroblast and control lots are quantitated for collagen content using a –b(4)----------
  - Involucrin content: Used as an identity test for keratinocytes. Involucrin expression is a marker for keratinocytes and is detected in cultured keratinocyte extracts by ELISA.

Testing performed on mature –b(4)- units made from cells:

- *In vitro comparability*:
  - Morphological analysis of final product: Used to evaluate if cells of WCB can produce a mature –b(4)- unit which passes pre-determined specifications.

Reviewer Assessment: The applicant's approach to qualify and demonstrate comparability for new cell banks used for Gintuit manufacture was discussed during the CTGTAC meeting on Nov. 17, 2011. The committee noted that each mature –b(4)- unit can be derived from two different donors (one donor for each cell banks) and therefore can be different donor pairings between keratinocyte and fibroblast cell banks used to form the mature units. The committee recommended collecting more quantitative biological data (e.g. amounts of cytokine expression) to support equivalent activity of product lots manufactured with different pairs of fibroblast and keratinocyte banks. Subsequent to the CTGTAC meeting, the applicant was asked to provide data to address the committee's observation if and/or how donor pairing of cell strains impacts product quality.

----b(4)---

-----*b(4)*---- ----------

----*b(4)*---- ----------

MASTER CELL BANK TESTING

This section is organized according to the type of test being performed. Tests are organized by the following categories:

- *In vitro* comparability
- *In vivo* comparability
- Microbiological and viral safety testing
- Neoplastic safety testing

For each category, there are descriptions and evaluations of the each individual analytical method and accompanying validation.

IN VITRO COMPARABILITY TESTING

Summary table of *In Vitro* Comparability Testing on Master Cell Bank [Adapted from BLA Table 3.2.S.2.3.3-3]

TEST	TEST METHOD	SPECIFICATION	JUSTIFICATION OF SPECIFICATION
Percutaneous Absorption (PA)	--b(4)----------	--b(4)--- ----------	---b(4)-- ----------
Mitochondrial Tetrazolium Testing (MTT)	QCP-112	-----b(4)--- ----------	--b(4)-- ----------

TEST	TEST METHOD	SPECIFICATION	JUSTIFICATION OF SPECIFICATION
Morphological analysis	QC-OPER-00547	----b(4)---------- -----b(4)---------- --b(4)---------- -----b(4)----------	*See section on Potency assay I
Cytokine Profile	QCP-119	Positive for: IL-α PDGF α TGβ1 -b(4)------- Negative for: IL-4	-----b(4)---- ----------
Cell purity	QC-OPER-00569	----b(4)--- ----------	---b(4)--- ----------
--b(4)-- -------- ----------	-b(4)------	----b(4)---------- --------	---b(4)---- ---------- ----------

Percutaneous Absorption (SOP –b(4)- SP630015, measured on –b(4)- construct)

---*b(4)*----------

----b(4)--- ----------

PA Method Validation [Validation report NCLVR-0358-001]

-----b(4)--- ----------

---
6 Pages determined to be not releasable:

b(4)

determined by established acceptance criteria defined for each parameter and as determined by the pathologist.

In Vivo Comparability Method Validation
Due to the *in vivo* nature of this assay, formal analytical method validation of this assay has not been performed.

MICROBIAL AND VIRAL SAFETY TESTING
Microbiological and viral safety testing for the MCBs is conducted at ----b(4)---------- ---------- using validated methods under GLP or GMP conditions where applicable (except –b(4)----------, testing performed by –b(4)---------- ---b(4)-------- has validated all methods in accordance with ICH Q2(R1) guidelines except –b(4)- and animal models. –b(4)------- has issued the applicant a letter authorizing FDA to access information found in –b(4)--- related to their test methods and validation. The letter is provided in BLA section 1.4.1.

Reviewer Note: This Master File has been confirmed to be active.

Microbiological and Viral Safety testing is conducted according to ICH Q5D and Q5B, 21 CFR 610 and the following FDA guidances: (1) *Guidance for Human Somatic Cell Therapy and Gene Therapy* (1998); (2) *Content and Review of Chemistry, Manufacturing and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs)*(2008); and (3) *FDA Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals* (1993).

Safety- Microbiological and Viral Testing on Master Cell Bank [BLA Table 3.2.S.2.3.3-3]

[b(4)]

5 Pages determined to be not releasable:

b(4)

[ ]

b(4)

WORKING CELL BANK TESTING

Relative to the MCB testing, WCBs are subjected to separate and overlapping panel of safety tests. Similar to the MCB, these include safety, morphological analysis, identity, growth, viability and functionality testing. Those tests used to release the WCB are indicated in the table below. Descriptions and evaluations of the each individual analytical method and accompanying validation are found following the summary table.

1 Page determined to be not releasable:

b(4)

Description of Cell Characterization Tests for Working Cell Banks

Cell Growth and Viability (SOP QCP-084 (fibroblast); SOP QCP-083 (keratinocyte))
Cell growth and viability is performed on the working cell banks to qualify the growth potential (population kinetic profile) and determine viability of the working cell banks.

Cell Growth and Viability Assay Method Validation
See cell counting validation outlined above for Master Cell Bank testing.

Collagen Biosynthesis (QCP-129)
Collagen Biosynthesis Methods Summary
----b(4)--- ----------

Collagen Biosynthesis Method Validation [Validation reports NCLVR-0250-001 and NCLR-0229-001]
----b(4)--- ----------

----b(4)--- ----------

[b(4)]

1 Page determined to be not releasable:

b(4)

[b(4)]

--b(4)---------- ----------

----------b(4)----------

--b(4)---- ----------
-----b(4)--- ----------

--b(4)----------

STABILITY OF CELL BANKS [3.2.S.2.3.4]

Reviewer Note: Apligraf® is currently marketed as a device (PMA#950032) and is subject to design controls under QSRs. cGMP regulations for BLAs require validation to be performed. The applicant has submitted prospectively designed, retrospective validations for the cell banking process and stability. According to the definition in ICH Q7 Section 12.4, Process Validation (PV) is the documented evidence that the process, operated within established parameters, can perform effectively and reproducibly to produce an intermediate or API meeting

its predetermined specifications and quality attributes. A <u>retrospective</u> process validation may be used for well established processes that have been used without significant changes in API quality due to changes in raw materials, equipment, facilities, or the production process.

Given the long manufacturing history of Apligraf® since its approval in 1998, the applicant was asked to provide a comprehensive table listing cell banks that have been used for the manufacture of Apligraf® to date and of Gintuit during the IDE studies, including supplement number, submission dates, and any changes to the cell bank manufacturing or testing (sent to applicant 8/12/11). These data were submitted to the BLA (8/18/11) and evaluated to determine if there were any significant changes that were implemented during the retrospective process validation period that could confound the results of the validation. All cell bank manufacturing and testing changes were relatively minor, approved by the FDA under PMA#950032, and not expected to result in changes in API quality. Therefore, according to definitions outlined in ICH Q7, a retrospective process validation may be utilized.

Retrospective validation for stability of cell banks [3.2.S.2.3.4]
----*b(4)*--- ---

------b(4)--- ---

- ---b(4)-- ---

- ---b(4)---- ------------------------------------

--b(4)----
---b(4)---- --

---b(4)-----
-------b(4)------ --
-- ----

---b(4)---- ----------

Conclusion of cell banking stability validation
The applicant concludes, based on this retrospective validation, the data currently supports a maximum stability of:

- ---b(4)---- ----------
- --b(4)-- ----------
- ---b(4)--- ----------
- ----b(4)--- ----------

Reviewer Note: The retrospective validation for stability of cell banks was reviewed as part of the BLA review and also during the pre-license inspection (Canton, MA). It was recommended to Organogenesis management during the pre-license inspection that the final maximum stability should be based off of more than one data point. The applicant noted the discussion item and responded they would take it under advisement. Additional information may be found in the EIR.

VALIDATION OF CELL BANKS

Retrospective process validation for cell banking
Validation of individual analytical methods used for cell bank testing and qualification are included with the analytical method descriptions in section 3.2.S.2.3.3 of the BLA. Validation of the cell banking process is described in section 3.2.S.2.3.5 of the BLA.

Summary of cell banking process validation [validation report –b(4)---Apligraf Manfuacturing-09-070]
The applicant conducted a prospectively developed, retrospective process validation for manufacturing of cell banks for the period from Jan 1999 to Jan 2010. The validation is intended to demonstrate controlled manufacturing of Gintuit cell banks in accordance with relevant regulations and standards relative to retrospective process validation. Specific aspects of control include documentation of in-process controls, QC release testing results, qualified equipment and reagents, SOPs and quality systems supporting manufacturing and testing. ---b(4)--- ----------
---

Conclusion of cell banking process validation
Core cell banking procedures have been FDA approved (under PMA#950032) and effective for an average of 13 years prior to the retrospective validation suggesting manufacturing controls that are in place are well established. In the retrospective validation, there was a low frequency of deviations in which in-process specifications were out of range. In these instances, the

deviations were deemed not to have product impact. The large number of historical data points, low percentage of OOS/Outlier data elements and the proper disposition of OOS cell banks indicate the cell banking process is operating under a state of control.

Reviewer Assessment: The two-tiered cell banking system used for the cellular components of Gintuit ----b(4)--- ---------- Given the limited yield of primary cells from the dissociated foreskin tissue and the limited expansion potential of these diploid cells, there is an ongoing need for the applicant to generate and qualify new cell banks (strains). ----b(4)-- ---------- ----------. The applicant anticipates submitting qualification data for several banks annually to the FDA for approval. The approach to qualify and demonstrate comparability for new cell banks used for Gintuit manufacture was discussed during the CTGTAC meeting on Nov. 17, 2011. Retrospective validations were executed to demonstrate control of the cell banking process and establish stability of the cell banks.

3.2.S.2.4 Controls of Critical Steps and Intermediates

The identification of critical steps and controls that are in place for Gintuit manufacturing has been gained from extensive experience gained after approval of the related PMA product in 1998. The following table summarizes the in-process controls that are in effect during manufacturing. It is noted that the in-process testing for microbiological safety is described separately in the review.

[b(4)]

[b(4)]

Environmental Controls

Please refer to the memo of the facilities reviewer for details.

Justification of In-Process Controls and Acceptance Criteria

The manufacturing process and controls that are in place for Gintuit manufacturing has been gained from extensive manufacturing experience from the commercially available product, Apligraf® since 1998. Other than the change of raw material and incorporation of additional safety tests, this manufacturing process and parameters have remained unchanged.

Reviewer Assessment: The in-process parameters that are tabulated above ensure reasonable consistency of manufacture of the drug substance, -b(4)- construct. Given the extensive manufacturing experience that the applicant has had to date (>13 years), the very low rate of product lot failures, and the lack of safety signals associated with Apligraf®, the in-process controls that are in place are sufficient. It is noted that during the PLI of the applicant's manufacturing facility, the inspection team observed the manufacturing process that is in place and added one deficiency (observation #5) regarding the need to validate critical in-process time limits for ----b(4)----------. This was adequately addressed in the applicant's 483 responses in STN 125400/0022 (Received 12/29/2011). The validation is provided in Section 3.2.S.2.5.

3.2.S.2.5 Process Validation and/or Evaluation

A retrospective validation of the manufacturing process was performed in January 2010 and validation of –b(4)---------- was performed in December 2011. In addition, a process re-qualification protocol that specifies re-qualification of the manufacturing process at a maximum of every –b(4)---------- is in place.

Manufacturing Process Validation

---b(4)-- ----------

- ----b(4)-- ----------
- ---b(4)--- ----------
- ---b(4)--- ----------
- ----b(4)--- ----------

---b(4)---- ----------

- **----b(4)**--- ----------
  - ------- ----------
  - ----------
  - ----------
  - ----------
  - ----------
  - ----------
- **--b(4)**---- ----------
  - ----------
  - ----------
  - ----------
  - ----------
  - ----------
- **-----b(4)**--- ----------
  - ----------
  - ----------
  - ----------
  - ----------
  - ----------
  - ----------
  - ----------
- **----b(4)**-- ----------
  - ----b(4)--- ----------

3 Pages determined to be not releasable:

b(4)

----b(4)------- ----------

--b(4)-- ----------

----b(4)--- ----------

--b(4)--- ----------

3.2.S.2.6 Manufacturing Process Development

The Gintuit manufacturing process is based on initial development work by –b(4)---------- in the 1980s and is largely equivalent to the process used for Apligraf® which received FDA approval for the venous leg ulcer indication in 1998. There have been incremental changes in the manufacturing process for Apligraf® over the years to improve the safety profile and product quality however these changes were in place prior to the generation of products used for the two investigational device exemption (IDE) studies for Gintuit supporting this BLA. The manufacturing process for Gintuit reflects the most up-to-date process used for the commercial lots of Apligraf® currently. This includes the use of the same ----b(4)---------- ---------- Some of the historical developments in the manufacturing process and media formulation are summarized in Section 3.2.S.2.6 of the BLA.

3.2.S.3 Characterization

3.2.S.3.1 Elucidation of Structure and Other Characteristics

The physicochemical characterization of the two layered structure of Gintuit is described in Section 3.2.S.1.3 of the BLA and is summarized below. An important consideration for this engineered product is the understanding that the lower and upper layers are unique and that certain characteristics and synergy are required for the successful development of the Gintuit structure. The basis of the characterization strategy is that:

- Lower layer (DE) acts as a matrix for the fibroblasts and as a substrate for the maintenance of the upper layer. As a result, the ability of the fibroblasts to secrete necessary ECM proteins and growth factors involved in wound healing are important.
- Upper layer acts as the element that provides the critical barrier properties upon formation of the cornified epithelial layer. The ability of the keratinocytes to secrete certain cytokines is important for appropriate maturation.

4 Pages determined to be not releasable:

b(4)

3.2.S.4 Control of Drug Substance

3.2.S.4.1 Specifications

Pease refer to the Section 3.2.P.4.1 of the BLA, specifications for drug product.

3.2.S.4.2 Analytical Procedure Summaries for Drug Substance Release Specifications

Please refer to Section 3.2.P.4.2 of the BLA, analytical procedures for drug product.

3.2.S.4.3 Validation of Analytical Procedures

Please refer to Section 3.2.P.4.3 of the BLA, validation of analytical procedures for drug product.

3.2.S.4.4 Batch Analyses

Please refer to Section 3.2.P.4.4 of the BLA, batch analyses for drug product.

3.2.S.4.5 Justification of Specifications

Please refer to Section 3.2.P.4.5 of the BLA, justification of specifications for drug product.

3.2.S.5 Reference Standards or Materials

---b(4)--- ----------

3.2.S.6 Container Closure System

Please refer to Section 3.2.P.6 of the BLA, Container Closure for Drug products.

3.2.S.7 Stability

Please refer to Section 3.2.P.7 of the BLA, Stability for Drug products.

3.2.P Drug Product

3.2.P.1 Description and Composition of the Drug Product

Section 3.2.S.1 contains a detailed description of the mature bi-layered –b(4)-- unit. This construct is maintained on a porous membrane within a culture insert, inside a shipping tray.

Agarose shipping medium ----b(4)----------, with exposure of this nutrient gel to the underside of the porous membrane (i.e. not directly onto product). It is noted that the membrane is removed prior to clinical application of Gintuit. This shipping tray is aseptically packaged into a heavy-gauge polyethylene transparent bag with 10% CO_2 using a heat sealer.

The final packaged drug product consists of the mature –b(4)- unit, culture insert, shipping tray, agarose and CO_2.

COMPONENTS	QUANTITY PER UNIT
Gintuit Unit composition:	
Human epidermal keratinocytes	--b(4)------
Human dermal fibroblasts	--b(4)------
Bovine Type I collagen	--b(4)------
Agarose Shipping Medium composition:	--b(4)------
--b(4)----------	--b(4)------
--b(4)----------	--b(4)------
--b(4)----------	--b(4)------
Carbon Dioxide (CO2), --b(4)----	10%

* These numbers represent the starting amounts of each material and may not affect be an accurate quantity of each material present in the final product.

3.2.P.2 Pharmaceutical Development

3.2.P.2.1 Components of the Drug Product

3.2.P.2.1.1 Drug Substance

Please refer to Section 3.2.S.1.3, general properties of drug substance

3.2.P.2.1.2 Excipients

There are two main excipients listed for the final product, the agarose shipping medium used for shipping (--b(4)----------) and CO_2 –b(4)---------- (10%, --b(4)----- grade). It is noted that these excipients are not part of the final product formulation as they are only present during package/transport and are not effectively included in the clinically applied product.

The composition of the agarose shipping medium is provided below.

1 Page determined to be not releasable:

b(4)

- *---b(4)---* ---
- *-------b(4)-----* ---
- *----b(4)----* ---

On the basis of this information, the inclusion of these excipients in the final drug product is not likely to introduce a significant safety risk. Please also refer to the adventitious virus safety discussions in Section 3.2.A.2.

3.2.P.2.2 Drug Product

3.2.P.2.2.1 Formulation Development

The formulation development for the -b(4)- bi-layered construct is described in Sections 3.2.S.2.6 and 3.2.P.2.3.

3.2.P.2.2.2 Overages

Overages are not applicable to this drug product.

3.2.P.2.2.3 Physicochemical and Biological Properties

3.2.P.2.3 Manufacturing Process Development

Section 3.2.S.2.6 contains description of the drug substance manufacturing process development.

3.2.P.2.4 Container Closure System

See Section 3.2.P.7.

3.2.P.2.5 Microbiological Attributes

Gintuit is a product with viable cells that cannot be terminally sterilized and thereby require aseptic processing to ensure product safety. The applicant has used FDA's Guidance for Industry – Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice (September 2004) regarding aseptic processing controls, microbial testing and environmental controls. Due to the limited 15 day shelf-life of Gintuit, release of the product

prior to completion of microbiological testing is required. The product is released and shipped based on in-process sterility and –b(4)- results that are available at time of release. These tests are shown in Table 3.2.P.2.5-1 below.

MATERIAL TESTED	ASSAY	SPECIFICATION	DAY SAMPLED	FINAL RESULT AVAILABLE AT SHIPMENT*
Acellular Cast Mix	Sterility QCP-002	Negative	0	Yes
Cellular Cast Mix			0	
HDF Cell Residue			0	
-b(4)-HEP Residue			6	
Differentiation Spent Media			10	
Cornification Spent Media II			15	Yes if shipment at day 22-29.
Cornification Spent Media III			17	Yes if shipment at day 24-29.
Cornification Spent Media III	-b(4)-- QCP-108	0 CFU		
Maintenance Spent Media I	Mycoplasma QCP-072	Negative	20	No
Maintenance Spent Media I Maintenance Spent Media II Maintenance Spent Media III Maintenance Spent Media IV	Sterility QCP-002	Negative	20-29	No
Ship Rinse Spent Media	-b(4)-- QCP-108	0 CFU	20-29	No

Sterility and ---b(4)---------- testing are used to assess the microbial attributes of the drug substance and drug product at critical stages of the manufacturing process. In-process sterility is performed using a 7-day BacT/ALERT 3D test (QCP-002). In addition, a sample of –b(4)---- ----------. Gross microbial contamination is also assessed via –b(4)--- ----------

Reviewer Assessments:

- *The rationale for shipping the product before availability of the final product sterility test (using the 14 day USP <71> method) is acceptable. Language regarding the potential for microbial contamination in Gintuit will be included in the final product labeling.*
- *The strategy of using in-process sterility and –b(4)- testing at critical steps is a reasonable way of mitigating the risk of positive sterility result. In addition, Recalls for Apligraf® since 1998 were investigated by Drs. Mark H. Lee and Eric Dollins during the PLI. It is observed that the frequency of microbial contamination results was not significant for the experienced manufacturing process and that the microbial ID suggested that the sources of the contamination were likely to be operators.*

- *Aseptic processing technique was also evaluated closely by the DMPQ inspectors during the PLI and multiple 483 observations were made. All of these inspection issues have been adequately addressed by the applicant as of February 3, 2012.*

3.2.P.2.6 Compatibility

There are four components present in the final packaging that have direct contact with the Gintuit unit: 1) Agarose Shipping Medium, 2) the porous polycarbonate membrane and 3) the culture insert to which the polycarbonate membrane is attached.

Agarose Medium (historical)

- A summary of the –b(4)--- formulations used for agarose shipping medium to date is provided in Table 3.2.P.2.6-1 of the BLA
- Final packaged product was exposed to accelerated stability testing at elevated temperatures up to –b(4)----------
- The currently used formulation of Agarose shipping medium met safety and histology requirements

---b(4)----------
----b(4)--- ----------

- ---*b(4)*---- ----------

- ----*b(4)*---- ----------

- ---*b(4)*---- ----------

----b(4)----------
-----b(4)--- ----------

----

1 Page determined to be not releasable:

b(4)

- *----b(4)---* ----------

3.2.P.3 Manufacture

3.2.P.3.1 Manufacturers

Facilities involved in Gintuit manufacturing, release and stability testing, packaging, labeling and storage are listed below.

FACILITY	
Manufacturing & Quality Control Facility: Organogenesis, Inc. 150 Dan Road Canton, MA 02021 USA Establishment Registration Number: 1221816	Manufacturing; Incoming, in-process and final release testing
---b(4)---------- ---------- ---------- ----------	---b(4)----------
Warehouse Facility: Organogenesis, Inc. 85 Independence Drive Taunton, MA 02780 USA	Receipt, initial inspection and storage of incoming raw materials (not including chemicals or tissues), and manufacturing supplies.
----b(4)---------- ---------- ---------- ----------	----------b(4)----------
----b(4)---------- ---------- ---------- ----------	----b(4)----------

3.2.P.3.2 Batch Formula

----b(4)---------- ----------

COMPONENTS	QUALITY STANDARDS	BATCH SIZE	
		-b(4)---	-b(4)---
-b(4)- Construct	-b(4)---	-b(4)---	-b(4)---
Agarose Shipping Medium	-b(4)---	-b(4)---	-b(4)---
-b(4)- Carbon Dioxide (CO_2)	-b(4)---	-b(4)---	-b(4)---

	LOT NUMBER FORMAT	DESCRIPTION OF LOT NUMBER
Gintuit	GSYYMM.DD.ZZ.BB	---b(4)---------- -b(4)---------- ---------- ------ --b(4)---------- ---b(4)---------- ----------

The rationale for selection of sterility and endotoxin test samples as a function of lot size was described by the applicant in STN 125400/0027 (Received 2/17/2011). In general, units from the ------b(4)----------. The general sampling strategy was made to be consistent with the Federal Register Proposed Rule Docket No. FDA-2011-D-0429, Amendments to Sterility Test Requirements for Biological Products.

3.2.P.3.3 Description of Manufacturing Process and Process Controls

This section describes the manufacturing process that occurs after –b(4)- construct maturation (i.e. drug substance). Specifically, this includes the following steps.

Reviewer Note: The numbering scheme below is a continuation of the description of manufacturing process and process controls for the drug substance (Steps –b(4)---) in Section 3.2.S.2.2.

------b(4)----------

----b(4)--- ----------

----

2 Pages determined to be not releasable:

b(4)

- ***----b(4)---*** ----------

3.2.P.3.5 Process Validation and/or Evaluation

Elements of the manufacturing process for the drug product that were validated independent of the drug substance (-b(4)- unit) are addressed in this section. It is noted that re-qualification of the entire drug substance and drug product process will occur on a periodic basis as described in Section 3.2.S.2.5.

Nonclinical Laboratory Study Protocol NCLVR-0393-001 (dated December 14, 2011)

- Risk assessment was performed on the manufacturing process to identify critical manufacturing process steps that require process hold times. The –b(4)---------- process was identified as a critical manufacturing process step.
- Prospectively designed retrospective validation of the –b(4)---------- and maximum process hold time limits for –b(4)- lots using existing batch records. Minimum process hold limits have been established for the -----b(4)----------.
- An upper process limit was established for ----b(4)---------- process.
- Manufacturing lots that were included in the validation is provided in Table 1 of the report. Definitions of elapsed time calculations are provided in Table 3.

The manufacturing process time parameters that were analyzed from the batch production record are tabulated below.

[b(4)]

-----b(4)----------
----b(4)----------

- ----b(4)----------
- --------b(4)----------
- ----------b(4)----------

2 Pages determined to be not releasable:

b(4)

3.2.P.4.3 Validation of Analytical Procedures

See Section 3.2.P.5.3.

3.2.P.4.4 Justification of Specifications

See Section 3.2.P.5.4.

3.2.P.4.5 Excipients of Human or Animal Origin or Novel Excipients

There is an excipient of human or animal origin in the formulation of Gintuit. Human recombinant insulin is part of the formulation for the agarose shipping media. Please refer to Section 3.2.S.2.3.2 for additional details. There are no novel excipients used in Gintuit as the nearly identical formulation has been in commercial use since Apligraf® approval in 1998.

3.2.P.5 Control of Drug Product

3.2.P.5.1 Specifications

The release specifications for the final product, Gintuit, were largely based on the findings of early developmental phases of Apligraf ® (previously named Graftskin). Since the PMA approval in 1998, the testing strategy and specifications have remained largely unaltered, with the exception of additional safety tests.

The final set of release tests and acceptance criteria are tabulated below.

STAGE OF MANUFACTURE	ASSAY	METHOD	SPECIFICATION
In-Process Drug Substance	Sterility	BacT/ALERT 3D	Negative
	--b(4)---------- ----------	--b(4)----	--b(4)----
	Mycoplasma	Mycoplasma	--b(4)----
Gintuit in primary package (poly bag)	Sterility	USP <71>	Negative
	--b(4)----	--b(4)----	--b(4)----
	Endotoxin	--b(4)----	--b(4)----

STAGE OF MANUFACTURE	ASSAY	METHOD	SPECIFICATION
--b(4)---------- (--b(4)----------)	--b(4)---------- ----------	--b(4)-----	--b(4)----------: -----b(4)---------- --b(4)------- ---b(4)----------
--b(4)---------- ----------	--b(4)----	--b(4)----	-----b(4)---------- ----------
---b(4)---------- ----------	--b(4)----	--b(4)----	--b(4)---- ----------
Final Product (post-primary packaging and labeling)	Overall Quality of Packaged Product	Visual Inspection***	-----b(4)---- ----------

----b(4)----------
--b(4)----------
-----b(4)----------.
*** The individual parameters observed during Visual Inspection are specified in the analytical method description below.

Reviewer Assessment: While the majority of the analytical methods and acceptance criteria for Gintuit lot release remain consistent with that of Apligraf®, there were several major updates with respect to the incorporation ----b(4)---------- and the formal incorporation of Visual Inspection as a lot release test. Notably, the biological aspect of the product potency was discussed in detail at the advisory committee meeting on November 17, 2011. The advisory committee recommended that incorporation of metrics of cytokines/growth factors would be appropriate given the current understanding of product function. In addition to the incorporation –b(4)----------, the applicant has agreed to perform as a PMC (STN 125400/0026, Received 1/31/2012), --b(4)---------- after which the data will be analyzed using statistically appropriate methods to identify any meaningful trends or correlations relative to assessing Gintuit potency. This set of changes address the recommendations by the AC adequately.

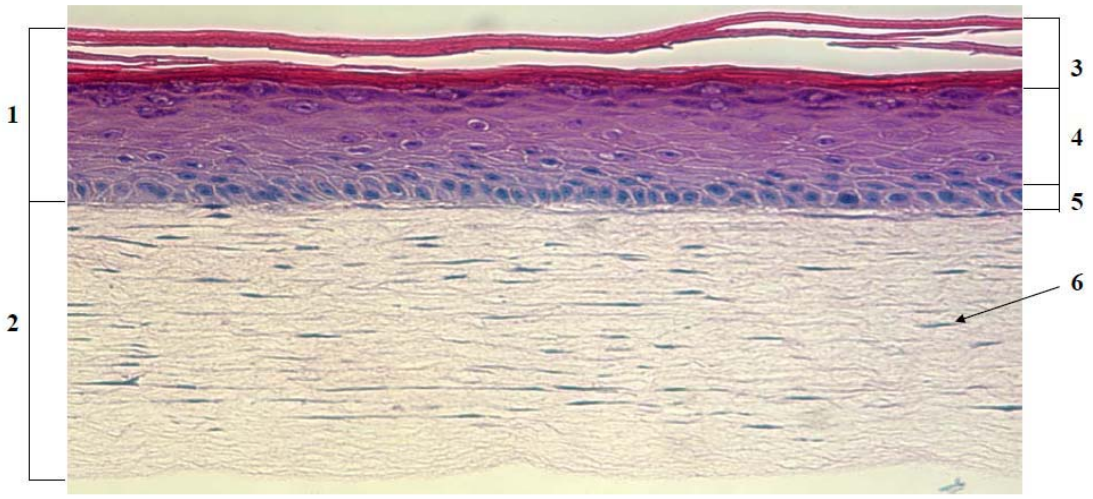
3.2.P.5.2 Analytical Procedures

The analytical methods proposed for commercial release of product, corresponding method identifier and validation report are summarized in the table below. Detailed descriptions of method validation for individual assays follow.

4 Pages determined to be not releasable:

b(4)

Histological Section of the Mature –b(4)-Unit [BLA Figure 3.2.P.5.2-2]



1.Epidermal Layer
2.Dermal Layer
3.Cornified Layer
4.Suprabasal Layer
5.Basal Layer
6.Fibroblast Layer

Specification:

1. Epidermal Coverage: > 95%. The percentage of the surface of the dermal matrix present on the slide, which is covered by epidermis, is determined.
2. Epidermal Development: ≥ 70%. The Epidermal Development data element includes two independent aspects of functional development each of which have the following specific acceptance criteria: ---b(4)----------. The 20x objective is used and the percentage of acceptable epidermal development is determined as per specifications; a basal layer of keratinocytes of cuboidal-columnar shape, 5 or more stratified suprabasal layers and ≥1 or more cornified layer on the apical surface.
3. Basal Cell layer: ≥ 95%. The 20x objective is used to determine the percentage of the epidermis present on the slide containing basal keratinocytes with a basophilic cytoplasm, lacking severe vacuolization or necrosis.
4. Suprabasal Cell layer: ≥ 80%. The slides are examined for the following: pink cytoplasm, pink nucleus, severe vacuolization, and necrosis. The percentage of the keratinocytes containing basophilic cytoplasm without vacuolization, necrosis or pyknosis (non-viable) is determined.
5. Dermal Matrix Thickness: ≥ 40 µm. The 20x objective is used and the thickness of the dermal matrix in 5 randomly selected fields across the length of the specimen is determined. The mean thickness of all five fields is calculated.
6. Fibroblast Density: ≥ 4 fibroblast nuclei per field. The 40x objective is used and the fibroblast density in 5 randomly selected fields of the dermal matrix present on the slides is determined. Pyknotic nuclei (non viable) are not included in the count.
7. Matrix Aspect: ≥ 95% uniform stain. The percentage of dermal matrix collagen present on the slide which stains uniformly without large holes or inclusions is determined.

Figures 3.2.P.2-3 through 3.2.P.5.2-8 of the BLA submission show representative histological cross-sections for passing (all acceptance criteria met) and non-passing (one or more acceptance criteria not met) samples.

-----**b(4)**----------

--*b(4)*----------
---------- ----------

----**b(4)**----------

--*b(4)*--- ----------

3.2.P.5.2.8 Visual Inspection

Method Summary (OCP-053):
One hundred percent (100%) visual inspection is performed on all packaged units just prior to final labeling and release. Visual inspection is a manual process performed in by qualified QC analysts to assess the following criteria:

- ---b(4)--- ----------

- ---b(4)--- ----------

- --b(4)--- ----------

- --b(4)--- ----------

- ----b(4)--- ----------

- --b(4)----------
- Date packaged and lot number labeling: Correct and legible

Any individual units failing to meet these criteria are rejected, segregated from the lot and discarded. If the number of individual rejects exceeds the maximum allowable limit, as a percentage of the packaging lot size, an out of specification investigation is initiated.

The applicant utilizes a multi-tier approach to consistently detect visual defects within the packaged –b(4)----unit. These include:

1) Standardized inspection procedures and criteria
2) Vision testing for analysts
3) Standardization of the inspection area
4) Training in visual inspection techniques
5) Qualification / requalification of visual inspection personnel
6) Reference defect libraries used for comparison

3.2.P.5.3 Validation of Analytical Procedures

3.2.P.5.3.1 Sterility- BacT/ALERT and USP<71>

Summary of Method Validation: BacT/ALERT 3D Microbial Detection System [Validation report NCLVR-0289- 001]:

----b(4)---- ---

1. ---b(4)----------
2. –b(4)----------
3. ----b(4)----------
4. –b(4)----------
    a. ----b(4)----------
    b. –b(4)----------
    c. –b(4)----------
    d. –b(4)----------
5. –b(4)----------

a. ---b(4)----------

10 Pages determined to be not releasable:

b(4)

Reviewer Assessment: The applicant has developed analytical assays, and designed and executed validation protocols to demonstrate the suitability of these assays to perform release testing of Gintuit. Validation protocols have been designed in accordance with principles and recommendations contained in various regulatory documents including CFRs, ICH, FDA Guidance, and compendia. The assays developed are appropriate for the characteristics of the product. Execution of validation experiments met system suitability and results satisfied predetermined acceptance criteria. Statistical analyses of assay parameters assessed under validation protocols are relevant in circumstances when the assay parameter evaluated supports a quantitative assessment. These validations were conducted for Apligraf® which is identical to Gintuit ----b(4)----------

3.2.P.5.4 Batch Analyses

A total of b(4) lots of Gintuit which were manufactured between September 18, 2007 and June 3, 2008 were used as clinical material for 92 clinical subjects in the pivotal clinical study in the dental indication, 06-PER-002-CTX. Lot specific information (i.e. cell banks and collagen batch used, etc.) from a sampling of b(4) manufacturing lots (b(4) of total) is provided in Table 3.2.P.5.4-1. The total number of units cast by a manufacturing lot was b(4) (i.e. two packaging lots of –b(4)---). For the b(4) clinical batches analyzed, each packaging lot ranged from –b(4)- units. All –b(4)-- batches were within specification for sterility, morphology, and –b(4)--, when data was available.

The complete listing of all lot numbers that were applied to each treated subject can be found in Clinical Study Report 06-PER-002-CTX Appendix 16.1.6.

3.2.P.5.5 Characterization of Impurities

Information regarding known and potential impurities in the drug substance, --b(4)----construct, derived from raw materials that are used in the cell banking stage or the manufacturing process of the product is provided in Section 3.2.S.3.2. This section addresses three critical reagents in more detail. It is noted that insulin and hydrocortisone are also present in the shipping medium, ---b(4)---------- manufacture.

Potential residual impurities in Gintuit final product was assessed using ICH Guideline Q3B(R2), Impurities in New Drug Products beginning at the point of the –b(4)----------. This assessment indicated that there were 3 notable formulations of potential risk which included --b(4)----------, insulin and hydrocortisone. The maximum impurity level (i.e. worst case scenario) for each component was below the thresholds established by ICH guidelines ----b(4)----------

4 Pages determined to be not releasable:

b(4)

----b(4)---------- ----------

---b(4)---- ----------

--b(4)---- ----------

3.2.P.6 Reference Standards or Materials

See Section 3.2.S.6.

3.2.P.7 Container Closure System

The packaging system for Gintuit is largely equivalent to the packaging system that was approved for Apligraf® in 1998. There have been packaging improvements submitted as supplements to the PMA (950032) that were reviewed and approved by FDA. The current custom design packaging consists of primary packaging, secondary packaging and tertiary packaging with each type playing a role in collectively maintaining the necessary chemical environment (nutrients and pH), sterility (protection from outside biological agents), and physical integrity during shipment.

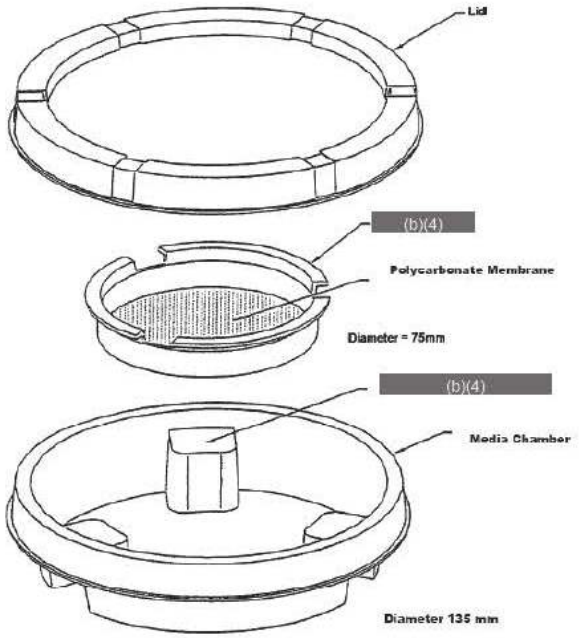
Reviewer Note: The facilities reviewer evaluated container closure from the standpoint of product sterility. This review is intended to provide a review of container closure from a product quality perspective.

Primary Packaging

The purpose of the primary packaging is to maintain the biological characteristics of the product. Each –b(4)- construct is manufactured on a sterile, synthetic (polycarbonate) membrane with --b(4)----- pores (diameter of 3µm, ----b(4)----------). The shipping tray base is circular with three supports upon which the culture insert ting rests. A mating lid sits on the lip of the base tray to form the gap which enables adequate CO^2 exchange. Figure 3.2.P.2.4-1 below shows a schematic of all components of the primary packaging. A detailed description of the manufacture of the permeable membranes is provided in Section 3.2.P.7.1.1. Table 3.2.P.7-2 below contains a general summary of information on the culture insert ring and membrane. The acceptance criteria for the processing tray containing culture insert and the shipping tray can be

found in Tables 3.2.P.7-3 and 3.2.P.7-4 of the BLA. All components are provided sterilized to an –b(4)-------.

Container Closure System for Gintuit [BLA Figure 3.2.P.2.4-1]



General Information on the Culture Insert (CI) and Membrane [BLA Table 3.2.P.7-2]

CRITERIA	INFORMATION
Culture Insert Ring Name of plastic Grade of plastic Regulations to which plastic complies Chemical name of precursor monomer Approved Manufacturer of the plastic	 ----b(4)---------- -------- ---------- ---------- ---------- --------- ----------
Membrane Name of plastic Grade of plastic Regulations to which plastic complies Chemical name Approved Manufacturer of the plastic	 Polycarbonate --b(4)--- ----b(4)---------- Polycarbonate ----------b(4)---------- ------- ---------- ----------

Secondary Packaging

The main function of the secondary packaging is to protect the product from contamination. This secondary packaging is composed of a heavy-gauge transparent –b(4)----------

polyethylene (PE) barrier film. The packaging is supplied as a pouch, which is sealed –b(4)-------
---

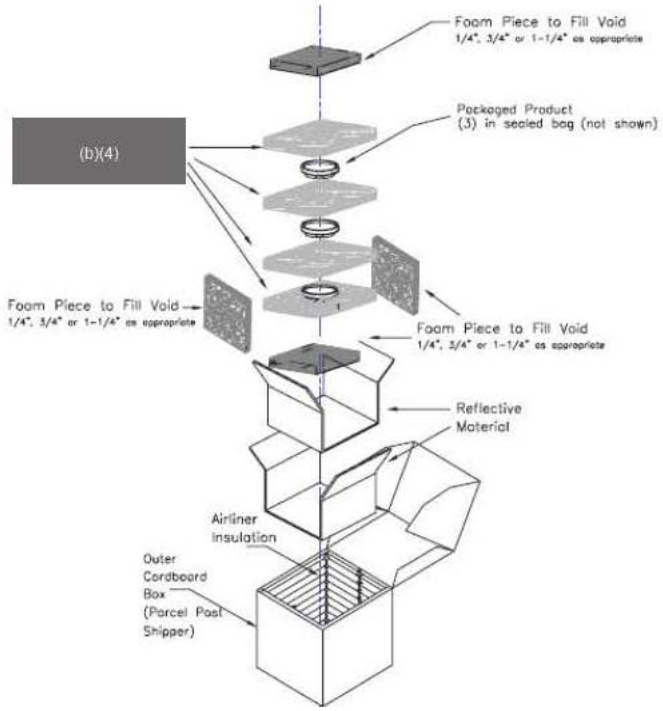
--------- are both cGMP compliant suppliers. Figure 3.2.P.2.4-2 shows the finished product in a secondary package. The pouches are -----b(4)---------- ----------
---------- The acceptance criteria for the bag are provided in Table 3.2.P.7-5 of the BLA.

Finished Product in Secondary Package [BLA Figure 3.2.P.2.4-2]

--b(4)--- ----------

<u>Tertiary Packaging</u>

The tertiary packaging provides the required protection for Gintuit during transport and maintains the appropriate temperature conditions within the validated range. This tertiary packaging consists of a cardboard box, gas-filled air liner, reflective material, foam pieces, Phase Change Material (PCM), package insert, and Handling and Storage Instructions Sheet. Two shipping box configurations, 24-hour shipping and 72- hour shipping, are possible and differ ----b(4)----------. Both configurations allow shipment of up to-b(4)- individual units of Gintuit. A detailed description of the overall assembly of the tertiary packaging is provided in Section 3.2.P.2.4. Figure 3.2.P.2.4-3 below provides a representative configuration of the shipper box. Table 3.2.P.7-6 summarizes the acceptance criteria for all critical components comprising the tertiary packaging.



The tertiary packaging for Gintuit serves as the shipping container for packaged Gintuit but also provides an additional function of maintaining the product at the validated shipping conditions of 20 to 23°C. This temperature range is necessary to maintain viability of Gintuit during shipment and temporary storage prior to application. The ability of the tertiary packaging to maintain these conditions was validated for both winter and summer shipping profiles. It is noted that process validation for Gintuit packaging, -b(4)- Unit Lot Size was completed in May 2000.

Testing Performed to Verify/Validate Acceptability of Primary, Secondary and Tertiary Packaging

A summary of the verification testing performed for each level of container closure/packaging is shown below [BLA Table 3.2P.2.4-1].

PACKAGING	TESTING CONDUCTED	RESULTS/CONCLUSION
Primary Packaging	----b(4)----------	---b(4)---------- ----------
	----b(4)---------- ----------	----b(4)---------- ----------
Secondary Packaging	----b(4)---------- ---------- ----------	----b(4)---------- ----------

2 Pages determined to be not releasable:

b(4)

*----b(4)---------- ----------

---*

3.2.P.8 Stability

3.2.P.8.1 Stability Summary and Conclusion

Gintuit is a biologic product with viable cells with a limited shelf-life. Product stability is determined by the ability of the product to maintain structural integrity and histology. Initial stability studies during product development and the more recent 15-day stability study support the justification for a 15-day shelf life for Gintuit when stored under the specified conditions at 20°C-23°C (68°F- 73°F). This time period refers to the time after the packed drug substance -b(4)- construct) is manufactured.

The design of the stability study, justification for the acceptance criteria and the resulting data are reasonable and supports the proposed shelf-life for the Gintuit under the specified storage conditions. Please refer to Section 3.2.P.8.3 for additional details.

3.2.P.8.2 Post approval Stability Protocol and Stability Commitment

Validation Protocol NCLVP-0360 describes the post approval stability protocol and commitment. The plan is summarized below:

- ----b(4)----------
- -----b(4)--- ----------
- ----b(4)-- ----------
- --b(4)--- ----------
- --b(4)--- ----------

[b(4)]

2 Pages determined to be not releasable:

b(4)

3.2.A Appendices (MF-J)

3.2.A.1 Facilities and Equipment

Please refer to the review of the facilities reviewer.

3.2.A.2 Adventitious Agents Safety Evaluation

Much of the details of this section has been described in the raw materials section of the BLA review and is summarized here in the context of risk assessment for each material of human, animal or other biological origin (see table in Section 3.2.S.2.3.1 for details)

Materials of human origin in final product - Human Cells
Quality and safety of the cells is maintained through the tissue procurement program, screening of the donor's parents, testing of the donor mother and testing of the cells through the cell bank. This is described in Section 3.2.S.2.3.2 and 3.2.S.2.3.3 of the BLA review.

Materials of animal origin in final product – Bovine Collagen
The collagen is isolated from bovine –b(4)---. The necessary supporting information regarding the safety of the source, infectivity of bovine –b(4)--, documented procedures and controls in place have been provided. Section 3.2.S.2.3.9 contains viral safety/viral inactivation information regarding bovine collagen which was designed to conform with "CPMP Note for Guidance on Virus Validation Studies: The Design, Contribution and Interpretation of Studies Validating the Inactivation and Removal of Viruses (CPMP/BWP/268/95)" and the "FDA Points to Consider in the Characterization of Cell Lines used to Produce Biologicals (1993)". Section 3.2.S.2.3 contains discussion regarding the extremely low risk for the introduction of adventitious agents from collagen derived from bovine –b(4)-----

Raw materials used in product manufacture

- **----b(4)---** ----------
- **--b(4)---** ----------
- **---b(4)---** ----------
- **--b(4)---** ----------

- **--b(4)----** ----------
- **--b(4)---** ----------
- **--b(4)---** ----------
- **--b(4)----** ----------
- **---b(4)---** ----------

Reviewer Assessment: The manufacture of Gintuit contain many materials of human and animal origin however there are reasonable procedures in place to provide assurance of a low risk of adventitious virus transmission by the final product. Please refer to the review comments in Section 3.2.S.2.3.1 for details regarding each raw material. Furthermore, a product of virtually identical formulation has been commercially available in the United States since 1998, without any safety signals. In the PMC, language will be included so that the applicant will be encouraged to continue to ----b(4)----------

3.2.A.3 Excipients

There are no novel excipients in the manufacturing of Gintuit.

3.2.R Regional Information

3.2.R.1 Executed Batch Records

Example batch records for drug substance and drug product are provided below. A complete set of representative batch records for the manufacture process of –b(4)- drug substance lot # --b(4)---- is listed in Table 3.2.R.1-1 and the batch records for product manufacturing for lot -b(4)------ is listed in Table 3.2.R.1-2.

Reviewer Assessment: The batch records have been reviewed and are acceptable. It is noted that detailed inspection of select batch records were conducted by Drs. Mark H. Lee and Eric Dollins during the PLI inspection of the manufacturing facility.

3.2.R.2 Methods Validation Package

Please refer to Sections 3.2.S.4.3 and 3.2.P.5.3 for validation of analytical procedures. In general, the methods validation is performed in accordance with USP --b(4)-- USP --b(4)-- and ICH Q2 (R1) Guidelines for Validation of Analytical Procedures.

3.2.R.3 Recall Summary 1999 to 2011

Table 3.2.R.3-1 below contains a list of Gintuit recalled to date. For a summary of the recall protocol refer to Section 3.2.P.5.1. An analysis of the data shows the following reasons for product recalls.

REASON	#	RECALL DATES - # OF LOTS RECALLED	MICROBIAL ID	CORRECTIVE ACTIONS TAKEN
Microbial Contamination of Agarose	1	Sep 27, 2009 - 60	*Staphylococcus epidermidis*	• Additional Cleaning • Microbial Characterization of suite (Environmental Monitoring)
Microbial Contamination of Agarose	1	Dec 24, 2008 – 93	*Microbacterium Liquefaciens Microbacterium barkeri*	• Additional Cleaning • "Disposables" used in production removed and replaced
Microbial Contamination of Agarose	1	Dec 24, 2008 – 84	*Brevibacillus borstelensis*	• Additional Cleaning • "Disposables" used in production removed and replaced
Microbial Contamination of Agarose	3	Dec 07, 2001 Sep 11, 2000 - 46	*Staphylococcus cohnii*	• ---b(4)---------- • Retrain of operators • Smoke studies • Equipment/surfaces cleaned before and after
Microbial Contamination of Agarose	2	July 01, 1999 - 32 Dec 10, 1999 – 32	*Burkholderia cepacia*	• Remove –b(4)---------- • ----b(4)---------- • Immediate cleaning of all –b(4)---- doors and establishing daily program • Cleaning of class –b(4)----- floors • Phased in –b(4)---------- of process

REASON	#	RECALL DATES - # OF LOTS RECALLED	MICROBIAL ID	CORRECTIVE ACTIONS TAKEN
Microbial Contamination of the unit sampled for the –b(4)-testing	2	Feb 21, 2011 – 2 Jan 30, 2011 - 50	*Deinococcus radiopugnans*	Status Report submitted on April 4, 2011
pH Out of Specification	2	July 20, 1999 – 58 Sep 29, 2006 - 48	N/A	• Revised OP-0470, MBR 501-005, -b(4)---- to verify gas composition, compilation and verification • Replaced heat sealer • Additional PM for heat sealer
Postive Mycoplasma	1	Sep 13, 2002 - 76	*Acholeplasma Laidlawii.*	• Investigation identified the organism in lot ---b(4)----------) used • ----b(4)----------

Reviewer Assessment: Given amount of products that have been manufactured to date, the amount of recalls are relatively small. However, there have been some common sources of failures such as the microbiological contamination of agarose listed above. This item was reviewed in more detail as part of the PLI of the applicant's facility in October 2011. As agarose is known to be a reagent that is easily susceptible to microbial contamination, the source of the microbial species varied, appropriate CAPA procedures were performed and the relative frequency was low, no additional action is required.

Additional Review Considerations

I. Retention Program (--b(4)-------00909)

The retention program, as described in –b(4)-------00909 "Retention Program for Living-Cell Based Product," has been updated throughout the interactive BLA review. The most up-to-date plan is described below and in STN 125400/0044 (Received 3/7/2012).

- ----b(4)-----

- –b(4)---- ----------.
- ---b(4)---- ----------

- ---b(4)--- ----------
- ---b(4)--- ----------

--b(4)--- ----------

Reviewer Assessment: The absence of a formal retention program was observed during the PLI and was included as a 483 item (Observation #6). The sponsor submitted the response in STN 125400/0022 and has updated it to reflect the agreed upon potency assay matrix. Although 21 CFR 600.13 specifies that the retention samples shall be stored for a period of 1 year after lot release, this approach is not possible with this cell-based product with an expiry of 15 days. The scientifically based rationale for this exception is acceptable. The applicant's reserve program is adequate.

II. Product Segregation and Tracking

Reviewer Note: During the PLI of the Canton, MA facility, the inspection team discovered that an outside-of-US investigational product with ----------b(4)----------, is currently manufactured in the same facility as Gintuit and Apligraf®. –b(4)- is a product composed of ----------b(4)---------- The information below summarizes the procedures in place to distinguish these products.

Gintuit vs. Apligraf®

The manufacture and properties of Gintuit and Apligraf® are identical ---b(4)---------- Therefore, segregation of the products before labeling is not necessary.

Gintuit vs. –b(4)-

-----b(4)-- ----------

In STN 125400/0020 (Received 12/22/2011), the applicant provided validation of the identity assay (Histology, QC-OPER-00547) to distinguish Gintuit vs. –b(4)-- (NCLVR-0396-001, Validation of ---b(4)--- ----------

Validation Report NCLVR-0396-001 (dated December 13, 2011)

- --b(4)--- ----------
- --b(4)-- ----------
- --b(4)-- ----------
- ----b(4)---- ----------

Summary of Key Results:

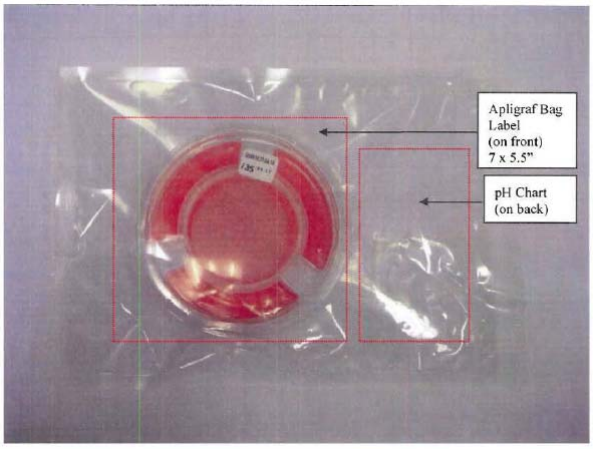
- The % correct was 100% for all three analysts

Reviewer Assessment: The identity assay has been properly validated to distinguish Gintuit and Apligraf® from the investigational product, --b(4)---. From a product safety and quality perspective, the segregation and testing procedures that are in place are sufficient. Please also refer to the memo of the facilities reviewer for additional details.

III. Product Package Labeling

The product package labeling consists of three components: 1) Bag Label, 2) pH Chart, 3) storage and handling instructions. Component 3 is placed in to the tertiary packaging (i.e. shipping box). Components 1 and 2 are placed on the secondary packaging (i.e. Poly bag) in the configuration shown below.

Placement of Gintuit bag label and pH chart on the secondary packaging



Gintuit Bag Label (Size = 7" x 5.5") – on secondary packaging (Poly bag)

Allogeneic cultured keratinocytes and fibroblasts in bovine collagen Gintuit™

- This bag contains a single unit of a 75 mm diameter cellular sheet consisting of human keratinocyte and fibroblast cells, human extracellular matrix proteins, and bovine collagen.
- This product is for topical oral application.
- **DO NOT FREEZE OR REFRIGERATE.** Store in shipping box (closed) at 68°F to 73°F (20°C to 23°C).
- **DO NOT OPEN THIS POLYBAG UNTIL IMMEDIATELY PRIOR TO USE. Use product within 15 minutes of opening.**
- The color of the agarose gel medium should be compared with the Agarose Gel Medium pH Chart provided in the shipping box. If the pH is not within the acceptable range (6.8-7.7) as indicated on the color chart, **DO NOT OPEN** and **DO NOT USE** this product.
- **DO NOT USE BEYOND ITS EXPIRY DATE (11:59 PM ET).**
- Product contains no preservatives. Handle aseptically.
- Do not use in patients with known allergies to bovine collagen.

LOT:

EXP.

Rx Only.

See enclosed package insert for additional information.

N 3 42606 04401 8

Peel off and apply to patient chart.

Manufactured and distributed by:

Organogenesis Inc.

LIVING TECHNOLOGY

150 Dan Road • Canton, MA 02021

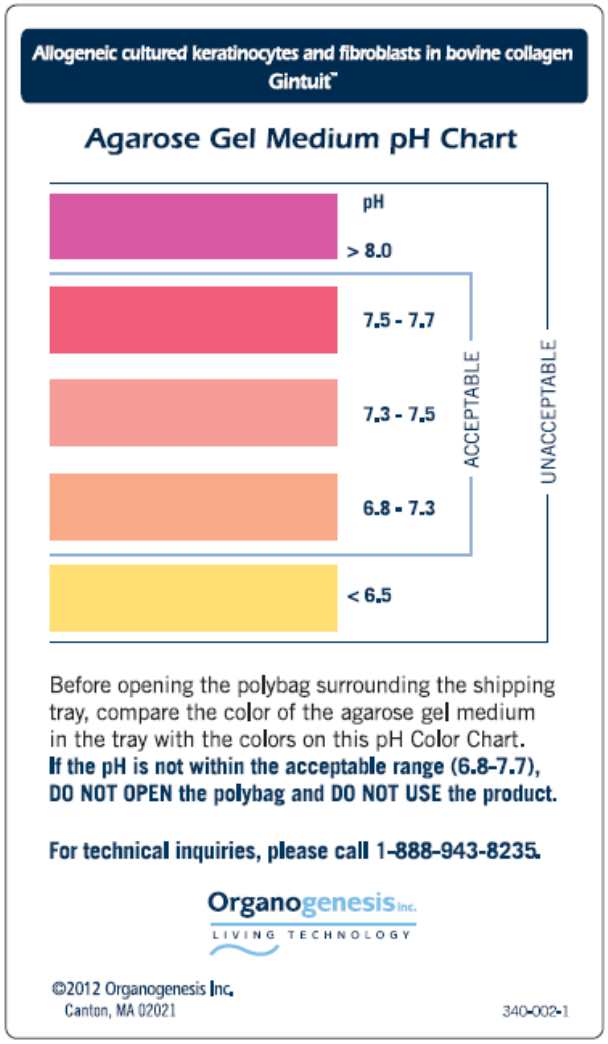
1-888-943-8235

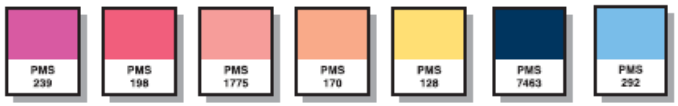
Manufacturer License: XXXX

 340-002

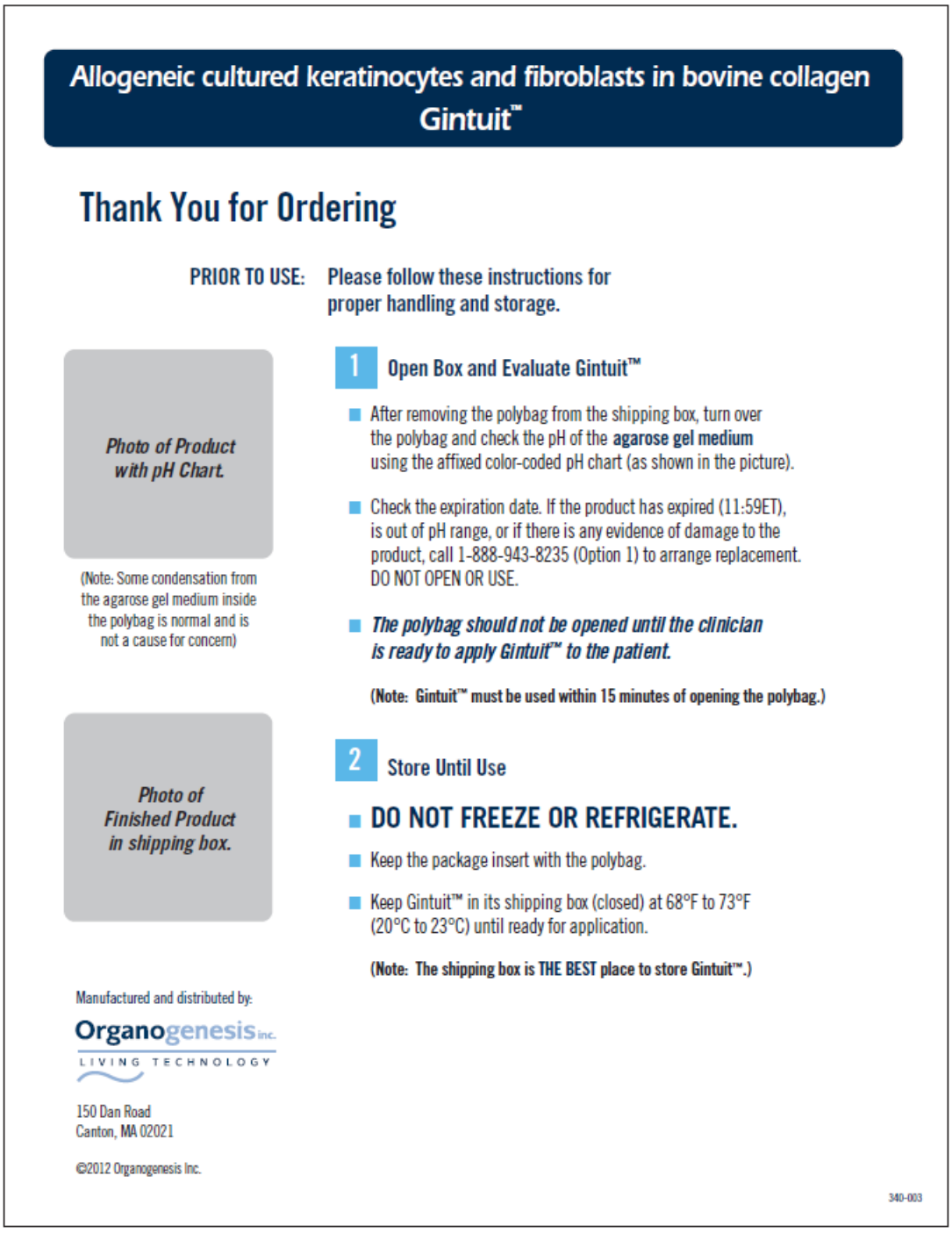
Allogeneic cultured keratinocytes and fibroblasts in bovine collagen Gintuit™	Allogeneic cultured keratinocytes and fibroblasts in bovine collagen Gintuit™
LOT:	LOT:
EXP.	EXP.

pH Chart (Size = 3 3/8” x 5 7/8”) – on secondary packaging (Poly bag)





Storage and Handling (Size = 8.5” x 11”) – included in tertiary packaging (shipping box)



Allogeneic cultured keratinocytes and fibroblasts in bovine collagen
Gintuit™

Thank You for Ordering

PRIOR TO USE: Please follow these instructions for proper handling and storage.

Photo of Product with pH Chart.

(Note: Some condensation from the agarose gel medium inside the polybag is normal and is not a cause for concern)

Photo of Finished Product in shipping box.

1 Open Box and Evaluate Gintuit™

- After removing the polybag from the shipping box, turn over the polybag and check the pH of the **agarose gel medium** using the affixed color-coded pH chart (as shown in the picture).
- Check the expiration date. If the product has expired (11:59ET), is out of pH range, or if there is any evidence of damage to the product, call 1-888-943-8235 (Option 1) to arrange replacement. DO NOT OPEN OR USE.
- ***The polybag should not be opened until the clinician is ready to apply Gintuit™ to the patient.***

(Note: Gintuit™ must be used within 15 minutes of opening the polybag.)

2 Store Until Use

- **DO NOT FREEZE OR REFRIGERATE.**
- Keep the package insert with the polybag.
- Keep Gintuit™ in its shipping box (closed) at 68°F to 73°F (20°C to 23°C) until ready for application.

(Note: The shipping box is THE BEST place to store Gintuit™.)

Manufactured and distributed by:
Organogenesis Inc.
LIVING TECHNOLOGY

150 Dan Road
Canton, MA 02021

340-003

Reviewer Assessment: These three product labeling components have been reviewed in conjunction with FDA labeling and promotional material experts at OCBQ/DCM/APLB and found to be acceptable.