I concur with this review. M. Serabian 01/11/21
I concur with this review. S. Sanduja 01/11/21

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Tissues and Advanced Therapies
Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch

BLA NUMBER:	STN #125730.000
DATE RECEIVED BY CBER:	March 31, 2020 (Pharmacology/Toxicology and Clinical modules); June 5, 2020 (Complete submission)
DATE REVIEW COMPLETED:	11/18/2020; amended 12/04/2020; 12/11/2020; 12/24/2020; 01/08/2021; 01/10/2021; 01/11/2021
PRODUCT:	STRATAGRAFT® (Allogeneic Cultured Keratinocytes and Fibroblasts in Murine Collagen)
APPLICANT:	Stratatech Corporation
PROPOSED INDICATION:	For treatment of adult patients with deep partial thickness thermal burns, containing intact dermal elements for which surgical intervention is clinically indicated.
PHARM/TOX REVIEWER:	Abigail Shearin
PHARM/TOX TEAM LEADER:	Sandhya Sanduja
PHARM/TOX BRANCH CHIEF:	Mercedes Serabian
PRODUCT (CMC) REVIEWERS:	Steven Bauer (Chair), Nahid Parvin, Kouassi AyiKoe, Laura Ricles, Takele Argaw
CLINICAL REVIEWERS:	Rosa Sherafat-Kazemzadeh
PROJECT MANAGER:	Candace Jarvis
DIVISION DIRECTOR:	Tejashri Purohit-Sheth
OFFICE DIRECTOR:	Wilson Bryan

EXECUTIVE SUMMARY:

STRATAGRAFT® is a cellular skin construct containing metabolically active cells that produce and secrete a variety of growth factors and cytokines. The construct is composed of an epidermal layer of fully-stratified allogeneic immortalized near-diploid human keratinocytes (NIKS® keratinocytes) grown on a dermal equivalent (DE) consisting of allogeneic normal human dermal fibroblasts (NHDFs) in a rat tail collagen-rich matrix. STRATAGRAFT® is a

sheet of approximately 100 cm^2 (about 8 cm by 12.5 cm) in size and is applied to a surgically prepared wound bed at an estimated ratio of 1 cm^2 STRATAGRAFT® per 1 cm^2 debrided wound. The number of STRATAGRAFT® constructs applied will vary depending on the size of the wound bed. The construct can be trimmed to accommodate the size and shape of the wound bed.

In vitro studies showed that NIKS® keratinocytes and NHDFs cultured for (b) (4) passages, respectively, are karyotypically stable, consistent with a Master Cell Bank for each cell line. NIKS® keratinocytes and NHDFs cultured for 43 and 6 passages, respectively, did not exhibit anchorage-independent growth (a standard assay that evaluates the potential for cellular transformation). Of note, the commercial STRATAGRAFT® product contains NIKS® keratinocytes and NHDFs that are at passage 40 and 7, respectively.

In vivo evaluation of STRATAGRAFT® included assessment of safety, tolerability, and tumorigenic potential following topical administration on full-thickness excisional wounds that were greater than 25% of the total body surface area (TBSA) of the immunodeficient mice. No tumor formation was observed for the 20-week study duration. In addition, following a single subcutaneous injection of NIKS® keratinocytes in immunodeficient mice, no tumor formation was detected for the 23-week study duration.

No animal reproductive or developmental toxicity (DART) studies were conducted with STRATAGRAFT®.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:
There are no nonclinical deficiencies identified in this submission. There are no outstanding requests for additional nonclinical data for evaluation of STRATAGRAFT®. The nonclinical information provided in the BLA submission supports approval of the licensure application.

Formulation and Chemistry:
STRATAGRAFT® consists of an off-white rectangular sheet (approximately 100 cm^2; 8 cm by 12.5 cm) containing viable, metabolically active cells derived from NIKS® keratinocytes (differentiated, multilayered, epidermal keratinocytes from a single human donor) cultured for (b) (4), then grown on a DE consisting of collagen (rat-tail collagen type I) matrix embedded with NHDFs obtained from a second human donor. The DE is generated by (b) (4) with feeding every (b) (4). The STRATAGRAFT® construct is then harvested and cryopreserved in (b) (4) glycerol.

Abbreviations

BCC	Basal cell carcinoma
CT	Computed tomography
DART	Development and reproductive toxicology
DE	Dermal Equivalent
(b) (4)	
F	Female
GFP	Green fluorescent protein
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
hCAP-18/LL-37	Human cathelicidin
(b) (4)	
(b) (4)	
HIF-1α	Hypoxia-inducible factor 1α
(b) (4)	
KA	Keratoacanthoma
(b) (4)	
M	Male
MCB	Master Cell Bank
MRL	Minimum risk level
NHDFs	Normal human dermal fibroblasts
NIKS®	Immortalized near-diploid human keratinocyte cell line
P	Passage
PDE	Permitted daily exposure
POD	Point of departure
PPM	Parts per million
SCC	Squamous cell carcinoma
SCID	Severe Combined Immunodeficient
TBSA	Total body surface area
(b) (4)	
VEGF	Vascular endothelial growth factor
WCB	Working Cell Bank

Related File(s):

IND #10113: Allogeneic Keratinocyte Cell Line (NIKS®), seeded on rat collagen ((b) (4)) conditioned with human dermal fibroblasts ((b) (4)); Indication: Treatment of complex skin defects in patients undergoing sequential skin reconstruction procedure; Sponsor: Stratatech Corp; **ACTIVE**

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INTRODUCTION

Intact skin is a critical barrier; inhibiting local infection of the dermis and/or underlying tissues, stopping excessive water loss, and preventing entry of toxins and irritants. The current standard of care for patients with deep partial thickness thermal burns is an autograft consisting of healthy skin obtained from an uninjured site. In patients with significant skin defects, sequential autograft harvesting may be necessary. This process can require the use of temporary skin replacements (biological skin substitutes from cadavers or synthetic skin substitutes) until adequate autograft tissue is available[1]. Patients may be hospitalized for months and require serial surgical procedures. In addition, significant morbidity can occur at the donor site(s) for the autologous skin grafts. STRATAGRAFT® is a viable skin construct that contains allogeneic NIKS® keratinocytes grown on a DE consisting of allogeneic NHDFs in a rat tail collagen-rich matrix. STRATAGRAFT® does not remain permanently engrafted because it is replaced by the patient's cells over time, thus reducing the need for autografting to achieve definitive wound closure.

[1] Wax MK, Meyers AD, Pittman AL, Ghanem TA, Split-Thickness Skin Grafts. Medscape. 2019. Available at: https://emedicine medscape.com/article/876290-overview.

NONCLINICAL STUDIES

PHARMACOLOGY STUDIES

The applicant states (Section 1 of Module 2.6) that STRATAGRAFT®: provides immediate wound coverage and a barrier function, and promotes expression and secretion of growth factors, cytokines, and wound healing factors that may contribute to regenerative healing and efficacy. However, no nonclinical pharmacology studies were conducted; please refer to the clinical review memo for confirmation of these claims.

Summary List of Pharmacology Studies

N/A

SAFETY PHARMACOLOGY STUDIES

No nonclinical safety pharmacology studies were conducted

Summary List of Safety Pharmacology Studies

N/A

PHARMACOKINETIC STUDIES (Cell Distribution)

No PK studies were conducted.

The applicant refers to the three completed clinical trials: STRATA2011, STRATA2014, and STRATA2016. During these clinical trials, the persistence of STRATAGRAFT® following application on the wound bed was evaluated. Three months after application of STRATAGRAFT® to subjects, a 2 mm punch biopsy was collected from the wound site. DNA was isolated and analyzed via (b) (4) analysis to determine the source of the DNA. The resulting data were compared to a reference sample obtained from the wound bed prior to STRATAGRAFT® administration to determine the percent of allogeneic DNA. Please refer to the clinical review memo for details of these analyses.

Summary List of Pharmacokinetic Studies

N/A

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology study was conducted to evaluate the safety profile of STRATAGRAFT® following administration in an athymic (immunodeficient) murine wound model:

Note: STRATAGRAFT® is composed of human cells, thus the potential for elicitation of a xenogeneic response following administration in immune competent animals exists. (b) (4) nude mice (b) (4) , thus greatly reducing their ability to mount a T-cell mediated response to the human cells.

Toxicology Study:

Study Number	Study Title	Report Number
1	Minimum Eight Week Transdermal Subchronic Toxicity Study with STRATAGRAFT® Skin Tissue in Nude Mice	NCREP.001

Study #1

Report Number		NCREP.001
Date Report Signed		June 28, 2019 **Note:** The revised study report provided in the BLA submission included: 1) the original study report (in Attachment #1) that was signed on June 27, 2002, 2) points of clarification and correction, and 3) amended tables (in Attachment #4).
Title		Minimum Eight-Week Transdermal Subchronic Toxicity Study with STRATAGRAFT® Skin Tissue in Nude Mice
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To evaluate STRATAGRAFT® for subchronic toxicity after surgical application to full-thickness wounds created on the dorsum of (b) (4) nude mice.
Study Animals	**Strain/Breed**	(b) (4) nude mice
	Species	*Mus musculus*
	Age	43-67 days old at the time of application
	Body Weight	22-24 grams at the time of application
	#/sex/group	**Test article:** 26 female mice **Control:** 11 female mice
	Total #	37

<table>
<tr><td>Test Article(s)</td><td>STRATAGRAFT® skin tissue (Batch Nos. (b) (4)

Cell passage information for NIKS® keratinocytes and NHDFs for each batch of STRATAGRAFT® used:

<table>
<tr><th>Batch Identification</th><th>NIKS® Keratinocytes Passage Number</th><th>NHDFs Passage Number</th></tr>
<tr><td colspan="3">(b) (4)</td></tr>
</table>
(*) Denotes cell passage information is not available.

Source: Report No. NCREP.001, located in Section: ‘Experimental Design’ in Module 4.2.3.1.1 of the BLA.</td></tr>
<tr><td>Control Article(s)</td><td>N/A; mice received no intervention</td></tr>
<tr><td>Route of Administration (ROA)</td><td>Topical application on the wound bed</td></tr>
<tr><td>Description of the Disease/Injury Model and Implant Procedure</td><td>A 14 cm^2 full-thickness wound was generated on the dorsum immediately before application.

Note: The wound size was greater than 25% of the TBSA for each mouse. This maximum tolerated wound size in mice is reported to be 30% TBSA[2].</td></tr>
<tr><td>Dosing Regimen</td><td>Single administration:

<table>
<tr><th>Group</th><th>No. of Female Mice</th><th>Length of Exposure</th><th>Dose Level</th></tr>
<tr><td>STRATAGRAFT® (Transplanted Test Group)</td><td>26</td><td>3 mice: 90 days
12 mice: 91 days
7 mice: 133 days
4 mice: 140 days</td><td>14 cm^2 patch (>25% TBSA)</td></tr>
<tr><td>Control (Untreated Control Group)</td><td>11</td><td>N/A (sacrificed at 129 days of age)</td><td>None</td></tr>
</table>
Source: Adapted from Report No. NCREP.001, located in Attachment #1 in Module 4.2.3.1.1 of the BLA.</td></tr>
<tr><td>Randomization</td><td>No</td></tr>
<tr><td>Description of Masking</td><td>Not specified</td></tr>
<tr><td>Scheduled Sacrifice Time Points</td><td>Test article group: 90, 91, 133, and 140 days post-dose (134-200 days old)
Control group: 129 days old

Note: No rationale for: 1) the sacrifice intervals for either group or 2) the single sacrifice time point for the Control group was provided.</td></tr>
</table>

[2] Abdullahi A, Amini-Ni, S, Jeschek, MG. Animal Models in Burn Research. *Cell Mol Life Sci* 71, No. 17 (2014).

Reviewer's Comments:

- This study is a retrospective analysis of previously completed studies. Thus, not all assessments were performed for each batch of STRATAGRAFT®.

- Per the applicant, the STRATAGRAFT® manufacturing process was being refined as the product development program progressed. The applicant cited the following differences between the manufacturing process of STRATAGRAFT® for Studies #1 and #14 and the commercial process. The specific product batch reflecting each manufacturing change was not specified.

  - During early STRATAGRAFT® development, NIKS® keratinocytes were expanded using a (b) (4) for the murine feeder line. This method was not used to manufacture STRATAGRAFT® for the STRATA2011 or STRATA2016 clinical trials and is not used for commercial manufacture of STRATAGRAFT®. Different (b) (4) of NIKS® keratinocytes and NHDFs in culture.

  - Minor changes were made in the production of the DE as the development program progressed. The STRATAGRAFT® construct administered in early nonclinical research studies and the STRATA2001 clinical trial used an (b) (4) . This concentration was (b) (4) for the construct administered in the STRATA2011 clinical trial and in later nonclinical studies. In addition, the density of NHDFs and NIKS® keratinocytes seeded on the construct administered in early nonclinical research studies and in the STRATA2001 clinical trial was (b) (4). At the time of initiation of the STRATA2011 clinical trial and for the STRATA2016 clinical trial and later nonclinical studies, the seeding density was (b) (4) for the NHDFs and to (b) (4) for the NIKS® keratinocytes.

  - Different approaches for cryopreservation of STRATAGRAFT® were explored. The constructs were not routinely cryopreserved but were transported at the time of maturation to the vivarium for surgical application. They were stored in a refrigerated shipping chamber containing (b) (4) until use. STRATA2001 and STRATA2011 clinical trials also used STRATAGRAFT® stored under refrigerated conditions. The cryopreservation procedure was further optimized and used for STRATAGRAFT® administered in the STRATA2016 clinical trial and for commercial manufacturing.

Key Evaluations and Results:

Skin tissue assessments: The morphology, tissue thickness, barrier function, and cell viability of the STRATAGRAFT® batches were evaluated (Table 1):

Table 1. Summary of tissue properties of STRATATECH®

Batch Identification	Tissue Architecture	Epidermal Thickness (µm)	Dermal Thickness (µm)	Barrier Function ((b) (4) Change)
(b) (4)	Normal	130	175	496
	Normal	70	75	569
	Normal	80	140	431
	Normal	175	175	514
	Normal	125	70	320
	Normal	35	55	154
	Normal	60	120	404
	Normal	60	60	408
	Normal	50	150	318
	Normal	80	80	356

N/A denotes assessment not performed.
Source: Report No. NCREP.001, located in Attachment #2 in Module 4.2.3.1.1 of the BLA.

In-life assessments:

- Daily observations: Animals were observed daily for mortality and moribundity. No test article-related clinical signs were observed.
- Clinical observations: Conducted for each animals at the time of sacrifice. All mice administered STRATAGRAFT® were healthy in appearance and behavior. No abnormalities were observed at the application site.
- Body weights (BWs): Obtained immediately prior to sacrifice.
  Note: BWs were not obtained for four mice in the control group and for the remaining seven mice, BWs were not consistently obtained prior to sacrifice. Thus this parameter provided no interpretable data.
- Clinical pathology: Serum was collected and frozen at the time of sacrifice for chemistry analyses; however, the samples were not analyzed.

Unscheduled deaths: No unscheduled deaths occurred.

Terminal sacrifice:

- Necropsy: The veterinary pathologist did not observe any test article-related abnormalities.

- Histopathology:
  - The graft site, kidney, lung, lymph nodes, and liver were evaluated microscopically.
  - The graft site in mice transplanted with STRATAGRAFT® showed evidence of collagen remodeling and angiogenesis, consistent with normal wound healing.
  - No test article-related findings were observed in the examined tissues.

[3] (b) (4) : This device was used to measure the (b) (4) of STRATAGRAFT®. (b) (4) is the (b) (4) . A low (b) (4) indicates reduced skin layers.

Reviewer's Conclusion: Within the significant limitations of the study design and data provided, no apparent local or systemic toxicity was observed out to 140 days following topical application of STRATAGRAFT® to immunodeficient mice with an existing wound.

Developmental and Reproductive Toxicology (DART) Studies:
No DART studies were conducted.

Genotoxicity Studies:
No genotoxicity studies were conducted.

Carcinogenicity/Tumorigenicity Studies:
These data are summarized with the respective toxicology study.

Study Number	Study Title	Report Number
2-6	In vitro tumorigenicity testing of NHDF Master and Working Cell Banks	NCREP.010
7-9	In vitro tumorigenicity testing of NIKS® Master and Working Cell Banks	NCREP.011
10	Initial Assessment of Oncogenic Growth of the Human NIKS® Keratinocyte Cell Line in Nude and (b) (4) Mice	NCREP.002
11	Assessment of Oncogenic Growth of NIKS® and NIKS®-derived Keratinocyte Cell Lines	NCREP.005
12	Assessment of Oncogenic Growth of NIKS® and NIKS®-derived (NIKS(b) (4)) Keratinocyte Cell Lines	NCREP.006
13	Assessment of Oncogenic Growth of NIKS® and NIKS®-derived (NIKS(b) (4)) Keratinocyte Cell Lines	NCREP.007
14	Retrospective Summary of Tumor Development in Nude Mice Engrafted with STRATAGRAFT® Skin Tissues (2001-2002)	NCREP.008
15	Retrospective Summary of Tumor Development in Nude Mice Engrafted with STRATAGRAFT® Skin Tissues (2010-2014)	NCREP.009

Studies #2-6

(b) (4)

11 pages have been determined to be not releasable: (b)(4)

(b) (4)

Study #14

Report Number	NCREP.008
Date Report Signed	7/25/2019 **Note:** The experiments described in this study report were completed between 11/9/2000 and 10/30/2001.
Title	Retrospective Summary of Tumor Development in Nude Mice Engrafted with STRATAGRAFT® Skin Tissues (2001-2002)
GLP Status	No

Testing Facilities		(b) (4) Stratatech Corporation, Madison, WI **Note:** The applicant stated that the studies were conducted at both sites; however, the studies that were conducted at each site were not identified.
Objective(s)		To assess the tumorigenic potential of NIKS® keratinocytes and the other components of STRATAGRAFT®.
Study Animals	**Strain/Breed**	(b) (4) mouse
	Species	*Mus musculus*
	Age	6-9 weeks old at application
	Body Weight	Not specified
	#/sex/group	44F; See Table 2 below for Experiment Nos.
	Total #	44 **Note:** This study report describes a total of 70 mice that received topical application of STRATAGRAFT®. However, 26/70 animals (Experiments #12, 14, 18) were part of the study assessing the subchronic toxicity of STRATAGRAFT® (Study #1; Study Report No. NCREP.001). Thus, for Study #14 (Study Report No. NCREP.008), the applicant focused on the remaining 44/70 mice (Table 2).
Test Article(s)		Two generations of STRATAGRAFT® were tested: • Experiments #12 and #14: An NHDF cell line, (b) (4), that was used during product development, but is not included in STRATAGRAFT®. • Experiments #16-20: The commercially sourced NHDF cell line (Lot #(b) (4)) purchased from (b) (4). This NHDF cell line is used to manufacture STRATAGRAFT®. See Table 3 for the Batch numbers for each product tested.
Control Article(s)		No concurrent negative or positive control animals were included
ROA		Topical application on the wound bed
Description of the Disease/Injury Model and Implant Procedure		A full-thickness excisional wound was generated on the dorsum immediately before application of STRATAGRAFT®. **Note:** The size of the wound was not specified.
Dosing Regimen		Single administration
Randomization		No
Description of Masking		Not specified
Scheduled Sacrifice Time Points		Days 7-421 post-application (see Table 2)

Reviewer's Comments:

- This retrospective study reported data collected from studies conducted to evaluate various properties of STRATAGRAFT®, such as ease of handling and overall engraftment quality. This study report includes a series of seven experiments, in which the wound site was harvested on or after, Day 7 post-application.

- Per the applicant, the experiments were intended to optimize the grafting of STRATAGRAFT® in an animal wound model. The resulting murine data were retrospectively evaluated for the presence of any aberrant growth (benign or malignant), as well as the extent of wound healing.

- During the conduct of the experiments analyzed in this Study #14 and for Study #15 (below), the method of preservation of STRATAGRAFT® was being optimized. The batches used in Experiments #12, #14, #16, #19, and #20 of Study #14 were maintained in culture until they were packaged and transported to the vivarium for application. The batches used in Experiments #17 and #18 of Study #14 were cryopreserved by (b) (4) . This method of cryopreservation has since been further optimized for the conduct of the STRATA2016 clinical trial and the commercial manufacturing process. Batches used in STRATA2016 and commercial batches of STRATAGRAFT® are cryopreserved in (b) (4) .

- The applicant did not specify the rationale for the large number of batches of STRATAGRAFT® administered in Studies #14 and #15.

Table 2. Information for the animals included in each experiment

Experiment	No. of Animals	No. of Animals in Study #1 (Study Report NCREP.001)	No. of Animals in Study #14 (Study Report NCREP.008)	Age of Animals (weeks) at the Time of Application	Sacrifice Time Point Post-application (Days)
12	10	0	10	6	Up to 421
14	10	0	10	8	Up to 387
16	12	8	4	8, 9	Up to 21
17	6	3	3	7	300
18	5	0	5	9	22
19	15	12	3	6	258
20	12	3	9	6, 7	Up to 259

Source: Report No. NCREP.008, located in 'Experimental Design' in Module 4.2.3.4.1.3 of the BLA.

Table 3. Cell strains and passage number used to generate the STRATAGRAFT® batches

Experiment	Batch Identification (non-GMP)	Keratinocytes	NHDFs	
		NIKS®	(b) (4)	(b) (4)
12	(b) (4)			
14				
16				
17				
18				
19				
20				

(-) Denotes specified cell or strain was not used in the indicated experiment.
(*) Denotes cell passage information is not available.

Source: Report No. NCREP.008, located in 'Experimental Design' in Module 4.2.3.4.1.3 of the BLA.

Key Evaluations and Results:

Tumor formation:

- Animals were examined visually for mass formation at the application site a minimum of twice weekly and a maximum of once daily. No masses were visually observed at any sites.
- The wound sites on the dorsum were collected at sacrifice for histopathology. (b) (4) staining was used to evaluate samples for basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and keratoacanthoma (KA; a tumor that may precede SCC), from 19/44 animals (sacrificed from Days 7-421 post-application):
  - Experiments #12 and #14 - 7/10 animals/experiment
  - Experiment #16 - 4 animals
- No animal exhibited evidence of tumor formation.

Reviewer's Comment:

- The applicant did not provide the rationale for not conducting histopathology on the remaining 25/44 mice.

Reviewer's Conclusion: While these experiments were initiated to evaluate aspects of STRATAGRAFT® safety and activity in addition to tumorigenic potential, the long-term follow-up up to 421 days post-application indicates that STRATAGRAFT® is non-tumorigenic.

Study #15

Report Number		NCREP.009
Date Report Signed		10/15/19 **Note:** The experiments described in this study report were initiated between 2010 and 2014.
Title		Retrospective Summary of Tumor Development in Nude Mice Engrafted with STRATAGRAFT® Skin Tissues (2010-2014)
GLP Status		No
Testing Facility		(b) (4)
Objective(s)		To assess the tumorigenic potential of the NIKS® keratinocyte cell line and the other components of STRATAGRAFT®
Study Animals	**Strain/Breed**	(b) (4) mouse ((b) (4)) (b) (4)
	Species	*Mus musculus*
	Age	6-15 weeks old at application
	Body Weight	17-29g at study initiation
	#/sex/group	136F and 2M (see Table 4below for the Experiment #)
	Total #	138

Test Article(s)	STRATAGRAFT® Non-GMP Batch #s: (b) (4) GMP Batch #s: (b) (4)
Control Article(s)	No concurrent negative or positive control animals were included
ROA	Topical application on the wound bed
Description of the Disease/Injury Model and Implant Procedure	A 3-7.5 cm^2 full-thickness excisional wound was generated on the dorsum immediately before application of STRATATECH®
Dosing Regimen	Single administration
Randomization	No
Description of Masking	Not specified
Scheduled Sacrifice Time Points	Days 21-28 post-application

Reviewer's Comment:

- This retrospective study reported data collected from studies conducted to evaluate various properties of STRATAGRAFT®, such as ease of handling, duration of STRATAGRAFT® retention, and overall engraftment quality. This study report includes a summary of a series of 15 experiments conducted between 2010 and 2014, in which the wound site was harvested on or after, Day 21 post-application. The resulting murine data were retrospectively evaluated for the presence of any aberrant growth (benign or malignant), as well as the extent of wound healing.

Table 4. Information for the animals included in each experiment

Experiment	No. of Animals	Age of Animals (weeks) at the Time of Application
1	8	6
2	19	8
3	19	10
4	2	14
5	2	14
6	4	15 9
7	6	10
8	5	11
9	5	12
10	12	8
11	13	10
12	11	10
13	8	8
14	20	10
15	4 (2/sex)	9

Source: Report No. NCREP.009, located in 'Experimental Design' in Module 4.2.3.4.2.5 of the BLA.

Table 5. STRATATECH® batches administered

Experiment	Batch #	Storage Information	Mouse ID #	Graft Size (cm^2)
1	(b) (4)	Immediate use	S506 - S509	5
		Immediate use	S510 - S513	
2		Immediate use	1287 - 1289	3
		Cryopreserved	1290-1298, 1300, 1302-1307	
3		Immediate use	1337-1339, 1347, 1348	3
		Cryopreserved	1340-1345, 1349-1352, 1354-1357	
4		Refrigerated (13 Days)	1384, 1385	3
5		Refrigerated (14 Days)	1387, 1388	3
6		Refrigerated (13 Days)	1386, 1389, 1418, 1419	3
7		Refrigerated (13 Days)	1420-1425	3
8		Refrigerated (13 Days)	1445-1449	3

9	(b) (4)	Refrigerated (8 Days)	1520, 1521, 1524, 1525, 1527	3
10		Refrigerated (4 & 6 Days)	1557-1563, 1565-1568, 1572	3
11		Refrigerated (11 Days)	1600-1604, 1607, 1609, 1610, 1612-1614, 1616, 1618	3
12		Refrigerated (1 day)	1697-1707	3
13		Cryopreserved	2046-2049	5
		Refrigerated (1 day)	2058-2061	
14		Cryopreserved	2140-2142, 2144, 2145	5
		Refrigerated (8 Days)	2149-2153	
		Cryopreserved	2154-2158	
		Refrigerated (8 Days)	2159-2163	
15		Cryopreserved	2487-2490	7.5

Source: Report No. NCREP.009, located in 'Experimental Design' in Module 4.2.3.4.2.5 of the BLA.

Reviewer's Comment:

- Per the applicant, the STRATAGRAFT® manufacturing process was being refined as the product development program progressed. The applicant cited the following differences between the manufacturing process of STRATAGRAFT® for Study #15 and the commercial process:

  - Three batches used in Experiments #13-15 ((b) (4) , respectively) contained an (b) (4) rather than from rat-tail collagen; this is not the commercial manufacturing process.

  - Batches used in Experiments #2 and #3 ((b) (4) respectively) were cryopreserved by (b) (4) Cryopreserved batches used in Experiments #13-#15 (Table 5) were cryopreserved using (b) (4)

Key Evaluations and Results:

Tumor formation:

- Animals were visually examined for mass formation at the application site at least twice weekly and a maximum of once daily. No masses were visually observed at any sites.
- The wound sites were collected at sacrifice for histopathology. (b) (4) staining was used to evaluate samples for BCC, SCC, and KA from 74/138 animals:
  - Experiments #1 and #13 - 8/8 animals

- Experiments #4 and #5 - 2/2 animals/experiment
- Experiment #7 - 6/6 animals
- Experiment #8 - 4/5 animals
- Experiment #11 - 13/13 animals
- Experiment #12 - 11/11 animals
- Experiment #14 - 20/20 animals

No animal exhibited evidence of tumor formation.

Reviewer's Comment:

- The applicant did not provide the rationale for not conducting histopathology on the remaining 64/138 mice.

Reviewer's Conclusion: While these experiments were initiated to evaluate aspects of STRATAGRAFT® safety and activity in addition to tumorigenic potential, the number of mice included in this retrospective analysis provide support that STRATAGRAFT® is non-tumorigenic.

Other Safety/Toxicology Studies:

Study Number	Study Title	Report Number
16	Toxicological profile, risk assessment, and development of a PDE for Glycerol	CAS #: 56-81-5
17	Toxicological profile, risk assessment, and PDE calculation for (b) (4) in STRATAGRAFT® skin tissue	RPT-STDY-0380 (CAS #: 7429-90-5)
18	Toxicological profile, risk assessment, and PDE calculation for (b) (4) in STRATAGRAFT® skin tissue	RPT-STDY-0381 (CAS #: 7440-42-8)

Study #16: ***Toxicological profile, risk assessment, and development of a PDE for Glycerol; Conducted by*** (b) (4)

Glycerol at a concentration of (b) (4) is used in the cryopreservation of STRATAGRAFT®. Page 1 of Module 3.2.P.4.6, Novel Excipient-CPS (cryopreservation solution) states that the majority of glycerol is removed from the final product during the post-thaw hold step, with an estimated (b) (4) residual glycerol in STRATAGRAFT® at the time of application to the patient. If a maximum of 60 STRATAGRAFT® constructs, each measuring 100 cm^2, are applied to a patient, the maximum daily dose (MDD) of glycerol will be (b) (4) /human/day. To establish the permitted daily exposure (PDE)[7] for glycerol, the expert consultant assumed that once applied to humans, STRATAGRAFT® would cover up to 33% of the patient's TBSA, and that a maximum of three applications over five consecutive days would occur, resulting in a maximum cumulative dose of (b) (4) glycerol/human. Data from a non-GLP study conducted in rabbits, in which

[7] The PDE is a health-based limit which presents a dose that is unlikely to cause adverse health effects to an individual exposed, by a specific route or routes of exposure, at or below this dose, every day for a lifetime.

glycerol (covered by gauze) was topically applied to intact skin for 24 hours, were presented[8]. One rabbit exhibited mild irritation and the remaining 13 rabbits did not exhibit irritation at the application site. The expert consultant noted that undiluted glycerol was used for this study, while STRATAGRAFT® is estimated to have a maximum of (b) (4) residual glycerol. An additional study of glycerol exposure by repeat oral administration in dogs was cited by the expert consultant. This study identified a no-observed-adverse-effect-level of 950 mg/kg/day[9]. This dose level is equivalent to 47.5 g glycerol/human/day. If administered for 3 days, the total equivalent exposure would be 142.5 g/human. The resulting data from the dermal study in rabbits were used to calculate the PDE for glycerol. Based on the data generated in the rabbits and using various adjustment factors (such as interspecies differences) as specified in the ICH Q3C(R5) Guideline[10], the PDE was estimated to be (b) (4) of glycerol/human/day. Therefore, for the dermal route of exposure, based on the calculated PDE, there is a 6-fold safety margin for glycerol exposure from STRATAGRAFT® application.

Reviewer's Comment:

- The estimated amount of residual glycerol present at the time of STRATAGRAFT® application calculates to an MDD of (b) (4) This amount is below the estimated PDE for the dermal ROA of (b) (4) of glycerol/human/day.

Study #17: ***Toxicological profile, risk assessment, and PDE calculation for*** (b) (4) ***in STRATAGRAFT® skin tissue; Conducted by*** (b) (4)

(b) (4)

[8] Weil CS, Scala RS (1971) Study of Intra- and Interlaboratory Variability in the Results of Rabbit Eye and Skin Irritation Tests. Toxicol Appl Pharmacol 19(2):276-360.

[9] Office of Economic Co-Operation and Development (2002). SIDS Initial Assessment Report for SIAM 14: Glycerol. Available at: https://hpvchemicals.oecd.org/ui/handler.axd?id=4b0a2d87-3183-40d4-84f5-0e118c647b19.

[10] International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) (2016) Impurities: Guideline for Residual Solvents Q3C(R5). Available at: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q3C/Step4/Q3C_R5_Step4.pdf.

[11] (b) (4)

(b) (4)

Study #18: ***Toxicological profile, risk assessment, and PDE calculation for*** (b) (4) ***in STRATAGRAFT® skin tissue; Conducted by*** (b) (4)

(b) (4)

(b) (4)

(b) (4)

Drug Interactions:

Section 7 of the proposed label includes pharmaceutical agents that may interact with/affect the efficacy of, STRATAGRAFT® following application according to the label in the indicated patient population.

Section 7.1 Mafenide Acetate:
Mafenide acetate is a topical antibiotic. The applicant provided a literature citation to support the statement that administration of mafenide acetate is not recommended because this drug "has been shown in vitro to reduce keratinocyte viability and disrupt the integrity the skin tissue". The cited publication[16] reports that:

- When applied to a tissue-engineered human skin substitute generated using primary human keratinocytes or NIKS® keratinocytes for the epidermis and primary dermal fibroblasts for the dermis, mafenide acetate led to degeneration of keratinocytes in all layers.
- Administration of mafenide acetate also led to separation of basal keratinocytes from the underlying dermis layer.

Reviewer's Conclusion:

- This reviewer agrees with the applicant that mafenide acetate could interact with STRATAGRAFT® and reduce keratinocyte viability. However, the term 'skin tissue' is misleading and the label should instead use the term tissue-engineered human skin substitute.

Section 7.2 Silver-containing Antimicrobials:
The applicant provided a literature citation to support the statement that administration of silver-containing antimicrobials is not recommended because it "may decrease the viability of keratinocytes and fibroblasts". The cited publication[17] reports that:

[16] Gibson, A. L., M. J. Schurr, S. J. Schlosser, A. R. Comer, and B. L. Allen-Hoffmann. Comparison of Therapeutic Antibiotic Treatments on Tissue-Engineered Human Skin Substitutes. *Tissue Eng Part A* 14, no. 5 (May 2008): 629-38.
[17] Nesporova K, Pavlik V, Safrankova B, Vagnerova H, Odraska P, Zidek O, Cisarova N, Skoroplyas S, Kubala L, Velebny V. Effects of wound dressings containing silver on skin and immune cells. Sci Rep 10, 15216 (2020).

- *In vitro* studies showed that extracts produced from multiple commercially available wound dressings containing silver statistically significantly decreased the viability of NHDFs.
- When HaCaT keratinocytes, an immortalized human keratinocyte cell line, were in direct contact with silver-containing wound dressings, a statistically significant decrease in cell viability occurred.

Reviewer's Conclusion:

- This reviewer agrees with the applicant that use of silver-containing antimicrobials and dressings may decrease the viability of the cells. However, the term 'human dermal fibroblasts' should be used rather than 'fibroblasts'.

Section 7.3 Chlorhexidine:
The applicant provided a literature citation to support the statement that administration of "chlorhexidine solution (concentrations greater than 0.12%) directly on STRATAGRAFT® after placement is not recommended because of potential toxicity to the cells". The cited publication [18]reports that:

- When chlorhexidine concentrations of 0.005% to 0.5% were applied to cultures of keratinocytes and fibroblasts, cell growth was decreased, as compared to cell cultures that were not exposed to chlorhexidine.
- Interpretation of this study was confounded by precipitation of chlorhexidine at the highest concentrations tested, 0.15% and 0.5%. The author states that these precipitates led to an artifactual increase in measured cell growth at those concentrations, as compared to 0.005%, 0.015%, and 0.05% concentrations.

Reviewer's Conclusion:

- This reviewer agrees with the applicant that chlorhexidine could interact with STRATAGRAFT® and reduce keratinocyte and/or fibroblast growth. The cited study shows that all concentrations of chlorhexidine tested down to 0.005%, are potentially toxic to keratinocytes and/or fibroblasts, thus, the applicant should remove reference to a specific concentration of chlorhexidine from the proposed label.

APPLICANT'S PROPOSED LABEL

- Subsections 7.1-7.3 of Section 7 ('Drug Interactions') should be revised, as applicable, to accurately reflect the provided published studies.

[18] Boyce, S. T., G. D. Warden, and I. A. Holder. Cytotoxicity Testing of Topical Antimicrobial Agents on Human Keratinocytes and Fibroblasts for Cultured Skin Grafts. *J Burn Care Rehabil* 16, No. 2 Pt 1 (Mar-Apr 1995): 97-103.

- Subsections 8.1-8.3 of Section 8 ('Use in Specific Populations') should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14), as applicable[19].

- Section 13 ('Nonclinical Toxicology') should be revised, as applicable, to accurately reflect the available nonclinical data.

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns that could not be addressed in the product label. The nonclinical data support approval of the license application.

KEY WORDS/TERMS

STRATAGRAFT®, NIKS® keratinocytes, NHDFs, human dermal fibroblasts, immunodeficient, tumorigenicity, dermal equivalent, skin repair, thermal burns, thermal wounds, deep partial thickness, allogeneic, cellular, dermal elements, wound bed, glycerol

[19] FDA (2015) Guidance for Industry: Pregnancy, Lactation, and Reproductive Potential: Labeling for Human Prescription Drug and Biological Products - Content and Format. Available at: https://www fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm450636.pdf.