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CNAS L1446

Shenzhen Testing Center of Medical Devices

Test Report

Report No.:	WD20240508
Test Article:	Poly-L-lactic acid microspheres PLLA microspheres
Sponsor:	Shenzhen Esun Industrial Co., Ltd.
Manufacturer:	Shenzhen Jusing Biotechnology Co. , Ltd.
Test Type:	Commission Test
Date of Issue:	May 13,2024

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Test Report

Report No.: WD20240508

Test Article	Poly-L-lactic acid microspheres PLLA microspheres		
Test Type	Commission Test	**Identification No./ Lot No.**	/
Trade Mark	esun	**Model / Type**	Viscosity average Molecular Weight sixty thousand
Date of Manufacturing	Jan. 23, 2024	**Accepting Date**	Mar. 05, 2024
Sponsor	Shenzhen Esun Industrial Co., Ltd.		
Applicant Address	15A, Microsoft Ketong Building, No. 55 Gaoxinnan 9th Road, Hightech Community, Yuehai Street, Nanshan District, Shenzhen.		
Manufacturer	Shenzhen Jusing Biotechnology Co., Ltd.		
Production Address	Floor 3, No. 9, Yifenghua Innovation Industrial Park, Xinshi Community, Dalang Street, Longhua district, Shenzhen City		
Test Items	*In vitro* Cytotoxicity Test		
Test in Accordance with	ISO 10993-5:2009 *Biological evaluation of medical devices -- Part 5: Tests for in vitro cytotoxicity*		
Summary	The test article, Poly-L-lactic acid microspheres PLLA microspheres, was extracted by MEM with 10% fetal calf serum. The extract was evaluated for in vitro cytotoxicity test by MTT assay recommended by ISO 10993-5:2009 Biological evaluation of medical devices, Part 5: Tests for in vitro cytotoxicity. L-929 cells were seeded in 96-well assay microtiter plate. After the incubation of cells for 24 h at 37°C in air with 5% CO_2, blank control, negative control, positive control and four different concentrations of the test article extract were added to microtiter plate. After 24 h treatment, carefully removed the culture medium from the plates. 50µL of the MTT solution was then added to each test well and the plates were further incubated for 2 h. Then the MTT solution was discanted and 100µL of isopropanol were added in each well. Swayed this plate and subsequently transferred it to a microplate reader equipped with a 570 nm filter to read the absorbance (reference wavelength 650 nm). The reduction of viability compared to the blank (Viab%) was calculated. Under the conditions of this study, the Viab.% of the positive control group and negative control group were 16% and 100%; the Viab.% of 100%, 50%, 25% and 12.5% extracts were 96%, 99%, 99% and 102%.		
Comments	"/" in the report indicates that this item is blank.		
Authorized Signatory	刘亮	**Date Completed**	May.13,2024

深圳市医疗器械检验中心 检验检测专用章 4403055618

INTRODUCTION

The test article identified below was extracted and the extract was subjected to an *in vitro* cytotoxicity test by MTT assay recommended by the Annex C of the ISO 10993-5:2009 *Biological evaluation of medical devices- Tests for in vitro cytotoxicity*.

The test article was accepted on Mar. 05, 2024. The extraction was applied from Mar. 19, 2024 to Mar. 20, 2024, and the observations were concluded on Mar. 21, 2024.

MATERIALS

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

Test article:	Poly-L-lactic acid microspheres PLLA microspheres
Identification No./ Lot No.:	/
Storage conditions:	Store at 2-8℃
Cell line:	L929 mouse fibroblast cells, recommended by the ISO 10993-5:2009, was from China Center for Type Culture Collection. Cultures were incubated at 37℃ in air with 5% carbon dioxide.
MEM with phenol red:	Thermo Fisher Scientific corporation product, with Earle's salts, L-Glutamine and phenol red. (Without specification, "MEM" mentioned in this text mean MEM with phenol red).
MEM without phenol red:	Thermo Fisher Scientific corporation product, with Earle's salts, without L-Glutamine and phenol red.
Fetal calf serum:	Shanghai BasalMedia Technologies corporation product.
Penicillin-streptomycin, liquid:	Thermo Fisher Scientific corporation product.
MTT:	Merck corporation product. MTT is solute fresh in MEM without supplements and without phenol red at a concentration of 1 mg/mL. Solution is sterilized by sterile filtration using syringe filters (pore size ≤ 0.22 μm). The solution should be used the same day.
DMSO:	Merck corporation product.
Isopropanol:	Guangzhou Chemical Reagent factory product.
Extraction vehicle:	MEM with 10% fetal calf serum.(100IU/mL penicillin, 100μg/mL streptomycin)
Test article preparation:	Under aseptic conditions, according to the requirements of the sponsor, 1.25 g of the test article was covered with 6.25 mL extraction vehicle based on a ratio of 0.2 g/mL and extracted at 37℃ for 24 h with agitation (The extract was used immediately). The extract of test article was transparent with no presence of particulates. Prior to use, the extract was sterilized by membrane filtration (0.22 μm). The filtration extract of test article was diluted by MEM with 10% fetal calf serum. The 100 %, 50%, 25% and 12.5% extracts were tested.
Negative control preparation:	Under aseptic conditions, 2.00 g of high-density polyethylene was covered with 10.00 mL extraction vehicle based on a ratio of 0.2 g/mL and extracted at 37℃ for 24 h. Prior to use, the extract was sterilized by membrane filtration (0.22 μm).
Positive control preparation:	MEM (1×MEM) with 10% fetal calf serum and with 10% DMSO
Blank control preparation:	MEM (1×MEM) with 10% fetal calf serum

METHODS

Experimental Procedure:

1. L-929 cell suspension preparation
   a. L-929 cell monolayer confluence was cultured with 1×MEM for 48 h~72 h and removed from culture flasks by enzymatic digestion (trypsin/EDTA).
   b. The cells were then resuspended in culture medium and the cell suspension was adjusted at a density of 1×10^5 cells/ml.
2. MTT assay
   a. Using a multichannel pipette, dispensed 100 µL culture medium only (blank) into the peripheral wells of a 96-well tissue culture microtitre plate. In the remaining wells, dispensed 100 µL of a cell suspension of 1×10^5 cells/mL. Blank (both at the left side and the right side), negative control, positive control and the test article group were set up, and each group contained six wells.
   b. Incubated cells for 24h (5 % CO_2, 37 °C, > 90 % humidity) so that cells formed a half-confluent monolayer.
   c. After 24 h incubation, aspirate culture medium from the cells. Per well, added 100 µL of treatment medium containing either the appropriate concentration of test article extracts, or the negative control, or the positive control, or nothing but blank. Four different concentrations of the test item extracts were tested (100%, 50%, 25%, 12.5%).
   d. Incubated cells for 24 h (5 % CO_2, 37℃, > 90 % humidity).
   e. After 24 h treatment, examined each plate and cellular morphology under a phase contrast microscope. Recorded changes in the morphology of the cells due to cytotoxic effects of the test article extracts.
   f. After the examination of the plates, carefully removed the culture medium from the plates. Pipetted 50 µL of MTT solution into each well, and incubated the plate for 2 h at 37℃. Then the MTT solution was discanted and 100 µL of isopropanol were added in each well. Swayed this plate and subsequently transferred it to a microplate reader equipped with a 570 nm filter to read the absorbance (reference wavelength 650 nm). To calculate the reduction of viability compared to the blank Equation was used:

$$Viab.\% = \frac{100 \times OD_{570e}}{OD_{570b}}$$

   where
   OD_{570e} is the mean value of the measured optical density of the extract of the test article;
   OD_{570b} is the mean value of the measured optical density of the blanks.
3. Evaluation Criteria
   a. A test meets the acceptance criteria if the mean OD_{570} of blanks is ≥0.2.
   b. A test meets acceptance criteria if the left and the right mean of the blanks do not differ by more than 15 % from the mean of all blanks.
   c. If 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.

Referred to ISO10993-5:2009, qualitative morphological grading of cytotoxicity of extracts is given in Table 1.

Table 1 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.

RESULTS

The mean OD_{570} of blanks was ≥0.2 and the left and the right mean of the blanks did not differ by more than 15 % from the mean of all blanks, so the test met the acceptance criteria.

Referred to the table 1, the microscopic observation show the negative control was grade 0; the positive control was grade 4; the cytotoxicity of 100 %, 50%, 25% and 12.5% extracts were grade 1, grade 1, grade 1 and grade 0.

The results of optical density is given in Table 2:

Table 2 Absorbance and *Viab%*

Groups	Blank Control		Positive Control	Negative Control	100% Extract	50% Extract	25% Extract	12.5% Extract
	Left	Right						
Well 1	0.9360	0.8901	0.1473	0.9321	0.8838	0.8935	0.9209	0.9449
Well 2	0.9099	0.8991	0.1224	0.8915	0.8789	0.9126	0.9255	0.9277
Well 3	0.9220	0.9174	0.1338	0.9259	0.8973	0.9089	0.9033	0.9187
Well 4	0.9461	0.9553	0.1699	0.9340	0.9269	0.9655	0.9298	0.9649
Well 5	0.9566	0.9287	0.1638	0.9477	0.9114	0.9515	0.9296	0.9648
Well 6	0.9851	0.9999	0.1876	0.9799	0.8885	0.9091	0.9523	0.9937
Mean OD	0.9426	0.9318	0.1541	0.9352	0.8978	0.9235	0.9269	0.9524
	0.9372							
Viab.%	/		16%	100%	96%	99%	99%	102%

CONCLUSION

Under the conditions of this study, the Viab.% of the positive control group and negative control group were 16% and 100%; the Viab.% of 100%, 50%, 25% and 12.5% extracts were 96%, 99%, 99% and 102%.

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by our testing center. Any extrapolation of these data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with ISO 17025.

RECORD STORAGE

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

Test Report

Report No.: WD20240508

Test Article

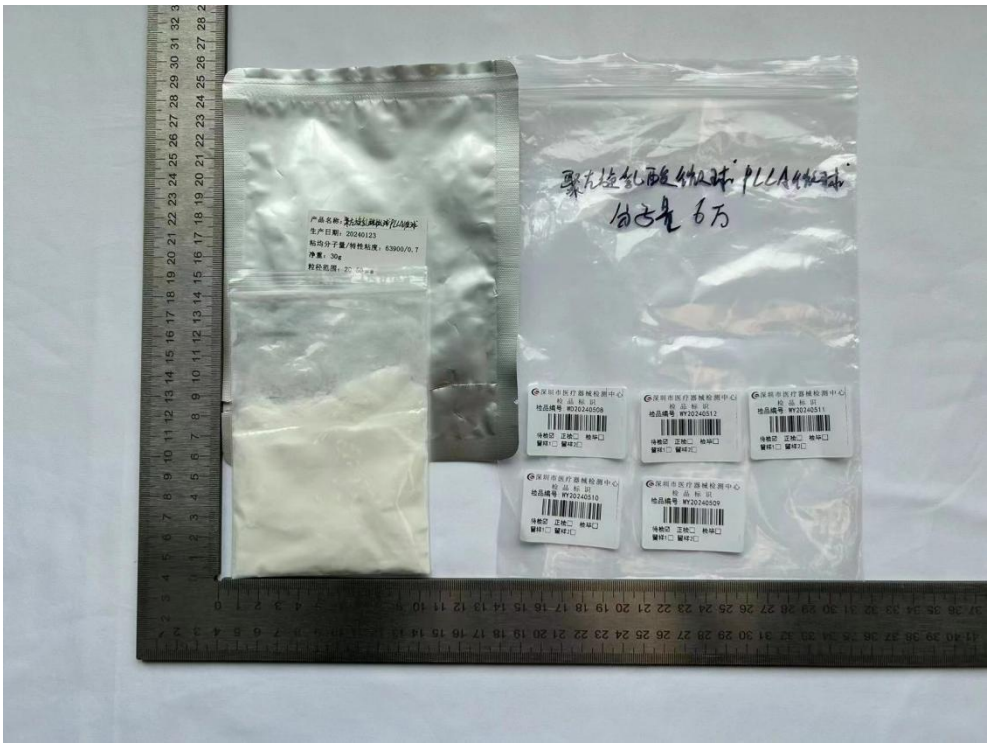

Fig.1 Test Article

Sample Specification

Instructions for Preparation of Extract for PCL PLLA Microsphere Biological Evaluation Test

Model / Type:

Serial Number	Classification Code	Management category	Product components	Material	Nature of body contact		Contact duration	Extraction surface	Total surface area or weight of components in contact with the human body (cm2/g)
					Category	Contact			
1					Implant_medical_device	Tissue/bone	Long-term exposure (>30 d)	Inner and outer surface	
2									
3									
Total	/								
Extraction ratio	0.2g/mL				Extraction temperature, duration (In vitro Cytotoxicity		37℃ 24h		
					Extraction temperature, duration (others)		37℃ 72h		

Zero point two grams/ml

37 degrees Celsius 24hours
37 degrees Celsius 72hours

Figure 1 Product structure diagram

Company Name (Stamp)
Date: March 4, 2024

Fig.2 Sample Specification

Model / Type

/

Blank Below