Transducer Protector

In Vitro Cytotoxicity Test

FINAL REPORT

Client: Finetech Research and Innovation Corporation
Testing Institution: SGS Taiwan Ltd.
Report No. : UB/2013/70737
Report Date: 2013/08/07

Note:

1. The content of this report is invalid if it is not presented as the entire report.
2. Any unauthorized alteration, forgery or falsification of the content or appearance of this report is unlawful and offenders may be prosecuted to the fullest extent of the law.
3. The results shown in this test report refer only to the test article(s) tested.
4. The report is the Chinese version of translations UB/2013/70737A-01

STUDY SCHEDULE

In Vitro Cytotoxicity Test

Transducer Protector

Report No.:	UB/2013/70737
Study Initiation date:	2013/07/25
Experimental starting date:	2013/07/26
Experimental completion date:	2013/08/02
Study completion date:	See Study Director's signature date in the report
Name of study Personnel:	Jeff Chen

Testing Institution

Name: SGS TAIWAN LTD.

Address: No. 38, Wu Chyuan 7th Rd., New Taipei Industrial Park, Wu Ku Dist., New Taipei City 24890, Taiwan (R. O. C.)

Client / Sponsor

Name: Finetech Research and Innovation Corporation

Address: No.29, Anle St., Xiushui Township, Changhua County 504, Taiwan (R.O.C.)

TEST ARTICLE INFORMATION

INFORMATION FOR ☒ TEST ARTICLE/ ☐ CONTROL ARTICLE

Sponsor Company Name	Finetech research and innovation corporation
Sponsor Address	No.29, Anle St., Xiushui Township, Changhua County 504, Taiwan (R.O.C.)
Contract study item	☒ Base on the contract ☐ Others _____
Name of Test article/ Control article	Transducer Protector
Batch/Lot number	☐ Base on the specific number on the package : _____ ☐ Base on the date on the package : _____ ☐ Base on the arrived date ☑ Others : N/A
Specification & Amount	10pcs/pack * 7packs (e.g.10ml / bottle * 6 bottles)
Retention amount (Note 2)	The amount of the same lot is sufficient for ☐ One test ☐ Two test (for retention)
External features	External features: ☐ liquid ☐ powder ☐ tablet ☐ capsule ☒ Other column Color : translucent white
Major components & Purity	Major components: Polypropylene meterial housing with membrane Purity:
Solvent and solubility	N/A
Storage condition	☒ Room temperature ☐ 4℃ ☐ Dry ☐ Light sensitive ☐ Others_____
Expiration date(Note 3)	☐ Date: ___ / ___ / ___ (YYYY/MM/DD) or ☒ Period : 2 year 0 month 0 day
Attachment(Note 4)	☐ Certificate of Analysis ☐ Material Safety Data Sheet ☐ Stability Test Result ☐ Other : _____ ☒ No attachment (Note4)
Sterilization	Has been sterilized ☐ YES ☒ NO (If Yes, please select the following item) Methods ☐ EOsterilization ☐ Gamma sterilization ☐ Steam sterilization ☐ Other_____
Categorization of devices (The column is only for device used)	1. ☒ Contact with intact skin or mucosa (cumulative contact duration) ☒ Short-term (no greater than 4 hr) ☐ Long-term (exceeding 4 hr) Maximum duration is_____hrs 2. ☐ Implanted device
Specific requirement (Note 5)	N/A

Sponsor Signature/ Date : Golden Li 2013. July 12th.

Note 1. Above all information is disclosure by the sponsor.
Note 2. 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.
Note 3. If the effective period is less than 5 years, the test article/control article will be retained till the expiry date. If the effective period is longer than 5 years, the test article/control article will be retained for 5 years only.
Note 4. Determination and documentation of identity, strength, purity, stability, composition, method of synthesis, fabrication, derivation or other characteristics of the test article/control article are the responsibility of the Sponsor.
Note 5. The test article/control article which has been destroyed or cutting will be discarded after the end of experiment. For retention or return of the kind of test article/control article, please indicate in the "special requirement". The human intake suggests or dose requested by the sponsor also can fill in the "special requirement". Note treatment method after test if the test article need to be retreated
Note 6. The code number of test article is the same as the report number.
Note 7. Note 'N/A' if not applicable. Do not leave blank.

版次：3.1 試驗-對照物質資料表 Information for test article-control article
發行日期：2013.06.14

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SGS

STATEMENT OF GLP COMPLIANCE

All study activities performed by SGS Taiwan were carried out in compliance with the GLP (Good Laboratory Practices) for Nonclinical Laboratory Studies (Department of Health, Taiwan, 2006), current OECD Principles of Good Laboratory Practice (Organization for Economic Cooperation and Development, Paris, ENV/MC/CHEM (98) 17) and U.S. Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58. (1987). The study was conducted in accordance with the protocol and standard operating procedures and monitored in conformity with the protocol. All laboratory data were accurately recorded and verified. SGS Taiwan made no GLP compliance claim for characterization and verification of the test article identity and properties; this was the responsibility of the sponsor.

Study Director:

Candy Tsou / SGS Taiwan Ltd. — Date Completed: 2013. 08. 16

Facility Manager:

Yuanmin Wen / SGS Taiwan Ltd. — Date Completed: 2013.08.16

SGS

QUALITY ASSURANCE STATEMENT

UB/2013/70737
Transducer Protector
In Vitro Cytotoxicity Test

This study was audited by Quality Assurance personnel of SGS Life Science Service. The QA inspection report includes review of study plan, result of a study-based audit and results of audit of raw data and study report. The audit report was issued upon the completion of each testing.

QA:

Melissa Lin / SGS Taiwan Ltd.

2013.08.07
Date Completed

Inspection Type	Inspection date	Study phase	Date to facility manager and study director
Study base	2013/07/25	Draft Protocol	2013/07/25
Study base	2013/08/02	Absorbance detection	2013/08/02
Study base	2013/08/07	Raw data & Draft Final report	2013/08/07

ARCHIVING

All the study-related raw data, records, protocol and the final report will be kept in archives room of SGS (TAIWAN) LTD for 5 years. Furthermore, retention of the test articles will be in Sample Storage Room for 5 years. All of the records and test articles are handled according to GLP guideline. Agent authorized by the sponsor can apply for the review according to SGS procedure.

Address:

No. 38, Wu Chyuan 7th Rd., New Taipei Industrial Park, Wu Ku Dist., New Taipei City 24890, Taiwan (R. O. C.)

Archiving List	
Final report	Final Report Copy
Raw Data	*In Vitro* Cytotoxicity Test - MTT data sheet
Records	Application Form Information for test article-control article GLP Test Article Control From and other supplementary record
Protocol	Protocol

ABSTRACT

In vitro cytotoxicity test was performed in this study to evaluate the biological compatibility of "Transducer Protector", which was provided by Finetech Research and Innovation Corporation. Extraction of test article and treatment of mouse lung fibroblast cells (L929 cells) with test article extracts were performed according to ISO10993-12 and ISO10993-5, respectively. Cell viability determined by MTT assay showed that the test article extract had in average <30% inhibitory effects to the viability of cells. Together with qualitative observations of cell morphology and monolayer confluency, these results suggested that the test article extract induced non-cytotoxicity effect in L929 cells.

PURPOSE

According to the nature and duration of the anticipated contact with human tissues when in use medical device should be carefully tested for biocompatibility to avoid potential physiological damage by toxic substances produced or contaminated during manufacturing. In this study, Transducer Protector is subjected to ***in vitro*** cytotoxicity test to evaluate toxicity of substances that could be extracted or released from the medical device. Therefore, the test system is Mouse lung fibroblast cells (L929 cells). The original source was from BCRC. Based on recommendations described in ISO10993-5, quantitative determination of cell viability by MTT assay and qualitative observation of cell morphology and growth density are carried out, followed by concluding level of cytotoxicity according to the scoring criteria listed in the document. These results provide practical information for assessing the ***in vitro*** cytotoxicity of the medical device.

EXPERIMENTAL DESIGN

1. Test System

A. Cell line: Mouse lung fibroblast cells (L929 cells). The original source was from BCRC60091.

B. Morphology: Fibroblast

C. Incubation condition Incubate in Minimum essential medium Eagle with 10% horse serum at 37°C in the presence of 5% CO_2

2. Reagents

A. Trypsin solution (Gibco, Cat No. 25200-056, Lot No.: 1211608)

B. Horse serum (Gibco, Cat No. 16050-122, Lot No.: 1131917)

C. L-Glutamine solution (Gibco, Cat No. 25030-081, Lot No.: 1115717)

D. Penicillin-Streptomycin solution (Gibco, Cat No. 15140-122, Lot No.: 1209968)

E. Minimum Essential Medium (MEM, Gibco, Cat No. 10370-021, Lot No.: 1237758)

F. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, Cat No. M5655, Lot No.: MKBHK674V)

G. Dimethyl sulfoxide (DMSO, Sigma, Cat No. D2650, Lot No.: 059K2300)

H. Sodium pyruvate, 100x (Sigma, Cat No. S8636, Lot No.: 1171639)

I. 10X Phosphate buffer solution (UniRegion Bio-Tech, Product No. UR-PBS001-5L, Lot No.: PBS001-5A)

3. Equipments

A. Orbital Shaker Incubator-3 (HILES, E-600)

B. Balance-13 (DENVER, TB-214)

C. Biological safety cabinet-1 (LABCONCO, 3450801)

D. CO_2 Incubator-1 (ASTEC, SCA-165DS)

E. Inverted Microscope-2 (OLYMPUS, CKX41SF)

4. Preparation of Test Article and Control Article

A. Test Article

The test article was handled under sterile environment and operated with aseptic technique during preparation. MEM complete medium was used as extraction buffer. The test article was extracted with a ratio of 0.2g/ml in MEM complete medium for 24±2 hours at 37±1°C with constant agitation at 150 rpm per criteria described in ISO10993-12. The pH adjustment, filtration and centrifugation were not conducted.

B. Control Articles

a. Blank control : MEM complete medium was as blank control,

b. Positive control: Polyurethane film – ZDEC (Polyurethane film containing Zinc Di-Ethyldithio-Carbamate, RM-A, Hatano Research Institute, Japan) extracted with 0.1g/1mL MEM complete medium was as positive control,

c. Negative control: HDPE film (High Density Poly-Ethylene film, RM-C, Hatano Research Institute, Japan) extracted with 0.1g/1mL MEM complete medium was as negative control.

d. Incubation Method: Extractions were performed at 37±1°C for 24±2 hours (ISO10993-12) with constant agitation at 150rpm.

5. *In vitro* cytotoxicity test-MTT

A. Cell incubation

a. Preparation of complete MEM cell culture medium

Complete cell culture medium was prepared by mixing 435 mL of MEM, 5 mL of Penicillin- Streptomycin solution, 5 mL of L-Glutamine solution, 5mL of sodium pyruvate and 50 mL of Horse serum. The completed medium was stored at 4°C.

b. Cell culture

Mouse lung fibroblast cells (L929 cells, Food Industry Research and Development Institute, Strain No. BCRC 60091) were used here for cytotoxicity test. The L929 cells

were grown on a 10-cm dish containing 10 mL of complete MEM medium and incubated at 37±1°C in the presence of 5% CO_2. Detachment of the cells was performed by washing the cells with 1 X PBS followed by treatment with 1.0 mL/dish of trypsin solution for 3 minutes at 37±1°C. Enzymatic activity of trypsin was terminated by adding complete MEM medium. Then transferred to new 10-cm dish for subculture.

B. *In vitro* cytotoxicity test

a. 100 µL of L929 cell suspension (1×10^5 cells/well) was transferred into each well of a 96-well cell culture plate. The cells were then incubated at 37±1°C for 24±2 hours in a humidified atmosphere containing 5% CO_2.

b. Culture medium was replaced with 100 µL of test article extracts or controls. The cells were then incubated for another 24 hours. Treatments of the cells with the extracts were performed in triplicates.

c. Morphology and monolayer confluency of cells were observed under microscope and scored in accordance with ISO10993-5. The scoring criteria were summarized in Table 2.

d. Following evaluation of cell conditions, the culture medium was aspirated from the plates. 50 µL of the MTT solution was then added to each well and the plate was further incubated for 2 hours ± 10 mins at 37±1 °C.

e. MTT solution was replaced with 100 µL of DMSO. The plate was incubated at room temperature for 25±5 minutes and subsequently subjected to a microplate reader equipped with a 570 nm filter for colorimetric measurement (reference 650 nm).

f. The triplicate results of MTT assay were presented as mean ± standard deviation (S.D.) and were scored in accordance with ISO10993-5 (as in Table 2). If the mean of cytotoxicity was less than 30%, the result will show "<30%".

g. Scores of cell morphology, confluency, and inhibition of viability were averaged to give final interpretation of cytotoxicity.

h. At end of the testing, all the test material, raw data, results, and reports were properly maintained under the guidance of Good Laboratory Practice (GLP).

6. Quality criteria

a. Positive control and negative control

(1) Positive and negative controls should be included in every cytotoxicity test.

(2) Positive control was Polyurethane film – ZDEC; Negative reference material was HDPE film.

b. Blank

(1) Measure the absolute value of optical density, $OD_{570,}$ The acceptance criteria of blank was $\geqq$ 0.2.

(2) Blanks were placed both at the left side (row 2) and the right side (row 11) of the 96-well plate.

(3) The left and right mean of the blanks do not differ by more than 15% from the mean of all blanks.

RESULTS

1. Appearance

The extracts of the test article was not different than the blank control.

2. Cell Morphology

As shown in Table 3 and Figure 1, the cells exposed to negative control showed no significant change in cell morphology compared to that of reagent control and resulted in a score as 0. Positive control extract caused severe cellular damage and obvious morphological alteration in almost all cells. Therefore, the positive control experiment was scored as 4. The cells treated with test article extract showed discrete intracytoplasmatic granules and no cell lysis. Therefore, the cell morphology was scored as 0.

3. Monolayer cell confluency

Table 4 and Figure 1 showed that the cells exposed to both reagent control and negative control extracts have similar growth density. Thus, we scored negative control as 0. Treatment of the cells with positive control extract abolished cell growth and resulted in approximately 30% of unhealthy cells remaining. We scored the effect of positive control on cell confluency as 3. In comparison to reagent control, monolayer confluency of the cells treated with test article extract was determined as 100% with a score as 0.

4. Inhibition of cell viability

The acquired readings of OD_{570} absorbance of reagent control were averaged and set as 0% inhibition of cell viability. In proportion to reagent control, we determined inhibition of cell viability of negative control, positive control, and test article as <30%, 96.74% ± 0.52% and <30%, respectively. The relative values of inhibition of cell viability and their scoring were summarized in Table 5.

CONCLUSION

The scores of the cytotoxicity test, including morphological evaluation, observation of monolayer confluency, and relative inhibition of cell viability, were averaged and listed in Table 6. Based on the averaged score was concluded that the "Transducer Protector" extract induced non-cytotoxicity to L929 cells according to ISO10993-5.

DATA MANAGEMENT

The quantitative data are showed as mean ± standard deviation (S.D.) and are scored using "Criteria for scores in cytotoxicity test" (Table 2). The qualitative data are scored using "Criteria for scores in cytotoxicity test". The individual score represents the average of triplicates. Mean score is the average of the quantitative and qualitative scores.

DEVIATIONS AND INVESTIGATIONS

There was no deviation and investigation during the test period of this study.

PROTOCOL AMENDMENTS

There was no protocol amendment during the test period of this study.

REFERENCES

1. Good Laboratory Practice for Nonclinical Laboratory Studies. Title 21 of the U.S. Code of Federal Regulations, Part 58 (1997) United States Food and Drug Administration.
2. ISO 10993 (2009) Biological evaluation of medical device－Part 5: Tests for *in vitro* cytotoxicity.
3. Mendes SC, Reis RL, Bovell YP, Cunha AM, Blitterswijk CA, Bruijn JD (2001) Biocompatibility testing of novel starch-based materials with potential application in orthopaedic surgery: a preliminary study. Biomaterials. 22, 2057-2064.
4. Abiraman S, Varma HK, Kumari TV, Umashankar PR, John A (2002) Preliminary *in vitro* and *in vivo* characterizations of a sol-gel derived bioactive glass-ceramic system. Bull. Mater. Sci. 25(5), 419-429.
5. ISO 10993 (2012) Biological evaluation of medical devices-Part 12：Sample preparation and reference materials.
6. Current OECD Principles of Good Laboratory Practice (Organization for Economic Cooperation and Development, Paris, ENV/MC/CHEM (98) 17).
7. EOMP-USL-0027 Operating procedures of the biosafety cabinet and laminar flow, UV-lamp verification and aerobic plate counts. Version 2.2
8. EOMP-USL-0030 Maintenance and operating procedures of the microscope. Version 1.0

Table 1 – Summary of extraction ratio for medical device

Thickness (mm)	Extraction ratio (surface area or mass/volume)	Examples of forms of materials
< 0.5	6 cm^2/mL	Film, sheet, tubing wall
0.5 to 1.0	3 cm^2/mL	Tubing wall, slab, small moulded items
> 1.0	3 cm^2/mL	Larger moulded items
> 1.0	1.25 cm^2/mL	Elastomeric closures
Irregularly shaped solid devices	0.2 g/mL	Powder, pellets, foam, non-absorbent, moulded items
Irregularly shaped porous devices (low-density materials)	0.1 g/mL	Membranes, textiles

NOTE: While there are no standardized methods available at present for testing absorbents and hydrocolloids, a suggested protocol is as follows:

-determine the volume of extraction vehicle that each 0.1 g or 1.0 cm^2 of material absorbs;

-then, in performing the material extraction, add this additional volume to each 0.1 g or 1.0 cm^2 in an extraction mixture.

Table 2 - Scoring criteria for cytotoxicity tests

Grade	Reactivity	Cell morphological change	Cell confluency	Inhibition of cell viability (MTT assay)
0	Non-cytotoxic	Discrete intracytoplasmatic granules and no cell lysis.	90-100%	<30%
1	Slightly-cytotoxic	Not more than 20 % of the cells are round, loosely attached and without intracytoplasmatic granules, or show changes in morphology; occasional lysed cells are present.	60-90%	30-40%
2	Mildly-cytotoxic	Not more than 50 % of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis.	40-60%	40-60%
3	Moderately-cytotoxic	Not more than 70 % of the cell layers contain rounded cells or are lysed; cell layers not completly destroyed.	20-40%	60-80%
4	Severely-cytotoxic	Nearly complete or complete destruction of the cell layers.	<20%	80-100%

Table 3 - Scores of cytotoxicity of test article extract in L929 cell morphology

Extracts	Exp 1	Exp 2	Exp 3
Reagent control	0	0	0
Positive control	4	4	4
Negative control	0	0	0
UB/2013/70737	0	0	0

Table 4 - Cytotoxic effect of test article extract in monolayer L929 cell confluency (%)

Extracts	Exp 1	Exp 2	Exp 3
Reagent control	100	100	100
Positive control	30	30	30
Negative control	100	100	100
UB/2013/70737	100	100	100

Table 5 - Cytotoxic effect of test article extract in inhibition of L929 cell viability (%)

Extracts	Exp 1	Exp 2	Exp 3	Mean±SD	Score
Reagent control	<30%	<30%	<30%	<30%	0
Positive control	97.11%	96.15%	96.97%	96.74%± 0.52%	4
Negative control	<30%	<30%	<30%	<30%	0
UB/2013/70737	<30%	<30%	<30%	<30%	0

Table 6 - Summary of cytotoxicity test results

Extracts	Cell morphology	Cell confluency	Inhibition of viability	Mean score	Cytotoxicity
Reagent control	0	0	0	0	None
Positive control	4	3	4	3.67	Severely
Negative control	0	0	0	0	None
UB/2013/70737	0	0	0	0	None

Cell Morphology

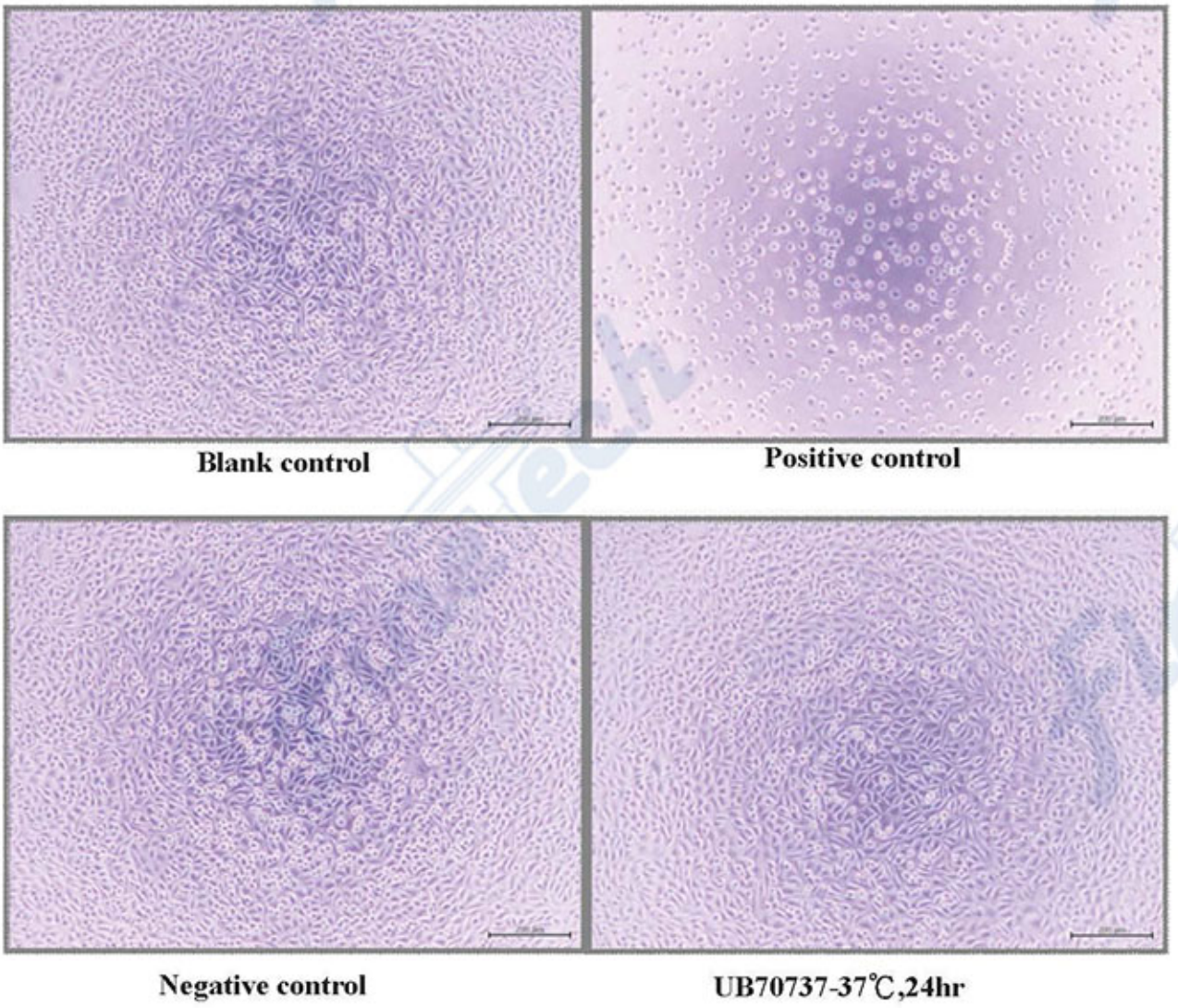

Figure 1 - Morphology and confluency of L929 cells after being exposed to test article or control extracts for 24 hour

TEST ARTICLE PHOTO