EASTDERM HYDROGEL WOUND DRESSING

<Lot No.: 2016.01.18.001>

Skin Sensitization Study (Maximization Test)

FINAL REPORT

Sponsor: **Easting Biotech Co Ltd**
Testing Institution: **SGS Taiwan Ltd.**
Ultra Trace & Industrial Safety Hygiene
Report No.: **UB/2016/30966A-05**

Note:
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STUDY SCHEDULE

Skin Sensitization Study (Maximization Test)
EASTDERM HYDROGEL WOUND DRESSING

Report No.:	UB/2016/30966A-05
Test Article Received Date:	2016.03.16
Experimental starting date:	2016.04.22
Experimental completion date:	2016.05.27
Study completion date:	See Study Director's signature date in the report

ADDRESS INFORMATION

Testing Facility/Test Site

Name: SGS TAIWAN LTD. Ultra Trace & Industrial Safety Hygiene
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Study Director

Name: Benson Liu
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Sponsor

Name: Easting Biotech Co Ltd
Address: 3F., No.49, Xiwei St., Sanchong Dist., New Taipei City 24155, Taiwan (R.O.C.)

INFORMATION FOR TEST ARTICLE

台灣檢驗科技股份有限公司
SGS Taiwan Ltd.

INFORMATION FOR ☒TEST ARTICLE/ ☐CONTROL ARTICLE

Sponsor Company Name	Easting Biotech Co Ltd
Sponsor Address	3F., No.49, Xiwei St., Sanchong Dist., New Taipei City 24155, Taiwan (R.O.C.)
Name of Test Article/ Control Article	EASTDERM HYDROGEL WOUND DRESSING
Amount (Note 2)	A、Quantity/Unit: 15/pcs (e.g.10ml / bottle * 6 bottles) B、☒One Test (No Retention)☐Two Test (For Retention) C、Packing Condition: ☐In Bulk ☒Intact Packing
Sterilization	Has been Sterilized ☐NO ☒YES, If Yes, Please Select the Following Method, ☐EO Sterilization☒Gamma Sterilization☐Steam Sterilization☐Other_____
Expiry Date (Note 3)	☒Expiry Date: 2019 . 01 . 18 (YYYY.MM.DD) ☐Not Provided.
Batch/Lot Number	☒Specific Number：2016.01.18.001 ☐Not Provided.
Sample Description	A、Major Components: Hydrogel, Polyurethane Film B、Purity: N.A. C、Concentration: N.A. D、Stability：N.A E、Color : Translucent F、External Features: ☐Liquid ☐Irregular ☐Powder ☐Granule ☒Flat ☐Other:_____ G、Solvent and Solubility ：N.A
Attachment(Note 4)	☐Certificate of Analysis ☐Material Safety Data Sheet ☐Stability Test Result ☐Others：_____ ☒No Attachment (Note4)
Storage Condition	☒Room Temperature☐2~8℃☐-10~-25℃☐Others_____
Others	N.A

Test Article No.
UB/2016/30966
It will be labeled by SGS sample receiving personnel.

Note1. Above all information including the blanks which sponsor cannot provide are disclosure by the sponsor.
Note2. If the sponsor doesn't provide the retention of test article/control article, the retention of a reserved test article/control article from each batch of test article /control article is the responsibility of the Sponsor.
Note3. Should prepare extra amount of the same lot test/control article for the retention. For retention, if the effective period is less than 5 years, the test article/control article will be retained till the expiry date. If the expiry date is longer than 5 years, the test article/control article will be retained for 5 years only. If the expiry date remained incomplete, it mentions that sponsor will agree that test facility will determine the earliest date, e.g. Exp. Date: 2015(YYYY), sponsor didn't identify the MM/DD, the expiration date should be 2015.01.01.
Note4. Sponsor should determinate, document and confirm the identity, strength, purity, stability, composition, method of synthesis, fabrication, derivation or other characteristics of the test article/control article before study. If the sponsor cannot provide the information, determination and documentation of the test article/control article are the responsibility of the Sponsor.
Note5. Note 'N/A' or 'N.A'if not applicable. Do not leave blank.
Note6. Test article and control article should be filled individually in "INFORMATION FOR TEST ARTICLE/CONTROL ARTICLE".
Note7. GLP test：(1) Corrections and additions to a final report should be in the form of amendments. Amendments should clearly specify the reason for the corrections or additions. Lot number, test article photos and raw data cannot be amended by sponsor's requirement. (2)One protocol only can generate one report except for translation version. If the report with more than two languages version, we only issue English version protocol. We only issue amendment or additional language GLP report within three years and non-GLP report within one year. (3)The GLP compliance statement will state that we follow TFDA GLP and TAF OECD GLP norm unless sponsor's requirement. SGS Taiwan Ltd. UTIS have acquired Good Laboratory Practice Statement of Compliance from TFDA and TAF.
Note8. Please write down it carefully and in detail. "INFORMATION FOR TEST ARTICLE/CONTROL ARTICLE" will be placed in the protocol and report along with a copy of this official document. If the information is not clear, we will exclude them from GLP statement.

Sponsor Signature/ Date：王以仁 2016/03/16

版次：3.5 試驗物質/對照物質資料表 Information For Test Article/Control Article
發行日期：2015.06.01 page 1of 1

SIGNATURE OF STUDY PERSONNEL

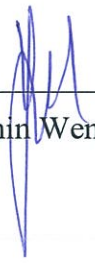
Skin Sensitization Study (Maximization Test)
EASTDERM HYDROGEL WOUND DRESSING

Study Director:

R Liu — 2016.06.14

Benson Liu / SGS Taiwan Ltd. — Date

Facility Manager:



2016.06.14

Yuanmin Wen / SGS Taiwan Ltd. — Date

OBJECTIVE

When direct contact with human tissues is anticipated, medical device should be carefully tested for biocompatibility according to the nature and duration of the contact to avoid potential physiological damage caused by hypersensitive substances produced or contaminated during manufacture. In this study, guinea pig skin sensitization study (Maximization test) was conducted to evaluate the possibility of skin sensitization after topical applications of the test article extracts on the skin of guinea pigs. The experiment was performed by following ISO 10993-10.

EXPERIMENTAL DESIGN

1. Test System

A. Species/ Strain: Guinea Pig/ Hartley
B. Resource: Wuxi Huishan Jiangnan Experimental Animal Center
C. Reason: According to ISO 10993-10
D. Body weights/Age: 300~500g
E. Sex: Male
F. Number: 30
G. Quarantine/ acclimation: Once animals are introduced in-house, they are subjected to quarantine and acclimatize for 7 days before treatment. Animals are selected based on health status by qualified staff.
H. Identification
(1)Individual identification: Animals were identified by hair-dyeing.
(2)Cage identification: Cages are properly labeled for identification including species/ strain, sex, in-housing date, animal I.D. number.
I. Housing condition
(1)Environment temperature: 18~26℃
(2)Humidity: 30~70%
(3)Lights: 12 hours light/dark cycle
(4)Fodder/ Supply: Guinea Pig Diet (Wuxi Huishan Jiangnan Experimental Animal Center); *ad libitum*
(5)Drinking water/ Supply: Tap water; *ad libitum*

2. Reagents

A. 0.9% normal saline (Anhui Double-Crane Pharmaceutical Co., Ltd, Lot No. 150326 6C)
B. Freund's complete adjuvant (Sigma, F5881, Lot No. SLBL3699V)
C. Sodium dodecyl sulfate (Sinopharm Chemical Reagent Co. Ltd, Lot No. 20150113)
D. Sesame oil (Ji'an luyuanxiangliao. Co., Ltd, Lot. No. 20151206)

3. Extraction

According to ISO 10993-12 guidelines, the ratio of the test article to the extractant was 6cm^2/mL. Extraction was conducted as the table below.

Extraction for	Group	Test article (cm^2)	Extractant	
			Solution	Volume (mL)
Induction phase I	Polar	204	0.9% normal saline	34
	Non-polar	204	Sesame oil	34
Induction phase II	Polar	204	0.9% normal saline	34
	Non-polar	204	Sesame oil	34
Challenge phase	Polar	204	0.9% normal saline	34
	Non-polar	204	Sesame oil	34

Extract condition of 72±2 hours at 37℃ with constant agitation were executed in this study. The pH adjustment, filtration and centrifugation were not conducted. After extraction, the appearances of extracts were not different from the control solution, clear. The extracts were not stored longer than 24 hours.

4. Grouping

Test group	Control group
20 animals (polar: 10, non-polar: 10)	10 animals (polar: 5, non-polar: 5)
Polar extract of test article	0.9% Saline
Non-polar extract of test article	Sesame Oil

5. Test Method

A. Induction phase I

(1) three kinds of solutions or emulsions were prepared from the control solution or test article extract as follow:

Polar group:

(A) Emulsion of Freund's complete adjuvant (Sigma F5881) in 0.9% saline and volume ratio 1:1 (50% FCA).

(B) Solution of either test article extracts or 0.9% saline.

(C) Emulsion of either test article extracts or 0.9% saline in 50% FCA in volume ratio 1:1.

Non-polar Group:

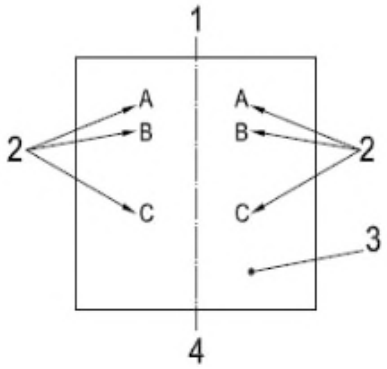
(A) Emulsion of Freund's complete adjuvant (Sigma F5881) in Sesame oil and volume ratio 1:1 (50% FCA).

(B) Solution of either test article extracts or Sesame oil.

(C) Emulsion of either test article extracts or Sesame oil in 50% FCA in volume ratio 1:1.

(2) Furs of animal's backside were clipped with an electric animal shaver. Animals with scratches or skin diseases on the clipped skin surfaces were rejected from the study. The clipped area will be about 8 cm^2.

(3) Injections sites were paired, and there were six injection sites in the clipped zone of each animal (see figure below). Each solution was injected into injection sites matches A, B and C. The injected volume was 0.1 mL at each injection sites.



1. Head side of guinea pigs
2. Injection sites
3. Clipped area on backside of guinea pigs
4. Caudal side of guinea pigs

B. Induction phase II
   (1) 6±1 days later, the injection sites were applied with 10% of sodium dodecyl sulfate for 24±2 h.
   (2) Then, an appropriate absorbent gauze patch was saturated (about 8 cm^2) with the test article extract or control solution, and applied to the clipped skin under an occlusive dressing secured by a wrap around the torso of the animal for another 48±2 h.

C. Challenge phase (14±1 days after induction phase II)
   (1) The furs of flank of the animals were clipped. An appropriate site of this hairless area were selected and applied by the patches that soaked with the control solution or test article extract and then secured with an occlusive dressing.
   (2) The dressings and patches were removed after 24±2 h.

D. Observation and evaluation
   (1) The appearance of the challenge skin sites of the test and control animals were observed at 24±2 h and 48±2 h after removal of the dressings.
   (2) Skin reactions for erythema and oedema were graded according to the Magnusson and Kligman grading given in Table 1.
   (3) Grades greater than 1.0 in the test group generally indicate sensitization while grades of control animals are less than 1.0. If grades greater than 1.0 are noted in control animals, the reactions of test animals which exceed the most severe reaction are presumed to be due to sensitization.

RESULTS

1. Approximately 24±2 hours and 48±2 hours after challenge phase, neither the control nor the test group showed significant skin response on the treated areas. None of the test or control groups had a mean score increase of 1.0 or more in the observation period.
2. Individual Animal Grades skin reaction
   Polar group:

Group	Sex	Number of animals	24 hrs. after challenge phase	48 hrs. after challenge phase
Control "0.9% saline"	male	P11	0	0
		P12	0	0
		P13	0	0
		P14	0	0
		P15	0	0
Mean score			**0**	**0**
Test "EASTDER HYDROGEL WOUND DRESSING Extracts (saline)"	male	P01	0	0
		P02	0	0
		P03	0	0
		P04	0	0
		P05	0	0
		P06	0	0
		P07	0	0
		P08	0	0
		P09	0	0
		P10	0	0
Mean score			**0**	**0**

Non-polar group:

Group	Sex	Number of animals	24 hrs. after challenge phase	48 hrs. after challenge phase
Control "Sesame oil"	male	NP11	0	0
		NP12	0	0
		NP13	0	0
		NP14	0	0
		NP15	0	0
Mean score			**0**	**0**
Test "EASTDER HYDROGEL WOUND DRESSING Extracts (oil)"	male	NP01	0	0
		NP02	0	0
		NP03	0	0
		NP04	0	0
		NP05	0	0
		NP06	0	0
		NP07	0	0
		NP08	0	0
		NP09	0	0
		NP10	0	0
Mean score			**0**	**0**

RELIABILITY CHECK

The positive control study was finished at 2016/03/17. According to ISO 10993-10 guidelines, positive control shall be performed at least once every six month. 2,4-Dinitrochlorobenzene (DNCB) (Xiya ReagentR, Lot. No. W5656) were used for positive control substances. The method for the positive control assay are identical to the method described above in this study. For the induction phase, 0.5% DNCB was used. For the challenge phase, 0.1% DNCB was used.

Animals in the positive control group exhibited discrete erythema to confluent erythema at the challenge site. All reactions in the positive control group scored of 2~3, had a 100% incidence are indicated that is positive sensitization reaction.

CONCLUSION

The results indicated that the polar and non-polar extracts of “EASTDERM HYDROGEL WOUND DRESSING” did not produce skin sensitization in guinea pigs.

REFERENCES

1. Skin Sensitisation, OECD guideline for the testing of chemicals. #406 (1992) OECD.
2. Biological evaluation of medical devices- Part 10: Tests for irritation and skin sensitization. ISO 10993-10:2010.
3. Biological evaluation of medical devices- Part 12: Sample preparation and reference materials. ISO 10993-12:2012.
4. Biological evaluation of medical devices- Part 2: Animal welfare requirements. ISO 10993-2:2006.

TABLES

Magnusson and Kligman Scale (ISO 10993-10)

Patch test reaction	Grading scale
No visible change	0
Discrete or patchy erythema	1
Moderate and confluent erythema	2
Intense erythema and swelling	3

TEST ARTICLE PHOTO

UB/2016/30966