GLP Final Report

Report No.: T51642018-002(E)

Exclusively prepared for:

SPONSOR
Solaplus biotech co., ltd.
No.75 FengFang Road, Ouhai Economic Development Zone, Wenzhou

STUDY TITLE
Cytotoxicity Study using MTT Method

TEST ARTICLE
Hemostatic Xerogel Sponge
Model: XLJ-I

ANAB ACCREDITED ISO/IEC 17025 TESTING LABORATORY
AT-2046

ilac-MRA

TESTING FACILITY
Mid-Link Technology Testing Co., Ltd.
B6-05, RongTong Building,
No. 80, Haiyun Street, TEDA
Tianjin, 300457, China

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Summary

The test article, Hemostatic Xerogel Sponge, XLJ-I, was extracted by Minimum Essential Medium (MEM) with 10% fetal bovine serum. L929 cells were seeded into 96-well plates and maintained in culture for 24 h to form a semi-confluent monolayer. They were then exposed to the test compound over a range of concentrations. After 24 hours exposure, the formazan formation was determined for each treatment concentration and compared to that determined in control cultures. For each treatment the percentage inhibition of growth is calculated.

Under the conditions of this study, the MEM extracts of test article would be considered no cytotoxicity potential. The negative controls, blank controls, and the positive controls performed as anticipated.

Approved by: _____ 12/27/2018

Shixia Wang, Study Director Date

GLP STATEMENT

This nonclinical laboratory study was conducted in accordance with the United States Food and Drug Administration Good Laboratory Practice Regulations, 21 CFR Part 58.

There was no deviation to the protocol or provisions of GLP Regulation noted during the course of the study.

Approved by: _____ 12/27/2018

Shixia Wang, Study Director — Date

1. Generals

1.1 Purpose

The purpose of this study was to evaluate the cytotoxicity of a test article extract.

1.2 Guidelines

This study was conducted based on the following documents:

1) ISO 10993-1, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process (2018).
2) ISO 10993-5, Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity (2009).
3) ISO 10993-12, Biological evaluation of medical devices - Part 12: Sample preparation and reference materials (2012).

1.3 Compliance

This study was conducted in accordance with:

1) Code of Federal Regulations (CFR), Title 21, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.
2) ISO 17025 - General requirements for the competence of testing and calibration laboratories (2005).

1.4 Dates

Test Article Received:	11/07/2018
Test Initiated:	11/26/2018
Observations Concluded:	11/28/2018

2. Materials

Test Article	Hemostatic Xerogel Sponge
Model	XLJ-I
Manufacturer	Same as sponsor
Identification Number	Not provided
Status	Sterile
Physical Description	White, Flake sponge, Solid
Composition	Chitosan, Sodium polyacrylate, Polyethylene glycol
Stability	Stability was determined by and on file with the sponsor.
Expiration Date (or Shelf Life)	Two years
Strength	Not applicable, no active ingredient
Purity	Not applicable, no active ingredient
Storage Condition	Room Temperature
Note	Information regarding the test article characterization was provided by sponsor in the Sample Submission Form.
Extraction Vehicle	Minimum Essential Medium (MEM)
Blank Control	
Manufacturer	Sigma-Aldrich. Inc
Lot Number	SLBV3954

Stability	Marketed product, stability is characterized by its labelling
Composition, Strength, Purity or other characteristics	Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum, 100IU/ml penicillin and 100μg/ml streptomycin
Storage Condition	2-8°C
Negative Control Article	High density polyethylene (HDPE)
Manufacturer	Kunshan Fei Yao Plastic Products Co., Ltd.
Lot Number	20180619
Stability	Marketed product, stability is characterized by its labelling
Composition, Strength, Purity or other characteristics	Not applicable, no active ingredient
Storage Condition	Room Temperature
Positive Control Article	Latex gloves
Manufacturer	TG MEDICAL SDN. BHD.
Lot Number	5112004347
Stability	Marketed product, stability is characterized by its labelling
Composition, Strength, Purity or other characteristics	Natural rubber latex
Storage Condition	Room Temperature
Reagent	MTT
Manufacturer	Beijing Solarbio Science & Technology Co., Ltd.
Lot Number	829Z056
Preparation	MTT was added into MEM without supplements and without phenol red at a concentration of 1 mg/ml. Solution was sterilized by sterile filtration using syringe filters (pore size ≤ 0.22 μm).

Sample Preparation

Prior to the extraction, test article was removed from the package, and was covered in 32ml extraction vehicle absorb to saturation. Then the test article was covered in the extraction.

Extraction Procedure

The test article, negative control, positive control and the control blank were subjected to the extraction conditions as described below. The extracts were continuously agitated during extraction.

Group	Test Article	Negative Control	Positive Control	Blank Control
Extraction Ratio	0.1 g: 1ml	60cm^2: 20ml	120cm^2: 20ml	/
Sample Amount	3.0 g	75 cm^2	120 cm^2	/
Extraction Vehicle Volume	30.0 ml	25 ml	20 ml	20 ml
Extraction Condition		37°C 24 hours		
Condition of Extracts	Clear	Clear	Clear	Clear
	No Particulate	No Particulate	No Particulate	No Particulate

Note: All extracts were not centrifuged, filtered or otherwise altered prior to testing. It was tested immediately after extraction.

Note: The MEM extraction method was conducted in the presence of serum to optimize extraction of both polar and non-polar components.

3. Test Systems

3.1 Test System and Justification

Mammalian cell culture monolayer consisting of L-929 mouse fibroblast cells was used. In vitro mammalian cell culture studies have been used historically to evaluate cytotoxicity of biomaterials and medical devices.

3.2 Test System Management

L-929 mouse fibroblast cells were propagated and maintained in culture flasks containing MEM at 37°C with 5% carbon dioxide (CO_2). For this study, 25 cm^2 culture flasks were seeded, labeled with passage number and date, and incubated at 37°C in 5% CO_2 to obtain subconfluent monolayers of cells prior to use. Aseptic procedures will be used in the handling of the cell cultures following approved MID-LINK Standard Operating Procedures.

4. Method

After thawing from stock, the cells were passaged two to three times before using in the test. Cell cultures were removed from culture bottles by enzymatic digestion (trypsin/EDTA) and the cell suspension was centrifuged at 200 G for 3 min. the cells were then resuspended in culture medium and the cell suspension was adjusted at a density of 1×10^5 cells/mL. Using a multichannel pipette, dispense 100 µL culture medium only (blank) into the peripheral wells of a 96-well tissue culture microtiter plate. In the remaining wells, 100 µL of a cell suspension of 1×10^5 cells/mL were dispensed. The cells were incubated for 24 hours (5% CO_2, 37 °C, > 90% humidity) so that cells from a half confluent monolayer. The plate was examined under microscope to ensure that cell growth was relatively even across the microtiter plate.

After 24 h incubation, the culture medium was aspirated from the cells. Per well, 100 µL of treatment medium containing either the appropriate concentration of sample extract or the negatives control, or the positive control, or blank control were added. The cells then were incubated for 24 h (5% CO_2, 37 °C, > 90% humidity).

After 24 h treatment, the plate was examined under a phase contrast microscope to identify systematic cell seeding errors and growth characteristics of control and treated cells. After the examination of the plates, the culture medium was carefully removed from the plates. Subsequently, 50 µL of MTT solution was added to each test well and the plate was further incubated for 2 h in the incubator at 37 °C. Then the MTT solution was discarded and 100 µL of isopropanol was added in each well. This plate was swayed and subsequently transferred to a microplate reader equipped with a 570 nm filter to read the absorbance (reference wavelength 650 nm).

5. Evaluation and Statistical Analysis

A decrease in number of living cells results in a decrease in the metabolic activity in the sample. This decrease directly correlated to the amount of blue-violet formazan formed, as monitored by the optical density at 570 nm (reference wavelength 650 nm). Following equation was used to calculate the reduction of viability compared to the blank:

$$\text{Viab\%} = \frac{R}{R0} \times 100, \text{ where}$$

R – The average absorbency reading of testing groups, positive control group and negative control group;

R_0 - The average absorbency reading of blank control group;

Acceptance Criteria

If the viability is reduced to <70% of the blank, it has a cytotoxic potential. The 50% extract of the test sample should have at least the same or a higher viability than the 100% extract; otherwise the test should be repeated.

Quality Control Criteria

The mean OD_{570} of blanks shall be ≥ 0.2. The mean of the blanks shall not differ by more than 15% from the mean of all blanks.

6. Results

See Attachment 2: Results.

7. Conclusion

Under the conditions of this study, the MEM test extracts would be considered no cytotoxicity potential. The negative controls, blank controls, and the positive controls performed as anticipated.

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other articles is the sponsor's responsibility.

8. Records

All raw data pertaining to this study and a copy of final report will be retained in designated Mid-Link's archive files in accordance with Mid-Link SOP.

Statement of Quality Assurance Activities

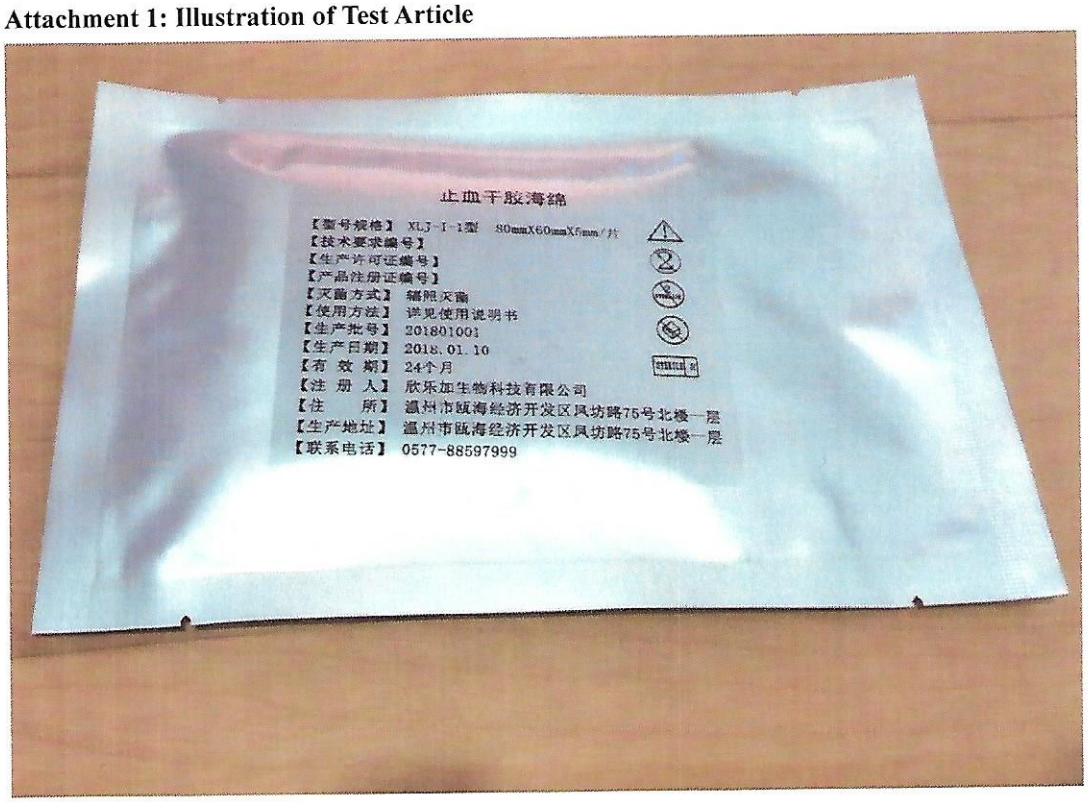
Phase Inspected	Date Inspected
Observation	11/28/2018
Study Data Review	11/28/2018
Final Report Review	12/07/2018

Based on a review of this study, it has been concluded that this report accurately describes the methods and standard operating procedures, and that the reported results accurately reflect the raw data of the study. This study has been reviewed in accordance with the provisions of the FDA Good Laboratory Practice Regulations (21 CFR, part 58). Results are included in the Periodic Status Report to Management and Study Director.

QA Representative 刘婷婷 12/27/2018

Authorized Signature Date

Attachment 1: Illustration of Test Article



Attachment 2: Results

Table 1 Results (Microscopic Observation)

Group	24 Hours	48 Hours
Blank Control 1	Normal/ Even	Normal/ Even
Blank Control 2	Normal/ Even	Normal/ Even
Test Extract 100%	Normal/ Even	Normal/ Even
Test Extract 75%	Normal/ Even	Normal/ Even
Test Extract 50%	Normal/ Even	Normal/ Even
Test Extract 25%	Normal/ Even	Normal/ Even
Positive Control	Normal/ Even	Abnormal / Lysis
Negative Control	Normal/ Even	Normal/ Even

Table A2 OD Value Results

No.	Blank Control 1	Blank Control 2	Test Extracts				Positive Control	Negative Control
			100%	75%	50%	25%		
1	0.440	0.445	0.378	0.417	0.403	0.438	0.002	0.438
2	0.482	0.509	0.418	0.359	0.396	0.441	0.005	0.459
3	0.468	0.462	0.382	0.408	0.402	0.489	0.005	0.442
4	0.487	0.483	0.364	0.406	0.440	0.449	0.006	0.459
5	0.453	0.467	0.391	0.395	0.432	0.448	0.005	0.438
6	0.459	0.476	0.424	0.401	0.420	0.453	0.003	0.422
Mean	0.465	0.474	0.393	0.398	0.416	0.453	0.004	0.443
	0.469							
Viab. (%)	99.06%	100.94%	83.72%	84.75%	88.55%	96.54%	0.92%	94.41%

The 50% extract of the test sample showed higher viability compared with that100% extract. The meanOD_{570} of blanks were ≥ 0.2. The mean of the blanks did not differ by more than 15% from the mean of all blanks. The results met all criteria of quality control. The positive control extract had cytotoxicity. While the negative control and test sample extracts did not show cytotoxic potential.