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Test Report

TEST ITEMS

Test for *in vitro* cytotoxicity (MTT cytotoxicity test)

TEST ARTICLE

silicone rubber
<Spec.: ZT-9773; Lot.: 202309040437>

IDENTIFICATION №

230922

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SUMMARY

An in vitro cytotoxicity study was conducted to assess the potential for cytotoxicity of the test article, **silicone rubber,** based on the International Organization for Standardization ISO 10993-5:2009 Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity; ISO 10993-12:2021 Biological evaluation of medical devices - Part 12: Sample preparation and reference materials.

Four concentrations (100%, 75%, 50%, and 25%) of the test sample extracts, the blank, 100% of the negative control and the positive control were prepared using Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum. The semi-confluent monolayers of L-929 mouse fibroblast cells were incubated with the test extract, the blank and other two controls, supplemented with 10% fetal bovine serum in a 96-well microplate respectively at 37℃ under the condition of 5% CO_2. At 24h, the MTT colorimetric assay was employed and the plate was read on a microplate reader at 570 and 650 nm. The viability of the cells was calculated.

Under the condition of this study, the viability of 100% extract of the test sample was 74 %. It can be considered that the test sample extracts had not a cytotoxic potential.

Study and Supervisory
Personnel: JI Lina
DING Tingting

Study Director: Sun Jiao SUN Jiao, Ph.D.

Nov 27. 2023
Date Completed

INTRODUCTION

The study was performed in order to determine whether leachables extracted from the test article would cause cytotoxicity. This test was conducted based on the requirements of ISO 10993-5:2009 Biological evaluation of medical devices - Part 5: Tests for *in vitro* cytotoxicity. The test article was received on Sep. 13, 2023. The cells were first exposed to the extract on Nov. 2, 2023, and the final observations were concluded on Nov. 3, 2023.

This study was completed in the Lab of Shanghai Biomaterials Research & Test Center (SBRTC). SBRTC was conducted in accordance with the provisions of the ISO/IEC 17025: 2017.

MATERIALS

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

Test Article:	silicone rubber <Spec.: ZT-9773; Lot.: 202309040437>
Identification №:	230922
Sterilization Status:	Non Sterile
Storage Temperature:	Room temperature
Extraction Vehicle:	gibco's Minimum Essential Medium (2395101) supplemented with L-glutamine and 10% fetal bovine serum (BG9605)
Test Extract Preparation:	According to the sponsor's requirement, the test samples were cut into small pieces and sterilized by ethylene oxide two weeks before the treatment. Based on the ISO 10993-12:2021, the ratio of $3cm^2/ml$ [Surface area of the test sample to volume of extraction vehicle] was adopted for testing. $30cm^2$ of the test samples (Sampling randomly according to the statement of the sponsor) were submersed in 10ml of extraction vehicle under aseptic conditions for preparing the test extract at 37℃ for 72 hours with continuously agitation during extraction. The extract was used immediately after extraction.
Blank Preparation:	The extraction vehicle not containing the test sample, retained in a vessel identical to that which holds the test sample and subjected to conditions identical to those to which the test sample is subjected during its extraction.
Negative Control Preparation :	High-density polyethylene film (221026), which was current SBRTC negative control (having been demonstrated to be noncytotoxicity) coming from Hatano Research Institute, FDSC. The ratio of $3cm^2/ml$ [surface area of the test sample to volume of extraction vehicle] was used and extracted at 37℃ for 72 h.

Positive Control Preparation:	Polyurethane film containing 0.1% zinc diethyldithiocarbamate (ZDEC) (221028) which was current SBRTC positive control (having been demonstrated to be severe citotoxicity) coming from Hatano Research Institute, FDSC. The ratio of $6cm^2/ml$ [surface area of the test sample to volume of extraction vehicle] was used and extracted at 37℃ for 72 h.
Condition of Extracts:	All the extracts of the test samples and the controls were clear, no suspended particulates and without any special treatments.

METHODS

Test System Management:

Mouse fibroblast cells (L 929, from the cell bank of Shanghai Institutes for Biological Sciences), were cultured in MEM supplemented with L-glutamine and 10% fetal bovine serum at 37°C in a gaseous environment of 5% carbon dioxide (CO_2). A 96-well microplate method was employed for the MTT colorimetric assay. Each well was seeded 100μl suspension of 1×10^4 cells, and incubated at 37°C in 5% CO_2 atmosphere for 24h prior to use.

Experimental Procedure:

After incubation, the growth medium was replaced with 100μl four concentrations (100%, 75%, 50% and 25%) of the test extract, 100% of the negative control and the positive control, the blank (row 2 and 11) respectively. Six replicates were prepared for each group. The 96-well plate were incubated at 37℃ in 5% CO_2 for 24h.

After 24 h treatment, the culture medium was removed carefully from the plates. 50μl of the MTT (1mg/mL) solution was then added to each test well and the plates were further incubated for 2 h at 37℃ in a 5% CO_2 atmosphere. Then the MTT solution was removed and 100μl isopropanol per well was added and shake for 10min by gently. The plate was read on a microplate reader at 570 nm (reference wavelength 650 nm). The viability of the cells was calculated according to the formula below:

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

Where

OD_{570e} is the mean value of the measured optical density of the extracts of the test sample;

OD_{570b} is the mean value of the measured optical density of the blanks;

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. If the viability of the test sample was reduced to <70% of the blank, it had 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.

DEVIATIONS

There were no deviations from the ISO 10993-5:2009.

RESULTS

Group	1	2	3	4	5	6	The optical density (570nm−650 nm)	Viab. %
100% of the negative control	0.879	0.781	0.848	0.736	0.806	0.857	0.818±0.054	100
100% of the test extract	0.585	0.581	0.649	0.596	0.615	0.626	0.609±0.026	74
75% of the test extract	0.666	0.671	0.661	0.622	0.644	0.628	0.649±0.021	79
50% of the test extract	0.689	0.706	0.651	0.645	0.688	0.705	0.681±0.026	83
25% of the test extract	0.761	0.786	0.724	0.688	0.684	0.742	0.731±0.040	89
100% of the positive control	0.039	0.041	0.041	0.038	0.039	0.043	0.040±0.002	5
The blank (row 2)	0.878	0.811	0.844	0.725	0.766	0.795	0.803±0.055	/
The blank (row 11)	0.829	0.888	0.849	0.819	0.776	0.852	0.836±0.038	/

Note: n=6

The mean value of optical density of the blank was 0.819±0.048. Both the left (row 2) and the right (row 11) mean of the blanks were less than 15% from the mean of all blanks.

Results and conclusions apply only to the test article tested. No further evaluation of these results was made by Shanghai Biomaterials Research & Test Center.

CONCLUSION

Under the condition of this study, the viability of 100% extract of the test sample was 74%. It can be considered that the test sample extracts had not a cytotoxic potential.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be stored in the designated archive files at Shanghai Biomaterials Research & Test Center.

PHOTOGRAPH OF THE TEST ARTICLE

