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苏州大学
卫生与环境技术研究所
最终报告

报告编号: SDWH-M201702770-1

参照 ISO 10993-5：2009 方法进行

泡棉海绵胶的细胞毒性试验

MTT 法

含 10%胎牛血清的 MEM 浸提液

委托单位

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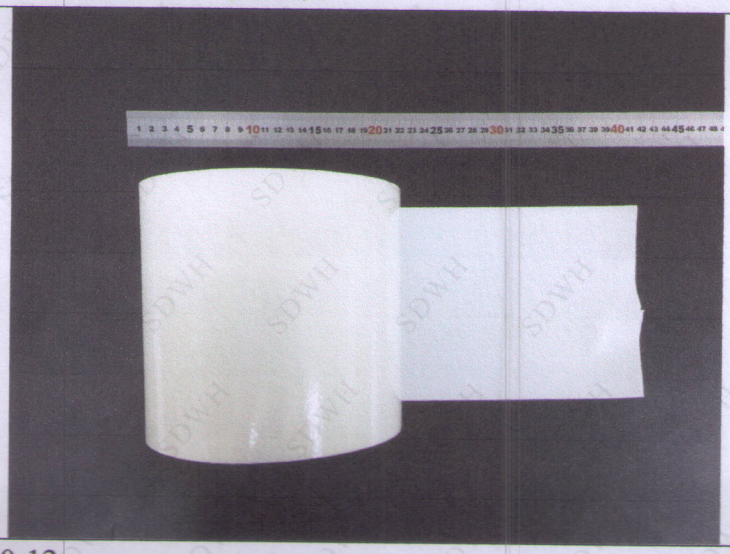
目 录

试验确认与签名

试验样品	
接样日期：	2017-10-12
试验计划书编号：	SDWH-PROTOCOL-M201702770-1
试验计划书生效日期：	2017-10-24
试验操作开始日期：	2017-10-30
试验操作结束日期：	2017-11-01
报告完成日期：	2017-11-03

编制：秦萍萍 2017-11-03 日期

审核：朱雨婷 试验负责人 2017-11-03 日期

签发：[illegible] 授权签字人 2017-11-03 日期

苏州大学卫生与环境技术研究所

1.0 摘　要

试验样品浸提液与生长旺盛的 L929 细胞培养（37℃ ,5% CO_2）24h 后，观察细胞形态，细胞裂解情况，采用 MTT 法测定供试品的潜在细胞毒性。结果显示 100%样品浸提液的细胞活力为 70.2%，对照组结果显示本次试验结果有效。

在本次试验条件下，样品泡棉海绵胶浸提液对 L929 细胞无潜在毒性影响。

2.0 目　的

该试验目的是为了评价试验样品对 L929 哺乳动物成纤维细胞的生物学反应。该测试是根据样品浸提液而设计的。

3.0 参考标准

医疗器械的生物学评价-第 5 部分：细胞毒性测试-体外法 ISO 10993-5：2009
医疗器械的生物学评价-第 12 部分：样品制备和参照样品 ISO 10993-12：2012

4.0 执行规范

ISO/IEC 17025:2005《检测和校准实验室能力的通用要求》CNAS-CL01 检测和校准实验室能力认可准则（中国合格评定国家认可委员会实验室认可证书 No.CNAS L2954）
实验室资质认定评审准则（江苏省质量技术监督局资质认定计量认证证书 CMA 151000100270）

5.0 对照和试验样品确定

5.1 试验样品名称：泡棉海绵胶
来样原始状态：未灭菌
CAS 编号：未提供
型号：未提供
规格：未提供
批号：H20170612001
样品材料：PE 泡棉
包装材质：未提供
性状：固体
颜色：未提供
密度：未提供
稳定性：未提供
溶解度：未提供
保存条件：室温
以上试验样品信息是由样品委托单位提供。
浸提液：含 10%胎牛血清的 MEM 培养液

5.2 阴性对照
名称：高密度聚乙烯
制造商：美国药典委员会
规格： 3 片装
批号：K0M357

性状：固体
颜色：白色
稳定性：室温下稳定
保存条件：室温
浸提液：含 10%胎牛血清的 MEM 培养液

5.3 阳性对照样品名称：Zinc diethyldithiocarbamate
制造商：Sigma
规格：25g
批号：MKBD516V
浓度：1%
溶剂：10%胎牛血清的 MEM 培养液
配制日期：2017-10-30
性状：固体
颜色：白色
保存条件：4±2 ℃

5.4 空白对照样品名称：含 10%胎牛血清的 MEM 培养液
配制日期：2017-10-30
性状：液体
颜色：粉红色
保存条件：4±2 ℃

6.0 试验系统鉴别

该试验用小鼠成纤维细胞 L929,细胞系来自美国菌种保存中心。

7.0 试验系统确认

小鼠成纤维细胞 L929 用来检测细胞毒性试验是因为其对试验样品浸提液反应灵敏。

8.0 给药途径确认

试验样品通过浸提液（用一种与试验系统相容的载体浸提）与试验系统接触，被认为是最佳给药途径，也是为标准推荐的。

9.0 试验设计

9.1 试验和对照样品制备

无菌操作按下表的比例（样品：浸提液体积）用含 10%胎牛血清的 MEM 培养液浸提样品，于 37℃浸提 24 小时。浸提前后浸提液状态未发生改变。浸提液立即用于实验。浸提液 pH 值未经调整，未经过滤，离心，稀释等处理过程。

无菌操作取样		灭菌方式	惰性容器内无菌浸提			最终浸提液	
取样方式	实际取样	Autoclave (121℃, 30min)	取样比例	浸提液	条件	pH	是否澄清
随机取样	60cm²		3cm² : 1ml	20.0ml	37℃，24h	7.4	澄清

同法制备空白对照、阴性对照样品和阳性对照样品。

9.2 仪器设备

高压灭菌器（SDWH2097）校正有效期（2017-11-23）；
CO_2 培养箱（SDWH021）校正有效期（2018-08-31）；
恒温摇床（SDWH2109）校正有效期（2017-11-23）
倒置显微镜（SDWH037）校正有效期（2018-08-28）；
钢直尺（SDWH463）校正有效期（2018-09-10）；
电子天平（SDWH056）校正有效期（2018-02-13）；
超净工作台（SDWH454）校正有效期（2018-09-04）；
酶联免疫检测仪（SDWH312）校正有效期（2018-08-27）。

9.3 试剂

MTT(3-（4，5-二甲基噻唑-2）-2，5-二苯基四氮唑溴盐)（SIGMA，批号：MKBX0151V）；
胎牛血清（CORNING，批号：35076116）；
胰酶（GiBco，批号：1780400）；
青霉素链霉素（GiBco，批号：1665735）；
MEM（HyClone，批号：AC10232463）；
异丙醇（江苏强盛功能化学股份有限公司，批号：20170206）。

9.4 试验方法

试验过程无菌操作；

将 L929 细胞培养在含 10%胎牛血清和抗生素（青霉素 100 U/ml,链霉素 100 μg/ml）的 MEM 培养液中，置于 37℃，5% CO_2 培养箱中培养。用 0.25%胰酶（含 EDTA）消化细胞制备成单细胞悬液，细胞悬液离心（200g，3min），然后将细胞重新分散于培养基中，调整细胞密度为 1×10^5 个/ml 的细胞悬液；

接种上述细胞悬液到 1 个 96 孔培养板中，每孔 100 μL，置 37℃培养箱中（5% CO_2，37℃，>90%湿度）培养 24 小时；

待细胞长成单层后，吸出原来的培养液，分别加入 100 μl 不同浓度的试验样品浸提液（100%、75%、50%、25%）、空白对照液、阳性对照（100%）和阴性对照液（100%），37℃，5% CO_2 培养 24 小时。每组做 5 个平行样；

培养 24h 后，取出 96 孔板先做细胞形态学观察，然后吸出原来的培养液，每孔加 50μl MTT（1mg/ml），培养 2 小时，吸弃上清，加 100μl 99.9%纯度的异丙醇溶解结晶；

在酶标仪上以 570nm 为主吸收波长，650nm 为参考波长测定吸光度值。

9.5 细胞形态结果

表 1 细胞形态学观察

组别	接种细胞前	加浸提液前	加浸提液 24h 后
空白对照	个别细胞有颗粒，细胞无裂解，生长状态良好。	个别细胞有颗粒，细胞无裂解，生长状态良好。	个别细胞有颗粒，细胞无裂解，生长状态良好。
阴性对照			个别细胞有颗粒，细胞无裂解，生长状态良好。
阳性对照			细胞裂解死亡。
100%样品浸提液			偶见圆形细胞及有颗粒或细胞形态改变，偶见裂解细胞，仅轻微的生长抑制。
75%样品浸提液			个别细胞有颗粒，细胞无裂解，生长状态良好。
50%样品浸提液			个别细胞有颗粒，细胞无裂解，生长状态良好。
25%样品浸提液			个别细胞有颗粒，细胞无裂解，生长状态良好。

9.6 细胞活力结果

表 2 细胞活力%

组别	Mean±SD	细胞活力%
空白对照	0.3970±0.007	100.0%
阴性对照	0.3900±0.007	98.2%
阳性对照	0.0198±0.013	5.0%
100%样品浸提液	0.2786±0.008	70.2%
75%样品浸提液	0.3478±0.009	87.6%
50%样品浸提液	0.3658±0.007	92.1%
25%样品浸提液	0.3714±0.005	93.6%

9.7 质量检查

阴性对照无细胞毒性影响，阳性对照造成细胞毒性影响。

在两天的试验过程中，未经处理的空白对照的光密度绝对值 OD570 表明了每个孔接种的 1×10^4 个细胞以正常倍增时间成指数增长。

空白对照的 OD570 平均值不能小于 0.2。

检查系统的细胞接种误差，96 孔板的左侧（第 2 列）和右侧（第 11 列）作为空白对照（第 1 列和第 12 列不用作空白对照）。左右两侧空白对照的平均值不能与所有空白的平均值相差超过 15 %。

9.8 统计方法

均数±标准差（Mean±SD）

细胞活力%=试验（或阳性及阴性）样品组[$\overline{OD_{570}-OD_{650}}$]/空白对照组[$\overline{OD_{570}-OD_{650}}$]×100%.

9.9 评价标准

50%的样品浸提液至少和 100%的细胞活力相同或者比 100%的细胞活力更高，否则应该重复试验。
细胞活力%越低，潜在的细胞毒性越大；
细胞活力＜空白组 70%，说明样品具有潜在的细胞毒性；
100%试验样品浸提液的细胞活力%为最终结果。

9.10 结　论

在本次试验条件下，样品泡棉海绵胶浸提液对 L929 细胞无潜在毒性影响。

10.0 记录存储

所有与本次试验有关的原始数据和记录都被保存在指定的 SDWH 档案文件中。

11.0 保密协议

签订检测委托合同即认为双方接受保密协议。

12.0 试验偏离声明

本次试验严格按照方案执行，未发生影响实验数据有效性的偏离。

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Sanitation & Environment Technology Institute, Soochow University, Final Report

Report Number: SDWH-M201702770-1

In Vitro Cytotoxicity Test of
FOAM TAPE
using ISO 10993-5 : 2009 Test Method
MTT Method
MEM with 10%FBS extract

Sponsor

shanghai Rui Quan medical instrument Co.,Ltd

Manufacturer

shanghai Rui Quan medical instrument Co.,Ltd

Sanitation & Environment Technology Institute, Soochow University
Tel: 0512-65880038 Fax: 0512-65880034 Email:sudaweihuan@mail.suda.edu.cn PC:215123
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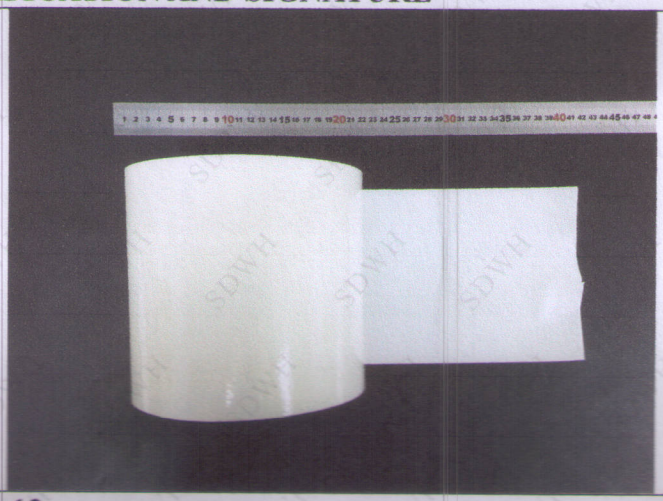
SUPPLEMENTARY EXPLANATION

1. Please apply for rechecking within 15 days of receiving the report if there is any objection.
2. Any erasure or without special testing seal renders the report null and void.
3. The report is only valid when signed by the persons who edited, checked and approved it.
4. The result relate only to the articles tested.
5. The report shall not be reproduced except in full without the written approval of the institute.

STUDY VERIFICATION AND SIGNATURE

Test Article	
Test Article Receipt	2017-10-12
Protocol No	SDWH-PROTOCOL-M201702770-1
Protocol Effective Date	2017-10-24
Technical Initiation Date	2017-10-30
Technical Completion Date	2017-11-01
Final Report Completion Date	2017-11-03

Edited by : Qin Pingping 2017-11-03
Date

Checked by : Zhu Yuting 2017-11-03
Study Director Date

Approved by : Wang Rui 2017-11-03
Authorized signatory Date

Sanitation & Environment Technology Institute, Soochow University

1.0 Study Summary

The test article extract (100, 75, 50, and 25% in growth medium) was added to L-929 cells in 96 well plates and then incubated at 37℃ in 5% CO_2 for 24h to determine the potential cytotoxicity. The MTT method results showed that the cell viability% of the 100 % test article extract was 70.2% and the results of control groups showed the test was valid.
Under the conditions of this study, the test article FOAM TAPE extract did not show potential toxicity to L-929 cells.

2.0 Purpose

The purpose of the test is to determine the biological reactivity of a mammalian cell culture (mouse fibroblast L-929 cells) in response to the test article.

3.0 Reference

Biological evaluation of Medical Devices Part 5: Tests for In Vitro Cytotoxicity (ISO 10993-5：2009)
Biological evaluation of Medical Devices-Part 12: Sample preparation and reference materials (ISO 10993-12：2012)

4.0 Compliance

ISO/IEC 17025:2005 General requirements for the competence of testing and calibration laboratories (CNAS-CL01 Accreditation Criteria for the competence of testing and calibration laboratories)
China National Accreditation Service for Conformity Assessment Laboratory Accreditation Certificate No.CNAS L2954
Accreditation Criteria for the competence of the laboratories (Quality and Technical Bureau of Jiangsu Province Metrology Accreditation Certificate CMA 151000100270)

5.0 Identification of test and control articles

5.1 Test article name: FOAM TAPE
Test article initial state: Not Sterilized
CAS Code: Not supplied by sponsor (N/S)
Model: N/S
Size: N/S
Lot/ Batch: H20170612001
Test Article Material: PE FOAM
Packaging Material: N/S
Physical State: Solid
Color: N/S
Density: N/S
Stability: N/S

Solubility: N/S
Storage Condition: Room Temperature
The information about the test article was supplied by the sponsor wherever applicable.
Extracting solvent: MEM medium, with addition 10% FBS

5.2 Negative Control Article Name: High Density Polyethylene
Manufacturer: U.S. Pharmacopeial Convention (USP)
Size: 3 Strips
Lot/ Batch#: K0M357
Physical State: Solid
Color: White
Stability: Stable at room temperature
Storage Conditions: Room temperature
Extracting solvent: MEM medium, with addition 10% FBS

5.3 Positive Control Article Name: Zinc diethyldithiocarbamate
Manufacturer: Sigma
Size: 25g
Lot/ Batch#: MKBD516V
Concentration: 1%
Solvent: MEM medium, with addition 10% FBS
Date prepared: 2017-10-30
Physical State: Solid
Color: White
Storage Condition: 4 ± 2 ℃

5.4 Blank Control Name: MEM medium, with addition 10% FBS
Date prepared: 2017-10-30
Physical State: Liquid
Color: Pink
Storage Condition: 4 ± 2 ℃

6.0 Identification of test system

L-929 mouse fibroblast cells obtained from ATCC (American Type Culture Collection), USA.

7.0 Justification of the test system

Historically, mouse fibroblast L929 cells have been used for cytotoxicity studies because they demonstrate sensitivity to extractable cytotoxic articles.

8.0 Route of administration

The test article was extracted and administered in vitro to mouse fibroblast L929 cells through a solvent compatible with the test system. This was the optimal route of administration available in this test system as recommended in the guidelines.

9.0 Experiment design

9.1 Sample and Control Preparation

Aseptic extracting the test article (test article to volume of vehicle) by MEM medium(10%FBS) according to the table below.Sealed and incubated at 37℃ for 24h.There is no change in the extraction solvent (pre- and post-extraction).Extracts were used immediately after extraction without the process of pH value adjustment, filtering, centrifugation, dilution, etc.

Aseptic Sampling		Sterilization method	Aseptic Extraction In Inert Container			Final Extract	
Sampling Manner	Actually sampling	Autoclave (121°C, 30min)	Ratio	Extracts	Condition	pH	Clear or Not
Random sampling	60cm^2		3cm^2 : 1ml	20.0ml	37℃，24h	7.4	Clear

The blank control (vehicle), negative and positive controls were similarly prepared.

9.2 Equipment

Autoclaves (SDWH2097),Calibration Expire(2017-11-23),
CO_2 Incubator (SDWH021),Calibration Expire(2018-08-31),
Constant temperature shaking table (SDWH2109),Calibration Expire(2017-11-23),
Inverted microscope (SDWH037),Calibration Expire(2018-08-28),
Steel Straight Scale (SDWH463),Calibration Expire(2018-09-10),
Electronic Balance (SDWH056),Calibration Expire(2018-02-13),
Clean bench (SDWH454),Calibration Expire(2018-09-04),
Power Wave XS Microplate Reader (SDWH312),Calibration Expire(2018-08-27).

9.3 Reagents

MTT
(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyletrazolium bromide)(SIGMA ,Lot No: MKBX0151V）
FBS (CORNING , Lot No: 35076116)
Trypsin (GiBco , Lot No: 1780400)
Penicillin, Streptomycin sulfate (GiBco, Lot No: 1665735)
MEM (HyClone , Lot No: AC10232463)
99.9%Isopropanol (Chinasun Specialty Products Co.,Ltd , Lot No:20170206).

9.4 Test Method

Aseptic procedures were used for handling cell cultures.

L929 cells were cultured in MEM medium (10% FBS, Penicillin 100 U/ml, Streptomycin sulfate 100 μg/ml) at 37℃ in a humidified atmosphere of 5% CO_2, then digested by 0.25% trypsin containing EDTA to get single cell suspension. And obtain a 1×10^5 cells/ml suspension by centrifuging (200g,3min) and re-dispersing in MEM medium finally.

The suspended cells were dispensed at 100μl per well in 96-well plate, and culture it in cell incubator (5% CO_2，37℃，>90%humidity), Cell morphology was evaluated to verify that the monolayer was

satisfactory.

After the cells grew to form a monolayer, original culture medium was discarded. The 96-well plates were then treated with 100μl of extract of test article (100%、75%、50%、25%), control article, negative article (100%) and positive article (100%) respectively. Incubate the 96-well plate at 37℃ in cell incubator of 5% CO_2 for 24 h. Five replicates of each test were tested.

After 24h incubation, observe the cell morphology first and then discard the culture medium. A 50μl aliquot of MTT (1mg/mL) was added to each well and then incubated at 37℃ in a humidified atmosphere of 5% CO_2 for 2 hours. The liquid in each well was tipped out and 100 μl 99.9% isopropanol was added to each well to suspend the cell layer.

Evaluate the suspension above with a dual-wavelength spectrophotometer with the measurement wavelength at 570 nm and reference wavelength at 650 nm.

9.5 Results of Cell Morphology

Table 1 Observation of the Cell morphology

Group	Before inoculation	Before treated with extract	24h after treatment
Blank control	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
Negative control			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
Positive control			Nearly complete or complete destruction of the cell layers.
100% Test article extract			Occasional cells were round and with intracytoplasmatic granules, or showed changes in morphology; occasional lysed cells were present; only slight growth inhibition observable.
75%Test article extract			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
50% Test article extract			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.
25% Test article extract			Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth.

9.6 Results of the Cell Vitality

Table2 Results of the Cell Vitality

Group	Mean±SD	Viability%
Blank control	0.3970±0.007	100.0%
Negative control	0.3900±0.007	98.2%
Positive control	0.0198±0.013	5.0%
100% test article extract	0.2786±0.008	70.2%
75% test article extract	0.3478±0.009	87.6%
50% test article extract	0.3658±0.007	92.1%
25% test article extract	0.3714±0.005	93.6%

9.7 Quality Check

No cytotoxic effect is observed for the negative controls and a cytotoxic effect is elicited by the positive controls.

The absolute value of optical density, OD570, obtained in the untreated blank indicates the 1×10^4 cells seeded per well have grown exponentially with normal doubling time during the two days of the assay.

The mean OD570 of blanks is not less than 0.2.

Check for systematic cell seeding errors, blanks are placed both at the left side (row 2) and the right side (row 11) of the 96-well plate (row 1 and row 12 shall not be used). The left and the right mean of the blanks do not differ by more than 15 % from the mean of all blanks.

9.8 Statistical Method

Mean±standard deviation（Mean±SD）

The Cell Viability % = [$\overline{OD_{570}-OD_{650}}$] of test (or positive and negative) article group/ [$\overline{OD_{570}-OD_{650}}$] of blank control group×100%.

9.9 Evaluation Criteria

The 50 % extract of the test article should have at least the same or a higher viability than the 100 % extract; otherwise the test should be repeated.

The lower the Viab.% value, the higher the cytotoxic potential of the test article is.

If viability is reduced to < 70 % of the blank, it has a cytotoxic potential.

The Viab.% of the 100% extract of the test article is the final result.

9.10 Conclusion

Under the conditions of this study, the test article FOAM TAPE extract did not show potential toxicity to L-929 cells.

10.0 Record Storage

All raw data pertaining to this study and a copy of the final report are retained in designated SDWH archive.

11.0 Confidentiality Agreement

Statements of confidentiality were as agreed upon prior to study initiation.

12.0 Deviation statement

There were no deviations from the approved study protocol which were judged to have any impact on the validity of the data.