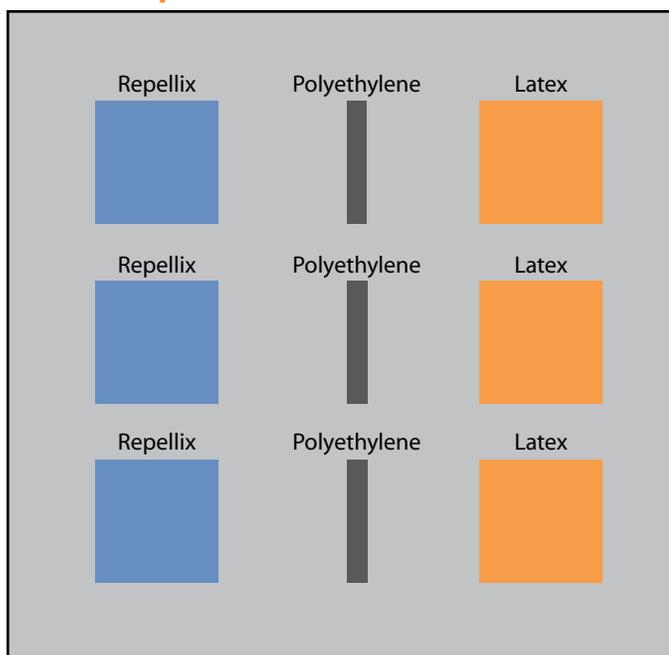




Repellix™ Cytotoxicity Test

An *in vitro* biocompatibility study, based on the requirements of the International Organization for Standardization (ISO 10993-5), was conducted on Repellix™ provided by Integrated Surface Technologies™ (IST). The test was conducted by NAMSA to determine the potential for cytotoxicity. Under the conditions of the study, Repellix showed no evidence of causing cell lysis or toxicity. Triplicate wells were dosed with a 1 cm x 1 cm portion of Repellix. Triplicate wells were dosed with a 1 cm length of high density polyethylene as a negative control. Triplicate wells were dosed with a 1 cm x 1 cm portion of latex, as a positive control. Each was placed on an agarose surface directly overlaying a subconfluent monolayer of L-929 mouse fibroblast cells. After incubating at 37°C in 5% CO₂ for 24 hours, the cell structure was examined macroscopically for cell decolorization around the test article and controls to determine the zone of cell lysis (if any). The culture was then examined microscopically (100X) to verify any decolorized zones and to determine cell morphology in proximity to the articles.

Test Description



Test Trays

Test Results

	Repellix	Polyethylene (Negative Control)	Latex (Positive Control)
Test Article			
Zone of Lysis (mm)	0 0 0	0 0 0	12 12 12
Grade	0 0 0	0 0 0	4 4 4
Reactivity	none none none	none none none	severe severe severe

Repellix Test Results:

Cytotoxicity is the quality of being toxic to cells

Lysis refers to the death of a cell by breaking of the cellular membrane

No Evidence of Lysis or Toxicity

Biocompatibility Test Details

Test System

Test System Management

L-929, mouse fibroblast cells, (ATCC CCL 1, NCTC Clone 929, of strain L, or equivalent source) were propagated and maintained in open flasks containing single strength Minimum Essential Medium supplemented with 5% serum and 2% antibiotics (1X MEM) in a gaseous environment of 5% carbon dioxide (CO₂). For this study, 10 cm² wells were seeded, labeled with passage number and date, and incubated at 37°C in 5% CO₂ to obtain subconfluent monolayers of cells prior to use. Aseptic procedures were used in the handling of the cell cultures following approved NAMSA Standard Operating Procedures.

Test Procedure

The test article was placed on the solidified agarose surface in three separate cell culture wells. Similarly, the negative control and the positive control were each placed on the solidified agarose surface in three cell culture wells. The wells were labeled with the corresponding lab number and dosing date, and incubated at 37°C in 5% CO₂ for 24 hours.

Following incubation, the cultures were examined macroscopically for cell decolorization around the test article and controls to determine the zone of cell lysis (if any). After macroscopic examination, the cell monolayers were examined microscopically (100X) to verify any decolorized zones and to determine cell morphology in proximity to the article.

For the suitability of the system to be confirmed, the negative control must have been a grade of 0 (reactivity none) and the positive control must have produced a zone of lysis (reactivity moderate to severe). The test article passed the test if all three monolayers exposed to the test article showed no greater than a grade of 2 (reactivity mild).

Conclusion

Under the conditions of this study, the test article showed no evidence of causing cell lysis or toxicity. The test article met the requirements of the test since the grade was less than a grade 2 (mild reactivity). The negative control and the positive control performed as anticipated.

Preparation of Agarose Overlay

The culture wells contained a subconfluent cell monolayer. The growth medium in each well was replaced with 2 mL of equal amounts of double strength Minimum Essential Medium supplemented with 10% serum and 4% antibiotics (2X MEM), supplemented with neutral red, and 2% agarose (final concentration 1% agarose, 1X MEM). The MEM-agarose mixture (2mL) was then placed in the cell culture wells and allowed to solidify over the cells to form the agarose overlay.

Criteria for Cytotoxicity Scoring

Grade	Reactivity	Condition of Cultures
0	None	No detectable zone around or under specimen
1	Slight	Some malformed or degenerated cells under specimen
2	Mild	Zone limited to area under specimen and up to 4 mm
3	Moderate	Zone extends 5-10 mm beyond specimen
4	Severe	Zone extends greater than 10 mm beyond specimen

Results and conclusions apply only to the test article tested. Any extrapolation of these data to other samples is the sponsor's responsibility. All procedures were conducted in conformance with good manufacturing practices and certified to ISO 13485:2003