证书编号：2018100305S

TESTING
CNAS L2954

Testing Center of Radiological Medical Research Institute, Soochow University

Test Report

Report Number: SDFY-2018-2351

Sample Name: PP SPUNBOND NONWOVEN FABRIC

Testing Item: Biocompatibility Test

Sample Supplier: SHANDONG JOFO NONWOVENS CO., LTD.

Supplementary Explanation

1. If the analysis report requires modification please raise the matter within 15 days of receiving the report, otherwise the request will not be accepted.
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3. The test report is only valid when signed by the persons who analyzed, reviewed and approved it.
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5. The test result is only valid for the sample provided.
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Cytotoxicity Test

Summary

The test article, PP SPUNBOND NONWOVEN FABRIC , were evaluated for cytotoxicity test in accordance with the ISO 10993-5,1999: Tests for in vitro cytotoxicity ASTM F813-07 and USP(87): Biological Reactivity Test, In Vitro-Elution test. The testing sample solution was mixed with growing-well L-929 cell, and then incubated for 24h and 48h at 37℃ in 5% CO_2. Intracytoplasmic granulethe and cell lysis were observed. The result showed that the Reactivity Grade of negative control was 0 grade; the Reactivity Grade of positive control was 4 grade，The cytotoxicity ratio were 83.8%（Reactivity Grade: 1grade）and 92.4% （Reactivity Grade :1 grade）respectively by calculation on the basis of cell concentrations of different groups. This meant that the test was valid and the sample article had no toxicity to L-929 cell.

Date completed: May 23, 2018

Tested by: Checked by:

Introduction

The test article, PP SPUNBOND NONWOVEN FABRIC, were evaluated for cytotoxicity in accordance with 10993-5,1999: Tests for in vitro cytotoxicity ASTM F813-07 and USP (87) : Biological Reactivity Test, In Vitro-Elution test. The purpose of this study was to determine the potential cytotoxicity of the testing article to L-929 cell.

Test system and test system Management

L-929 mammalian fibroblast cell will be grown in RPMI 1640 supplemented with 10% serum. Cells will be seeded into the 96-well cell culture plates,and incubated at 37°C in a humidified incubator with 5% CO_2 to obtain confluent monolayers of cells prior to use. Aseptic procedures will be used in the handling of cell culture.

Personnel: Associates involved were appropriately qualified and trained.

Justification for selection of the test system

Mammalian cell culture monolayer, L-929 mouse fibroblast cells (American Type Culture Collection CCLI (NCTC clone 929), will be used. In vitro mammalian cell culture study has been used to historically evaluate the cytotoxicity of biomaterial of medical device.

Materials

1. Test Sample:

1.1 Sample Name: PP SPUNBOND NONWOVEN FABRIC

1.2 Model: Not supplied

1.3 Lot No: Not supplied

1.4 Receiving Date: May 12 2018

2. Equipment: Autoclaves,CO_2 Incubator, Inverted microscope, Super clean working desk, Refrigerator, culture plate, Enzyme Link Immunosorbent Assay, etc.

3. Reagents: Phenol (Lot No: 20170401), MTT (Lot No: 13241337), Calf Serum (Lot No: 071116), Trypsinase (Lot No: 1983B39), RPMI 1640 (Lot No: 1320625), Penicilline (Lot No: Y0709317), Streptomycin sulfate (Lot No: 071107), DMSO (Lot No: 201710712),etc.

4. Cell Strain: Recommended cell lines are American Type Culture Collection CCLI (NCTC clone 929).

5. Control Preparation:

5.1 Negative control: RPMI 1640 medium, with addition 10% calf serum. （37°C 24h）

5.2 Positive control: RPMI 1640 medium, with addition 10% calf serum and 0.5 % phenol（37°C 24h）.

6. Storage Conditions: Room temperature

Test method

1. Cell Strain: Recommended cell lines are American Type Culture Collection CCLI (NCTC clone 929).

2. .Preparation of sample Extracts

The sterilized sample was put into a culture flask., and serum-free culture medium is then added according to the ratio of $6cm^2$: 1ml ,finally, the flask is sealed,and the sample is extracted at 37°C for 24h to obtain the sample extract.

3. Cell culture

L929 cells were cultured in RPMI 1640 medium, supplemented with 10% calf serum at 37°C in a humidified atmosphere of 5% CO_2. L929 cells were digested by using 0.25% trypsin and the single-cell suspension formed. 7×10^4 cells/mL suspended cells were cultured in 96-well plates with 100μl per well. After the cells were cultured for 24 h, discard the original culture medium, and then add 100μl the sample extraction ,positive control solution and negative control solution to each well respectively.

4. Cell morphological observation and evaluation of cytotoxicity

. After 24h and 48h incubation respectively,take out a 96-well plate for cell morphological observation first,and then add 10μl MTT (5mg/ml) to each well. The cells were further cultured for 4 hours when all liquid in each well was tipped out and100 μl DMSO was added to dissolve the precipitate. Absorbance at 570 nm was determined by ELISA spectro $\bar{A}_{570}$ of negative control group is treated as the 100%,the cell cytotoxicity ratio of the positive control group and sample group are determined by the following formula:

The cell cytotoxicity ratio = $\bar{A}_{570}$ of sample group(or $\bar{A}_{570}$ of positive control group)/ $\bar{A}_{570}$ of negative control group×100%

The morphology evaluation:the reactivity will be graded as following Table 1.

Table 1. Reactivity Grades for Elution Test

Grade	Reactivity	Conditions of all Cultures
0	None	Discrete intracytoplasmic granules; no cell lysis
1	Slight	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Not more than 50% of the cells are round and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	Moderate	Not more than 70% of the cell layers contain rounded cells or are lysed
4	Severe	Nearly complete destruction of the cell layers

According to USP , test articles scoring "0", "1",or "2" will be considered NON-TOXIC. Test articles scoring "3" or "4" will be considered TOXIC. The positive control sample must have a score of "3" or "4" and the negative control sample must have a score of "0" for a valid test.

Results

Results in this experiment are showed Table 2 and Table 3.

Table 2: Descriptive statistics of the cell vitality

24h

	N	Mean	Cell vitality(%)	Maximum	Minimum	Std Deviation
Negative control	5	1.088	100	1.111	1.064	0.021
Positive control	5	0.068	4.8	0.072	0.066	0.002
Sample group	5	0.912	83.8	1.005	0.849	0.065

48h

	N	Mean	Cell vitality(%)	Maximum	Minimum	Std Deviation
Negative control	5	1.512	100	1.561	1.461	0.038
Positive control	5	0.083	5.5	0.093	0.077	0.006
Sample group	5	1.406	92.4	1.484	1.341	0.054

Table 3: the result of the morphology evaluation

Incubate the cultures for 24h			
Group	Conditions of all Cultures	Reactivity	Grade
Negative control	Discrete intracytoplasmic granules; no cell lysis	None	0
Positive control	Nearly complete destruction of the cell layers	Severe	4
sample group	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present	Slight	1
Incubate the cultures for 48h			
Group	Conditions of all Cultures	Reactivity	Grade
Negative control	Discrete intracytoplasmic granules; no cell lysis	None	0
Positive control	Nearly complete destruction of the cell layers	Severe	4
sample group	Not more than 20% of the cells are round, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present	Slight	1

The testing sample solution is mixed with growing-well L-929 cell, and then incubated for 24h and 48h. Observe the morphology and measure the cytotoxicity ratio by MTT method. The cytotoxicity ratio are 83.8% and 92.4% respectively .

Conclusion

In the experiment, the Grade was 4 in positive control and 0 in negative control. So the test is regarded to be valid.

The Grade of sample article was 1. This means the testing sample has no toxicity to L-929 cell.

Delayed Contact Sensitization Study (A Maximization Method) In the Guinea Pig

Summary

A guinea pig maximization test (ISO 10993-10/Amd.1) of sample PP SPUNBOND NONWOVEN FABRIC was conducted to evaluate the potential for delayed dermal contact sensitization. The method of Magusson and Kligman (1970) was adapted for alcohol in a 0.9% sodium chloride USP solution (AS) test article extract.

The AS extract of the test article was intradermally injected and occlusively patched to ten guinea pigs in an attempt to induce sensitization. Following a recovery period, the original ten test and five previously untreated control animals received a challenge patch of the test article extract and the control vehicle. In addition the test article was applied to the same animals. All sites were scored at 24h and 48h after patch removal.

Under the conditions of this study, the AS test article extract and the test article showed no signification evidence of causing delayed dermal contact sensitization in the guinea pig.

Date completed: Jun 7, 2018

Tested by: 5.1.2e Checked by: 5.1.2e

Introduction

A guinea pig maximization test of the material identified below was conducted to evaluate the potential to cause delayed dermal contact sensitization. The test article was received on May 12, 2018. The method of Magnusson and Kligman, as reported in Allergic Contact Dermatitis in the Guinea Pig, 1970, was employed with adaptations for a test article extract. The susceptibility of the Hartley guinea pig strain to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB), has been substantiated at TCRSU with this method under lab number SDFY--2018-2151 completed on Apr 11, 2018.

Materials

The sample provided by the sponsor was identified and handled as follows:

Test Article: PP SPUNBOND NONWOVEN FABRIC

Model: Not supplied

Lot No: Not supplied

Equipment: Incubator

Storage Conditions: Room temperature

Vehicle: alcohol (83.8%)in saline 1:20 solution (AS)

Preparation:

For each phase of this test, a ratio of 6cm^2: 1ml (test article to volume of vehicle) was used for the test extract. The test article was extracted in AS at (70±2) ℃ for (24 ±2) hours. For the challenge phase,

the vehicle (without the test article) was similarly prepared to serve as the control .In addition, the test article (as received) was cut into 2×2cm sections at the challenge phase .

Condition of Extracts:

	TEST	CONTROL
Induction I	clear with test article particulates*	Not applicable
Induction II	clear with test article particulates	Not applicable
Challenge	clear with test article particulates	Clear

*Filtered with a 0.8 μm filter disc to yield a clear particulate free extract

Additional Materials:

Freund's Complete Adjuvant (FCA) was used at induction I, and a 10% (w/w) sodium lauryl sulfate (SLS) suspension in petrolatum was used for induction II. These materials were provided by the test facility.

Method

Test System:

Species: Albino Guinea pig

Source: Provided by Animal Center, TCRSU〈Permit Code: SCXK (SU)〉

Acclimation Period: Minimum 5 days

Number of Animals: 15

Justification of Test System:

The albino guinea pig has been used historically for sensitization studies (Magnusson and Kligman, 1970). The guinea pig is believed to be the most sensitive animal model for this type of study .The susceptibility of the guinea pig to a known sensitizing agent, 1-chloro-2,4-dinitrobenzene (DNCB) has been substantiated at TCRSU with this method .

Animal Management:

Husbandry : Refer to ISO 10993-10 Annex C: Animal and husbandry.

Food: All-nutrient animal food provided by Suzhou (Twin-lion) Experimental Animal Food Science & Technology Service Co .Ltd.

Water: Drinking water met the sanitary standard

Housing: Animals were housed in groups in stainless steel suspended cages identified by a card indicating the lab number, animal numbers, test code, sex, animal code and first treatment date.

Personnel: Associates involved were appropriately qualified and trained.

Selection: Only healthy, previously unused animals were selected.

Intradermal induction phase Ⅰ:

A pair of 0.1ml intradermal injections was made for each of the following, into each animal, at the injection sites (A, B and C) as shown in Figure 1 in the clipped intrascapular region.

Site A: A 50:50 (volume ratio) stable emulsion of Freund's complete adjuvant mixed with the chosen solvent. Physiological saline (equivalent) was used for water-soluble material.

Site B: The test sample (undiluted extract); the control animals were injected with the solvent alone.

Site C: The test sample at the concentration used at site B, emulsified in a 50:50 volume ratio stable emulsion of Freund's complete adjuvant and the solvent (50%) was injected into the control animals with an emulsion of the blank liquid with adjuvant.

Topical induction phase Ⅱ :

Seven days (±1day) after completion of the intradermal induction phase, the test sample was administered by topical application to the intrascapular region of each animal, using a patch of area approximately 8cm^2 (filter paper or absorbent gauze), so as to cover the intradermal injection sites. The concentration selected in Intradermal induction phaseI for Site B was used. The maximum concentration that could be achieved in Intradermal induction phaseIdid not produce irritation, when the area was pretreated with 10% sodium dodecyl sulfate massaged into the skin 24h±2h before the patch was applied. The patches were secured with an occlusive dressing. The dressings and patches were removed after 48h±2h.

The control animals were treated similarly, using the blank liquid alone.

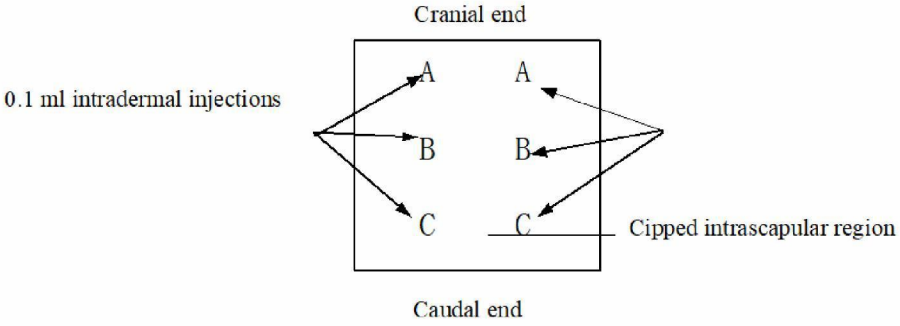

Figure 1-Location of intradermal injection sites

Challenge phase :

At 14 days (±1day) after completion of the topical induction phase, all test and control animals were challenged with the test sample. The test sample and a vehicle control were administered by topical application to sites that were not treated during the induction stage, such as the upper flank of each animal, using appropriate patches or chambers soaked in the test sample at the concentration selected in the intradermal induction phaseI for site C. Dilutions of this concentration were also applied to other untreated sites in a similar manner. Occlusive dressings were used to secure areas treated. The dressings and patches were removed after 24±2h..

Observation of animal:

The appearance of the challenge skin sites of the test and control animal were observed after 24h and 48h removal of the dressings. Natural or full-spectrum was used to visualize the skin reactions.

The skin reactions for erythema and oedema were observed and graded according to the Magnusson and

Animal Number/ Group	Topical induction phase II	Hours following patch removal（h）		Weight（g）	
		24	48	Before injection	After experiment
1 Test	0	0	0	317	358
2 Test	0	0	0	326	367
3 Test	0	0	0	308	350
4 Test	0	0	0	329	369
5 Test	0	0	0	334	373
6 Test	0	0	0	320	359
7 Test	0	0	0	312	354
8 Test	0	0	0	326	369
9 Test	0	0	0	341	380
10Test	0	0	0	335	376
11control	0	0	0	325	369
12control	0	0	0	336	317
13control	0	0	0	307	350
14control	0	0	0	318	358
15control	0	0	0	323	366

Kligman grading given in Table 1 for each challenge site and at each time interval .It is highly recommended that reading be done without knowledge of the treatment, in order to minimize bias in the evaluation of the results.

Evaluation of results:

Magnusson and Kligman grades of 1 or greater in the test group generally indicate sensitization, provided grades of less than 1 are seen in control animal. If grades of 1 or greater are noted in control animal, then the reactions of test animal which exceed the most severe reaction in control animals are presumed to be due to sensitization. If the response is equivocal, re-challenge is recommended to confirm the results from the first challenge. The outcome of the test is presented as the frequency of positive challenge results in the test and control animal.

Table 1 Magnusson and Kligman scale

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

Result

Individual results of dermal scoring for the challenge appear in Table 1. No evidence of sensitization was observed.

Clinical Observations: All animals appeared clinically normal throughout the study.

Conclusion

Under the conditions of this study, the test article extract and the test article showed no significant evidence of causing delayed dermal contact sensitization in the guinea pig.

Table 2 GUINEA PIG SENSITIZATION DERMAL REACTIONS – CHALLENGE

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Intracutaeous irritation Test

Summary

The test article, PP SPUNBOND NONWOVEN FABRIC was evaluated for primary Intracutaeous irritation in accordance with the ISO 10993-10/Amd.1. Tests for Irritation and Sensitization. The skin responses on injection sites in 24h,48h and 72h respectively after injection were observed and recorded. The tissue reaction was graded for erythema and oedema according to the classification system given in Table 1. According to what was observed, the response of skin on testing side does not exceed that on the control side. The primary irritation index for the test article was calculated to be 0. The test result showed that leached solution of sample does not induce irritation to rabbit skin.

Date completed: May 17, 2018

Tested by 5.1.2e

Jun 12 2018 . Jun 12 , 2018 Jun 12

Testing Center of Radiological Medical Research Institute, Soochow University

Introduction

The test article, PP SPUNBOND NONWOVEN FABRIC, was evaluated for primary Intracutaeous irritation in accordance with the guidelines of the ISO 10993-10/Amd.1.Tests for irritation and sensitization. This study was to determine the potential skin irritation after the injection of sample solution into the animal back. The test article was received on May 12, 2018. Injections were applied on May 13, 2018. and the observations were concluded on May 17, 2018.

Materials

Test Article: PP SPUNBOND NONWOVEN FABRIC

Model: Not supplied

Lot No: Not supplied

Equipment: Incubator

Storage Conditions: Room temperature

Sample and Control Preparation

The cut sample was placed in a container, the leaching solution (0.9% NaCl injection solution) was added in the proportion of 10ml leaching solution to 2g sample. The container was incubated at (70±2)℃ for (24±2) hours. The solution so prepared is termed as leached solution of sample. The control solution (0.9% NaCl) was obtained in same way but in the absence of a sample.

Method

Test System:

Species: Rabbits.
Breed: New Zealand white (single strain)
Source: Provided by Animal Center, TCRSU〈Permit Code: SCXK (SU)2002-0008〉
Body Weight Range: Not less than 2 kg.
Age: Young adult
Acclimation Period: Minimum 5 days.
Number of Animals: Three

Justification of Test System:

The rabbit is specified as an appropriate animal model for evaluating potential skin irritants by the current ISO testing standards. The rabbit is widely used for this purpose and relative ranking of irritant scores can be determined.

Animal Management:

Husbandry: Refer to ISO 10993-10 Annex C: Animal and husbandry

Food: All-nutrient animal food was provided by the Suzhou (Twin-lion) Experimental Animal Food Science & Technology service Co., Ltd.

Water: Provided by Sanitary Standard for drinking water.

Housing: Animals were individually housed in stainless steel suspended cages identified by a card indicating the lab number, animal number, test code, sex, and date dosed.

Personnel: Associates involved were appropriately qualified and trained.

Selection: Only healthy, previously unused, animals free from irritation or other dermatological lesions that could interfere with the test were selected.

Experimental Procedure

The rabbit's naked skin was cleaned with 75% alcohol. Ten points at 2 cm intervals were chosen on one side of the rabbit back and injected with 0.2ml leached solution at each point. Similarly, on the other side of the rabbit back. Five points were chosen at 2 cm intervals and injected with 0.2ml control solution at each point.

The skin responses of injection sites were observed and recorded in 24h,48h and 72h respectively after injection. The skin responses include erythema, odema and necrosis as well. From weak to serious, the responses were differentiated by grade 0, 1, 2, 3, 4 on the basis of its extent. See table 1.

Result

According to what observed, the response of skin on testing side does not exceed that on the control side. Thus, it is identified as grade 0. See table 2.

Conclusion

The test result shows that leached solution of sample does not induce irritation to skin.

Record Storage

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

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Table.1

Rabbit No.	Group	Reaction	Interval (hours)		
			24 h	48h	72h
1	Test	Erythema	0	0	0
		Oedema	0	0	0
	Control	Erythema	0	0	0
		Oedema	0	0	0
2	Test	Erythema	0	0	0
		Oedema	0	0	0
	Control	Erythema	0	0	0
		Oedema	0	0	0
3	Test	Erythema	0	0	0
		Oedema	0	0	0
	Control	Erythema	0	0	0
		Oedema	0	0	0

Classification System for Skin Reaction

Reaction

Erythema and Eschar Formation:	Numerical Grading
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet redness) to eschar formation preventing grading of erythema	4
Edema Formation:	
No edema	0
Very slight edema (barely perceptible)	1
Well-defined edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1mm)	3
Severe edema (raised more than 1mm and extending beyond exposure area)	4
Total possible score for irritation	8

Irritation Response Categories in the Rabbit

Response Category	Mean score
Negligible	0 to 0.4
Slight	0.5 to 1.9
Moderate	2 to 4.9
Severe	5 to 8

NOTE: Other adverse changes at the skin sites were recorded and are reported

Table 2. Dermal Observations