MedGaea Life Sciences Institute

Report NO.
RE25627CY01-1

In Vitro Cytotoxicity Test
TPU/70D Nylon Fabric

Study Report

GLP Compliance
Registered No. 0010

MedGaea Life Sciences Institute

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TPU/70D Nylon Fabric

The study complied with the Taiwan FDA GLP (2019), OECD-GLP (ENV/MC/CHEM(98)17, 1997) and U.S. FDA (21 CFR Part 58, 2024) on principles of Good Laboratory Practice for Nonclinical Laboratory Studies. The study was performed in accordance with the agreed protocol and described in this report providing a true and accurate record of the results. The test article characteristics and its related properties in vehicle including purity, concentration, stability and homogeneity are provided and confirmed by the sponsor, and are excluded from GLP.

Kai-Li Tseng

Kai-Li Tseng Ph.D.
Study Director

04/15/2026

Date

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QUALITY ASSURANCE STATEMENT

In compliance with the Taiwan FDA, OECD and U.S. FDA (21 CFR Part 58) "Good Laboratory Practice for Nonclinical Laboratory Studies", the Quality Assurance Unit conducted study-based type of audits on facility, equipment, personnel, test methods, raw data, and records regularly.

The study report had been reviewed and presented for internal approval. All parts of the experimentation involved were conducted according to the protocol. All original records, raw data, and documentations were truthfully transferred and addressed in the results of this report.

Inspection record：

Inspection Date	Inspection Phase	Date of Reports to	
		Study Director	Management
07/09/2025	Protocol	07/09/2025	07/09/2025
07/14/2025	Test article extraction and cell culture	07/14/2025	07/14/2025
07/15/2025	Test article treatment	07/15/2025	07/15/2025
07/16/2025	MTT assay	07/16/2025	07/16/2025
07/22/2025	Raw data	07/22/2025	07/22/2025
07/22/2025	Study report draft	07/22/2025	07/22/2025
04/14/2026	Study report amendment	04/14/2026	04/14/2026

Yi-Jie Liao

Quality Assurance Unit in Charge

04/15/2026
Date

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Study Director: Kai-Li Tseng
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Sponsor: JIUNDAR INDUSTRIAL CO., LTD.
Address: No. 9, Aly. 2, Ln. 252, Sanjun St., Shulin Dist., New Taipei City 23865, Taiwan

Study Announcement

The study report is valid for the test article used only.
The study report could not be recopied or extracted without the permission from MedGaea Life Sciences Ltd.
The study report is invalid without endorsement of MedGaea Life Sciences Ltd.

Protocol Number: PE25627CY01

Experimentation Staff

Test article preparation: Shih-Cheng Li, Tzu-Ying Cheng
Administration: Shih-Cheng Li, Tzu-Ying Cheng
MTT assay: Shih-Cheng Li, Tzu-Ying Cheng
Cell morphological grading: Shih-Cheng Li, Tzu-Ying Cheng
Quality assurance unit in charge: Yi-Jie Liao, Hao-Syuan Yu

Study Period

Experimental starting: 07/14/2025
Culturing of cell: 07/07/2025~07/14/2025
Cell quantity: 07/14/2025
Extraction of the test article: 07/14/2025~07/15/2025
Test article treatment: 07/15/2025~07/16/2025
Qualitative and quantitative (MTT assay) determination: 07/16/2025
Experimental completion: 07/21/2025

CONTENTS

SUMMARY

An *in vitro* biocompatibility study, based on the International Organization for Standardization 10993: Biological evaluation of medical devices — part 5: Tests for *in vitro* cytotoxicity (2009), was conducted on the test article "TPU/70D Nylon Fabric" to determine the potential cytotoxicity. The test article was extracted at 37 ± 1°C for 24 ± 2 hours in MEM medium with serum. After extraction, the test article extract was diluted with complete culture medium, and then four different concentrations of extract (100%, 50%, 25% and 12.5%) were subsequently applied to mouse lung fibroblast (L-929) cells in triplicate. After 24 hours of incubation, the morphological changes of L-929 cells were observed by microscope and the quantitative evaluations of cell viability were performed by MTT assay.

Under the condition of this study, the positive control was considered cytotoxic and the negative control was considered non-cytotoxic. Thus, the test was considered valid. The microscopic observation revealed that all the test article extracts did not induce cell morphological changes. Furthermore, the quantitative assay of all test article extracts showed no cell cytotoxicity. Therefore, the test article was considered non-cytotoxic and met the requirements of the ISO 10993-5:2009 guideline.

INTRODUCTION

Testing was performed following the requirements of the International Organization for Standardization 10993: Biological Evaluation of Medical Devices part 5: Tests for *in vitro* cytotoxicity (2009) and part 12: Sample Preparation and Reference Materials (2021). The test article "TPU/70D Nylon Fabric" was extracted at 37 ± 1°C for 24 ± 2 hours in complete culture medium. The *in vitro* cytotoxicity was performed in mouse lung fibroblast (L-929) cells by MTT assay. Morphology and viability of L-929 cells after 24 hours of incubation with the test article extract were recorded.

MATERIALS AND METHODS

1 Test Article Information
- 1.1 Name: TPU/70D Nylon Fabric
- 1.2 Batch/Lot No.: J685-70-F
- 1.3 Package: Bag
- 1.4 External features: Flat
- 1.5 Color: Dark Blue
- 1.6 Major components: TPU/70D Nylon Fabric
- 1.7 Storage condition: Room temperature
- 1.8 Receiving date: 07/04/2025
- 1.9 Expiration date: 07/01/2030

The above information was enclosed in Appendix 4.

2 Cell Line

Mouse lung fibroblast cells (L-929 cell line) (NCTC Clone 929, BCRC No.: RM 60091, Food Industry Research and Development Institute of Bioresource Collection and Research Center).

3 Materials
- 3.1 Chemicals
  - 3.1.1 Fetal bovine serum (Hyclone, Lot No. AK30770730)
  - 3.1.2 MEM (Eagle's minimum essential medium, GIBCO, Lot No. 2766673, 3066277)
  - 3.1.3 Sodium bicarbonate (SIGMA-ALDRICH, Lot No. BCCL7573)
  - 3.1.4 Penicillin/Streptomycin (GIBCO, Lot No. 242480)
  - 3.1.5 Sodium pyruvate (SIGMA-ALDRICH, Lot No. RNBM6678)
  - 3.1.6 MTT(3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide, thiazolyl blue, SIGMA-ALDRICH, Lot No. MKCR1832)
  - 3.1.7 Isopropanol (Honeywell, Lot No. M318A)
- 3.2 Culture medium (SOP: MG-CUS-002)

90% MEM medium with 2.0 mM L-glutamine, 2.2 g/L sodium bicarbonate, 0.11 g/L sodium pyruvate, 100 U penicillin/100 μg/mL streptomycin and 10% fetal bovine serum.

3.3 Controls
- 3.3.1 Negative control: High density polyethylene film (Hatano Research Institute, FDSC, Lot No. C-231)
- 3.3.2 Positive control: ZDEC Polyurethane film (Hatano Research Institute, FDSC, Lot No. A-231K)
- 3.3.3 Extraction solvent: MEM medium with 10% serum

4 Methods (ISO 10993-5:2009; SOP: MG-QP-1809, MG-QP-1803)

4.1 Sample preparation and group design

4.1.1 Test article: The test article "TPU/70D Nylon Fabric" has a thickness of 0.22 mm (provided by sponsor). According to the ISO 10993-12:2021, the extraction ratio of the test article is determined as 6 cm^2/mL. The extraction was carried out at 37 ± 1°C for 24 ± 2 hours with constant agitation. The test article extract was pinkish and clear and then the extract was diluted with complete culture medium to four different concentrations (100%, 50%, 25% and 12.5%). After dilution, the test article extracts were directly and immediately used in this study without centrifugation, filtering, adjusting pH and any additional processing or storage.

4.1.2 Negative control: The thickness of negative control material, High Density Polyethylene Film, was measured as 0.38 mm (thickness < 0.5 mm) by micrometer (Mitutoyo Corporation, BMD-25, JAPAN, SOP: MG-EQS-021). According to the ISO 10993-12:2021, the extraction ratio was determined as 6 cm^2/mL. 60 cm^2 of negative control was extracted by 10.0 mL of culture medium with serum. The extract condition was the same as test article.

4.1.3 Positive control: The thickness of positive control material, ZDEC Polyurethane Film, was measured as 0.40 mm (thickness < 0.5 mm) by micrometer. According to the ISO 10993-12:2021, the extraction ratio was determined as 6 cm^2/mL. 30 cm^2 of positive control was extracted by 5.0 mL of culture medium with serum. The extract condition was the same as the test article.

4.1.4 Extraction solvent: 10.0 mL of medium with serum was used and extract condition was performed in the same way as the test article.

4.2 Cell culture (SOP: MG-CUS-004)

L-929 cells were propagated and maintained at 37°C with 5% CO_2 in an open flask containing culture medium (contained 10% fetal bovine serum). Passage number: p=n+9. For qualitative assay, 1×10^5 cells were seeded in each well of 24-well plate. For quantitative assay, 1×10^4 cells were seeded into each well of 96-well plate.

5 Experimental Procedure

After an overnight culture, triplicate culture wells which contained a confluent cell monolayer were subjected to qualitative and quantitative assays. The culture medium was removed, and 1 mL (qualitative assay) and 100 μL (quantitative assay) of extraction solvent, extracts from the test article, negative and positive control materials were added to each well. Cells were incubated at 37°C with 5% CO_2 for 24 hours. The cytotoxic effect was evaluated microscopically and the cell viability was measured by MTT assay.

6 Assay

6.1 Qualitative assay: After 24 hours incubation, the confluence of the cell monolayer and

changes of cell morphology were observed under microscope (Appendix 3). (SOP: MG-EQS-026)

6.2 Quantitative assay:

6.2.1 After 24 hours incubation, the culture medium was removed, and then the MTT working solution was added into each well (1.0 mg/mL in culture medium) and incubated at 37°C with 5% CO_2 for 2 ± 0.5 hours.

6.2.2 At the end of the incubation period, removed the MTT working solution and added 100 μL of isopropanol to each well.

6.2.3 Pipetting each culture well to completely dissolve the formazan.

6.2.4 Swayed the plate and then the value of (OD_{570} − OD_{650} nm) was measured by using Microplate Absorbance Reader (Eon Microplate Spectrophotometer, BioTek Instruments, Inc., USA, SOP: MG-EQS-109).

6.2.5 MTT assay

The cell viability was calculated as follows:

$$\text{Cell viability (\%)} = 100 \times \frac{\text{Mean } (OD_{570} - OD_{650} \text{ nm}) \text{ of sample}}{\text{Mean } (OD_{570} - OD_{650} \text{ nm}) \text{ of extraction solvent}}$$

6.2.6 If viability is reduced to < 70% of the blank, indicating the test article has a cytotoxic potential.

DATA MANAGEMENT

Qualitative analysis will be evaluated according to Appendix 3. The achievement of a numerical grade greater than 2 is considered a cytotoxic effect. The quantitative analysis is assessed according to cell viability, when cell viability is reduced to < 70% of the blank, the test article is considered to be cytotoxic.

The cytotoxicity test is fulfilled for a valid test as follows: (1) Each positive control article replicate in qualitative analysis is ≧ Grade 3. (2) Each negative control article replicate in qualitative analysis is Grade 0. (3) Each vehicle control replicate in qualitative analysis is Grade 0.

AMENDMENTS / DEVIATIONS

Study report amendments and deviations:

This study was conducted in accordance with the approved protocol, with the exception of changes to the test article name and major components. At the request of the sponsor, the test article name and major components were changed from “TPU/70D Nylon Facbric” to “TPU/70D Nylon Fabric”. This change had no impact on the conduct or performance of the study, and did not affect the study results.

ARCHIVING

All the study-related raw data, documents, records, test article, study protocol, study report and specimens are kept at MedGaea Life Sciences Institute archives D-4 and 106 for a defined period. During this period, agents authorized by the sponsor can apply to review the records.

RESULTS

In qualitative assay, the morphological changes of L-929 cells were examined after treatment (Table 1 and Appendix 1). The criteria used for grading is described in Appendix 3. Cells exhibited normal morphology in the negative control group. Under this experimental condition, negative control was graded as 0, while positive control was graded as 4. The results revealed that all the test article extracts did not induce cell morphological changes and were graded as 0.

In quantitative assay, the cell viability was determined by MTT assay (Table 2 and Appendix 2). After 24 hours of incubation, cell viability in negative control, 100%, 50%, 25% and 12.5% test article extract groups were 94.61%, 99.31%, 100.00%, 100.00% and 100.00%, respectively. Consequently, under these experimental conditions, no cytotoxic effects were observed in cells treated with any of the test article extract.

On the basis of afore-said, there was no evidence of cytotoxicity induced by the test article. Therefore, the test article “TPU/70D Nylon Fabric” met the criteria of biocompatibility guided by ISO 10993-5:2009.

CONCLUSION

Under the conditions of this study, based on ISO 10993-5:2009 “Tests for *in vitro* cytotoxicity” guideline, the test article showed no evidence of causing cytotoxicity. Therefore, the test article “TPU/70D Nylon Fabric” met the criteria of biocompatibility guided by international standards, ISO 10993-5:2009.

REFERENCES

1. Biological evaluation of medical device－Part 5: Tests for *in vitro* cytotoxicity. ISO 10993 (2009) International Standards Organization.

2. Biological evaluation of medical device － Part 12: Sample preparation and reference materials. ISO 10993 (2021) International Standards Organization.

3. Good Laboratory Practice for Nonclinical Laboratory Studies. Title 21 of the U.S. Code of Federal Regulations, Part 58 (2024) United States Food and Drug Administration.

4. Good Laboratory Practice for Nonclinical Laboratory Studies. (2019) Taiwan Food and Drug Administration.

5. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. Journal of immunological methods 65 (1-2): 55-63

6. Principles on Good Laboratory Practice. (1997) Organization for Economic Cooperation and Development.

7. Biological reactivity tests, *in vitro*. In: United States pharmacopeia-National Formulary (USP-NF) <87>, (2024), Issue 3.

8. Biocompatibility Testing of Medical Devices - Standards Specific Information for the Accreditation Scheme for Conformity Assessment (ASCA) Pilot Program (2020).

Table 1. Morphological Grading of Cytotoxicity

Groups	Confluent monolayer[#]	Reactivity
Extraction solvent	0	None
Test article (100%)	0	None
Test article (50%)	0	None
Test article (25%)	0	None
Test article (12.5%)	0	None
Negative control	0	None
Positive control	4	Severe

[#] Biological reactivity was evaluated according to Appendix 3.

Table 2. MTT Assay Results

Groups	Cell viability (%)[#]	Cytotoxicity
Extraction solvent	100.00	No
Test article (100%)	99.31	No
Test article (50%)	100.00	No
Test article (25%)	100.00	No
Test article (12.5%)	100.00	No
Negative control	94.61	No
Positive control	1.41	Yes

Remark: The maximum cell viability is 100.00%.

$$^{\#}\text{Cell viability (\%)} = 100 \times \frac{\text{Mean } (OD_{570} - OD_{650}\text{ nm}) \text{ of sample}}{\text{Mean } (OD_{570} - OD_{650}\text{ nm}) \text{ of extraction solvent}}$$

Appendix 1. Morphological Grading of Each Culture

Groups	Cellular morphology (Grade 0-4)[#]			
	1	2	3	Mean
Extraction solvent	0	0	0	0
Test article (100%)	0	0	0	0
Test article (50%)	0	0	0	0
Test article (25%)	0	0	0	0
Test article (12.5%)	0	0	0	0
Negative control	0	0	0	0
Positive control	4	4	4	4

[#] Biological reactivity was evaluated according to Appendix 3.

Appendix 2. MTT Assay Result of Each Culture

Groups	MTT assay (OD_{570} - OD_{650} nm)				Cell viability[#]
	1	2	3	Mean	Percentage (%)
Extraction solvent	0.843	0.904	0.867	0.8713	100.00
Test article (100%)	0.818	0.874	0.904	0.8653	99.31
Test article (50%)	0.883	0.931	0.929	0.9143	100.00
Test article (25%)	0.901	0.950	0.928	0.9263	100.00
Test article (12.5%)	0.976	0.950	0.943	0.9563	100.00
Negative control	0.806	0.826	0.841	0.8243	94.61
Positive control	0.014	0.011	0.012	0.0123	1.41

Remark: The maximum cell viability is 100.00%.

$$^{\#}\text{Cell viability (\%)} = 100 \times \frac{\text{Mean } (OD_{570} - OD_{650} \text{ nm}) \text{ of sample}}{\text{Mean } (OD_{570} - OD_{650} \text{ nm}) \text{ of extraction solvent}}$$

Appendix 3. Scoring Criteria for Cytotoxicity Test

For the suitability of the system to be confirmed, the negative controls must have been a grade of 0 (reactivity none) and the positive controls must have been a grade of 3 or 4. The test article passes the test if qualitative morphological grading showed no greater than a grade of 2 (mild reactivity). The test would have been replaced if the controls did not perform as anticipated.

Qualitative morphological grading of cytotoxicity of extracts

Grade	Reactivity	Conditions of all cultures
0	None	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
1	Slight	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; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cells are round, devoid of intracytoplasmatic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

Appendix 4. Test Article Information Sheet

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INFORMATION FOR
■TEST ARTICLE/☐SAMPLE/SPECIMEN
☐CONTROL ARTICLE SHEET

管制文件章 品保室(麥)

Medical device

Sponsor's company name	JIUNDAR INDUSTRIAL CO., LTD.
Sponsor's address	No. 9, Aly. 2, Ln. 252, Sanjun St., Shulin Dist., New Taipei City 23865, Taiwan
Test article name	TPU/70D Nylon Fabric
Package	■Bag ☐Tube ☐Box ☐Bottle ☐Other:
External features	☐Irregular ☐Liquid ☐Powder ■Flat ☐Membrane ☐Tube ☐Other:
Color	Dark Blue
Major components	TPU/70D Nylon Fabric
Storage condition	■Room temperature ☐Refrigerated ☐Frozen
Expiration date	☐Labeled expiry date: MM/DD/YYYY ■Minimum validity for testing: 07/01/2030
Batch/Lot No.	J685-70-F
Surface area of test article	**Surface area per test article__-__cm²** **Thickness: 0.22 mm (Thickness < 0.5mm or ≧ 0.5mm)** *Please provide the surface area if applicable. (This area includes the combined area of all tissue contacting surfaces of the test article.)
Remark/Requirement	
Sponsor Signature	麥玉致 2026.3.27.

Note:

1. Please indicate" - "where not applicable.
2. MDG is not responsible and liable for any test article properties. The test article information listed above is provided and confirmed by the sponsor. Sponsor is take fully responsibility on the test article chemical and physical characteristics, and it's related analyses information.
3. Target Regulatory Authority(ies) (multiple selections allowed):

   ■TFDA ☐US FDA ☐CE Marking ☐NMPA ☐Others:

MG-QR-2201 10

Appendix 4. Test Article Information Sheet (Cont'd)