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"Risk-Based Biocompatibility Evaluation of a Pulmonary Clot Retrieval Device: Cytotoxicity, Skin Sensitization and Skin Irritation"

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Abstract:
Mechanical thrombectomy medical devices for pulmonary embolism (PE) provide a less invasive way to improve pulmonary perfusion and prevent mortality. The biocompatibility of these devices is essential for reducing undesired biological responses like cytotoxicity, hypersensitive reactions, or irritation. This investigation evaluates the in vitro and in vivo biocompatibility of an innovative pulmonary clot retrieval device constructed out of a nitinol framework with a polymeric coating that complies with ISO 10993 specifications. Cytotoxicity has been assessed by exposing L929 fibroblast cells to medical device extracts through cell viability measured using the MTT test and morphological examination performed under a microscope. The guinea pig maximization test was used for assessing skin sensitization potential, whereas rabbits were tested for skin irritation under semi-occlusive conditions. Cytotoxicity studies showed excellent cell viability and no morphological changes, indicating no cytotoxic impact. During a 14-day period, there have been no sensitization reactions in the guinea pig model, and Magnusson and Kligman scores remained constant. There have not been any symptoms of oedema or other negative consequences. The results of these studies suggest the pulmonary clot retrieval device offers excellent biocompatibility, encouraging its safe usage in medical procedures for thrombectomy in PE patients.

Keywords: *Pulmonary embolism, pulmonary clot retrieval device, mechanical thrombectomy, ISO 10993, Skin sensitization, Irritation, Cytotoxi*

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I. Introduction:

Pulmonary embolism (PE) is a dangerous, possibly life-threatening medical condition triggered by a blockage of the pulmonary circulation system due to thromboembolic material [1-3]. Prompt clot removal asmong individuals with a moderate- or serious-risk PE is crucial in preventing hemodynamic worsening, right ventricle dysfunction, and death [4, 5]. Clinical acceptability of mechanical pulmonary thrombi removal as an alternative treatment approach for patients who are not suitable for therapeutic thrombolysis or patients who do not respond to it has become more widespread [6-8].

As an intravascular medical device with limited exposure to blood and indirect tissue interaction, a pulmonary clot retrieval device must comply with specified regulatory standards for biological safety [9-11]. Medical devices may pose potential biological risks due to their constituent materials, outer finishes, manufacturing residues, or substances resulting from degradation [12-14]. These risks include cytotoxicity, tissue irritation, and sensitization manifestations [15]. Hence, a thorough biocompatibility assessment is a crucial aspect for the entire device risk assessment approach [16-18].

International and regional regulatory entities require biological assessment complying with the ISO 10993 series of standards, "Biological Evaluation of Medical Devices." ISO 10993-1 outlines a risk-based methodology to assess specific biological endpoints that should be evaluated depending on the type and time span of device interaction [19, 20]. Cytotoxicity, skin irritation, and sensitization are recognized as essential prerequisites for establishing baseline biological safety of devices designed for limited-duration intravascular application.

In vitro cytotoxicity testing, as required in ISO 10993-5, provides an initial screening method to assess potential harmful effects of device removal on L929 fibroblast cells [21, 22]. Irritation to skin and sensitivity of skin testing to materials or extracts from medical devices, as required in ISO 10993-23 and ISO 10993-10

standards, respectively, evaluate potential harmful effects due to local inflammatory or immune reactions to materials or extracts of medical devices [23-26].

The study device for clots in the lungs is made from nitinol and coated with a polymeric layer and comes pre-sterilized for one-time use only. The objective of the study is specifically focused on determining the device's biocompatibility through cytotoxicity testing, skin sensitization, and skin irritation tests, as per the ISO 10993 guidelines and Good Laboratory Practices whenever required and relevant [19,27]. The study analysis outcome is therefore intended to support medical device registration as it confirms the medical device does not produce unjustifiable biological responses and is fit for its intended medical utilization for treating Pulmonary Embolism through its intended application as planned and conceived at its initial device invention and development stage.

II. Materials and Methods

2.1. Device Description

The Pulmonary Clot Retrieval Device is a catheter-based, mechanical thrombectomy system indicated for percutaneous removal of thrombosis from the pulmonary arteries. It is divided into a proximal Luer hub and Y-hub for flushing, aspiration, and control, a shaft and 12 Fr. catheter with high push ability and a large working lumen, as well as reinforced distal segments with a high level of stiffness and a controlled degree of flexibility. In the description, the distance or may be the range between three DISCS can be mentioned. The distal part is equipped with multiple disc elements for engagement of clot material, as well as a soft, a traumatic tip (Figure 1). Manual Retraction Aspirator (hand lever) should be activated to start aspiration. The Pulmonary Catheter should be withdrawn into the Aspiration Catheter simultaneously to ensure that the clot can be trapped in the system. This will be confirmed by the aspiration flow and the fluoroscopic image. If there is any clot remaining in the vessel, the process should be repeated accordingly. Select the right size catheter depending on the size of the artery and the degree of thrombosis to avoid causing vascular damage and to ensure effective treatment. The device size matrix is given in the below table.

Table 1: Size matrix of the Pulmonary Clot Retrieval Device

Size (mm)	Guidewire	Catheter outer diameter (Fr)	Usable Length (mm)	Vessel Diameter (mm)	Sample total Length (mm)
S (6-10), M (11-14), L (15-18), XL (19-25)	0.035″ (0.889 mm)	12 Fr (4.0 mm)	1200	6-10, 11-14, 15-18, 19-25	59, 60, 62, 72

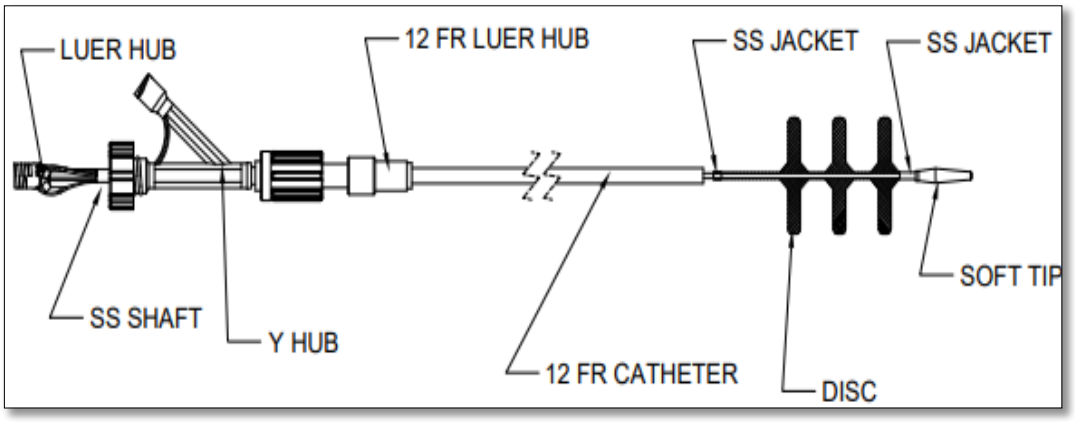

Figure 1. Schematic diagram of Pulmonary Clot Retrieval Device.

2.2. Test Extraction for Biocompatibility Tests/ Extraction Procedure

The process of extracting follows the ISO standard "Biological Evaluation of Medical Devices -Part 12: Sample preparation and reference materials ISO 10993-12:2021(E)" [28, 29]. The extracts have been prepared using polar (physiological saline) and non-polar (sesame oil) solvents separately under aseptic condition at extraction ratio of either 0.2 g/mL or 6 cm^2/mL with the vehicles. The needed proportion of the test specimen was measured and cut into small pieces (if needed) to facilitate its submersion in the sufficient amount of solvent vehicles. The extraction process was performed under constant stirring at 37 ± 1°C for 72 ± 2 hours. The extraction vehicles without the specimen being tested have been identically prepared to serve as controls. The overall condition of the extraction prior to and after the extraction will be monitored. The extracts will not be subjected to filtering, centrifuging, or any other procedures to remove dispersed particles. However, in case any such processing is required, its justification will be adequately documented. The extract's pH level has not been

changed. After the extraction, both extracts need to be utilized promptly and within 24 hours, which is deemed stable for the span of the period stated in ISO 10993-12.

2.3. Cytotoxicity Assessment

The Pulmonary Clot Retrieval Device's possible harmful effects have been evaluated using an in vitro cytotoxicity testing method. The cytotoxic activity has been evaluated in compliance with ISO 10993-5: 2009 "Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity" and ISO 10993-12: 2021 "Sample Preparation and Reference materials" [21, 22, 29].

2.3.1. Study Design

2.3.1.1. Preparation of Extracts

The extraction was performed using aseptic techniques in sterile, chemically inert, closed containers in compliance with ISO 10993-5:2009(E) and ISO 10993-12:2021(E). The test item, positive control (polyurethane film containing 0.1 % Zinc Diethyldithiocarbamate (ZDEC)), and negative control (High density Polyethylene film (HDPE)) were prepared aseptically for extraction at a ratio of 0.2 g/mL. Minimum Essential Medium Eagles (MEM) supplemented with 10% Fetal Bovine Serum (FBS), L-Glutamine, Non-essential amino acids (NEAA), and antibiotics (1% Penicillin Streptomycin) has been used as a blank control to assess the essential confounding impacts due to the extraction vessel, vehicle, and extraction process. The culture medium was maintained at a pH of between 7.2 and 7.4. Before being used, the medium was thawed to room temperature after being kept between 2 to 8 °C. To improve submersion in the extraction medium, the test item was weighed and broken into pieces before extraction. The prepared test item was put into the extraction medium (MEM with additives) needed for the extraction procedure. The amount of positive and negative control items had been weighed, autoclaved at 121°C, and cut into parts before extraction to foster submersion in extraction fluid. The extraction was carried out at 37 °C for 24 hours.

2.3.1.2. Test System Setup

L929 cells were grown in an open flask in MEM culture media for 24–36 hours at 37°C and 5% CO_2 level. Trypsin/EDTA enzymatic digestion was used to extract a monolayer of L929 cells in culture flasks. The cell suspension was then centrifuged at 200xg for three minutes. The cells were resuspended in growth media at a density of 1×10^5 cells/mL. About 100 µL of culture media, which was solely blank, was injected into the peripheral wells of a 96-well microtiter plate (these are the blanks). In the remaining wells, 100 µL of 1×10^5 cells/mL (=1 x 10^4 cells/well) cell suspension was added. Cells were incubated for 24 hours (5% CO_2, 37°C, >90% humidity). The period of incubation ensures the cells recuperate, adhere, and proceed to the exponential growth phase. The microtiter plate has been examined under the microscope to ensure the cell growth was comparatively uniform throughout.

2.3.1.3. Treatment Method

Four concentrations of the test item extraction and the positive control extraction have been evaluated (100%, 10%, 1%, and 0.1%). And a negative control extraction at 100% and a blank control containing medium alone were added as well.

2.3.1.4. Preparation of the Treatment Medium

Before treating the grown cells, each of the 100% test item extraction and the 100% positive control extraction will be periodically diluted in the treatment media to obtain three logarithmic dosages: 10%, 1%, and 0.1%. The procedure involved a neat concentration of negative control item extraction. The treatment medium was mixed properly.

2.3.1.5. Treatment of Culture

Day 1: As previously described in the culture setup, the cell suspension had been prepared, added to the plate, and incubated for 24 hours.

Day 2: After 24 hours of incubation, the cell culture media was evacuated and 100 µL of treatment medium with the appropriate amount of test item extract, negative control item extraction, or blank was added per well in triplicate. Cells had been incubated for 24 hours (5% CO_2, 37°C, and >90% humidity).

Day 3: After 24 hours of incubating, the treated cultures were inspected under a microscope to distinguish cell morphology between control and treated cells. The cytotoxic impacts of the tested item extraction have been determined by changes in cell morphology.

The culture media was then withdrawn from the plate after the examination. Following the introduction of 50 µL of MTT solution to each test well, the plate underwent incubation for 2 hours under normal culture conditions. Thereafter the MTT solution had been decanted, and 100 µL of isopropanol was introduced into each well to suspend the cell layer. The spectrophotometer was utilized to assess the suspension at a measured wavelength of 570 nm.

2.3.2. Data Analysis

As the number of live cells decreased, the sample's metabolic activity subsequently decreased. This decrease was precisely corresponding to the amount of blue-violet formazan produced, as measured by optical density at 570 nm. A decrease in viability compared to the blank has been calculated using the equation below.

$$\%\,Viability\ = \frac{100\ \times\ OD_{570e}}{OD_{570b}}$$

Where, OD_{570e} is the mean value of the measured optical density of the test item;
OD_{570b} is the mean value of the measured optical density of the blank.
Scoring for cytotoxicity was carried out based on the following criteria (Table 2).

Table 2: Criteria employed for the quantitative scoring of cytotoxicity responses

Grade	Reactivity	Conditions for 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 intra cytoplasmatic 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.

2.3.3. Acceptance Criteria

The 50% extraction of the test item must possess the identical or higher viability as the 100% extract; otherwise, the test will be repeated. The test item's cytotoxic potential increased with a lower viability % rating. If the viability was lowered to < 70% of the blank, this will be deemed to be a cytotoxic potential. If there is a difference in test findings over replicates, the experiment is considered invalid and the investigation is repeated.

2.4. Skin Sensitization

Skin sensitization was measured by employing the Guinea Pig Maximization Test (GPMT) in compliance with ISO 10993-10 [25, 26]. The healthy Hartley guinea pigs (300–500 g) were acclimated and separated into test and control groups. The animals were randomly assigned a group of fifteen animals per extract (ten tests and five controls). The evaluation was divided into two phases: induction and challenge. During the intradermal and topical induction stages, test group animals were administered polar or non-polar specimen extracts, whereas control group animals were administered just the polar or non-polar solvent. In the challenge phase, all the animals of the control and test groups were administered with both the respective polar or non-polar test item extract and the respective polar or non-polar solvent vehicle. After the challenge phase, the animals' skin responses had been evaluated using the Magnusson and Kligman scoring technique.

Table 3: Study design for skin sensitization test

Group	Phase	Administration
G1 (Control- Polar Vehicle)	Intradermal	Vehicle
	Topical	Vehicle
	Challenge	Test Item Extract & Vehicle
G2 (Control- Polar Test Item Extract)	Intradermal	Test Item Extract
	Topical	Test Item Extract

	Challenge	Test Item Extract & Vehicle
G3 (Control- Non Polar Vehicle)	Intradermal	Vehicle
	Topical	Vehicle
	Challenge	Test Item Extract & Vehicle
G4 (Control- Non Polar Test Item Extract)	Intradermal	Test Item Extract
	Topical	Test Item Extract
	Challenge	Test Item Extract & Vehicle

2.4.1. Animal Preparation

Intradermal and Topical Induction Phase: Approximately 24 hours before the experiment started, both the control and test animals intrascapular fur had been removed.

Challenge Phase: The untreated upper flank area on each side of both test and control animals was cut to remove fur about 24 hours before the challenge phase began.

2.4.2. Main Test

2.4.2.1. Day 0- Intradermal Induction Phase:

Control Group: Three doses of 0.1 mL intradermal injections were administered to each of the five (control group) animals at administration locations (A, B, and C) at the trimmed intrascapular area. The preparation of the intradermal induction injections was as follows. One pair of parallel injections of 0.1 mL/site was administered from the formulations.

Site A: 0.1 mL of a stable 50:50 volume proportional emulsion of Freund's complete adjuvant (FCA) blended with the solvent vehicle (1.5 mL of FCA and 1.5 mL of saline/sesame oil).

Site B: 0.1 mL of the specified solvent vehicle alone.

Site C: 0.1 mL of the stabilized emulsion of Freund's complete adjuvant in a 50:50 volume ratio using the selected solvent vehicle. (The 1.0 mL of FCA: saline/sesame oil preparation provided at site A was emulsified with the 1.0 mL of vehicle provided at site B.)

Test Group: All of the 10 animals in the test group were administered 0.1 mL intradermal administration at injection sites (A, B, and C) in the trimmed intrascapular portion. The injections used for intradermal induction were made as shown below. One pair of injections (0.1 mL/site) was administered from the preparations.

Site A: 0.1 mL of a stable 50:50 volume ratio emulsion of Freund's complete adjuvant (FCA) blended with the solvent vehicle (2.0 mL of FCA and 2.0 mL of saline/sesame oil).

Site B: 0.1 mL of the corresponding undiluted extract.

Site C: 0.1 mL of the extraction used at site B was blended in a stable 50:50 volume proportional emulsion of the solvent vehicle and Freund's complete adjuvant (1.5 mL of the extract provided at site B was emulsified with the 1.5 mL of the FCA: saline/sesame oil mixture provided at site A).

2.4.2.2. Day 7- Topical Induction Phase:

The day before topical induction, the test animals' fur had been removed from their intrascapular portion (the same portion as intradermal induction) and then moisturized with 0.5 g of 10% sodium lauryl sulphate (SLS) in Vaseline. The region was thereafter left uncovered. After 7 days of the intradermal induction phase, all residual SLS residue was carefully wiped away, followed by the topical induction phase.

Control Group: Animals in the control group were administered with the respective polar or non-polar vehicle by topical administration. Topical application of absorbent gauze patches (8 cm^2) containing 0.3 mL of vehicle to the intrascapular portion of all animals was performed. The patches were kept in place for 48 hours using occlusive dressing. The bandages and patches were taken off after being exposed for 48 hours.

Test Group: Animals of the test group were administered topical treatment of either polar or non-polar test item extracts. Absorbent gauze patches (8 cm^2) were filled with 0.3 mL of test extract and applied topically to each animal's intrascapular region. The patches were kept in place using occlusive dressing for 48 hours. After 48 hours of being exposed, bandages and patches were taken off. Warm water was used to remove the remaining test item extract without disturbing the epidermis' integrity.

2.4.2.3. Day 21- Challenge Phase:

Control and Test Group:
On day 21, 14 days after completing the topical induction phase, the challenge phase began. During the challenge phase, animals in both control and test groups were treated with topical injections of respective polar or non-polar test item extractions. Adsorbent gauze Patches of about 8 cm^2 were filled with 0.3 ml of test extract and applied to each animal's untreated portion (clipped left upper flank). Similarly, absorbent gauze patches measuring 8 cm^2 were then filled with 0.3 mL of vehicle solvent and applied on the untreated region (clipped right upper flank). The patches were kept in place using an occlusive dressing for 24 hours. After 24 hours, the occlusive bandage and the remaining test item extraction had been removed with warm water, without affecting the epidermis' existing reaction or integrity. The testing sites of test and control animals have been examined 24 and 48 hours after dressing removal. The Magnusson-Kligman scale was employed for assessing skin reactions.

2.4.3. Positive Control Response Validation

In order to validate the test procedure's reliability and sensitivity, investigations with positive controls have been carried out on a regular basis (at least once every six months). Powerful allergens like formaldehyde have been used as positive controls.

2.4.4. Observations

Morbidity and mortality had been monitored twice a day (morning and evening) during the course of the study. Body weight was measured when the animals arrived, on Day 0 prior to intradermal induction, and after the study was completed. Clinical symptoms have been observed every day. The Magnusson and Kligman grading scale was utilized to assess skin responses at challenge sites 24 and 48 hours after the bandage was removed.

2.4.5. Pathology

Necroscopy: The treated and control animals have been humanely put to death by CO_2 exposure at the end of the experiment. Each animal underwent a gross pathological examination.

2.4.6. Evaluation of Results

Sensitization is shown by Magnusson and Kligman grades of 1 or above in the test group, as long as the control animals exhibit grades of less than 1. If control animals get a grade of 1 or higher, test animals' reactions that are considerably more severe are likely attributable to sensitization. If the reaction is unclear, a rechallenge may be recommended to corroborate the results of the first. The test findings will be provided as the frequency of positive challenge outcomes in both test and control animals.

2.5. Skisn Irritation

2.5.1. Study Design

The skin-irritating potential of the Pulmonary Clot Retrieval Device has been examined in female rabbits as per ISO guideline ISO 10993-23:2021(E) [23, 24]. Since the test item had been expected to cause irritation, one animal underwent testing first. As a single animal failed to exhibit a clear positive reaction (erythema or oedema score more than 2), two more animals were studied. Each animal was administered injections with polar and non-polar test item extractions, as well as solvent controls, at different sites according to **Table 4**. The tissue outcome for erythema and oedema was scored up to 72 hours after being injected by applying the scale provided in **Table 5.**

Table 4: Intracutaneous Irritation Study Protocol Summary

Animal	Injection	Injection/Route	No. of Injections	Volume per Injection site
1.	Test Item Extract	Intradermal/Left Side of the spine	10	0.2 mL
	Solvent Control	Intradermal/Right Side of the spin	10	0.2 mL
2.	Test Item Extract	Intradermal/Left Side of the spine	10	0.2 mL
	Solvent Control	Intradermal/Right Side of the	10	0.2 mL

		spine		
3.	Test Item Extract	Intradermal/Left Side of the spine	10	0.2 mL
	Solvent Control	Intradermal/Right Side of the-spine	10	0.2 mL

Table 5: Scoring System for Skin Reaction

Reaction	Irritation Score
Erythema and eschar formation	
No erythema	**0**
Very slight erythema (barely perceptible)	**1**
Well defined erythema	**2**
Moderate to severe erythema	**3**
Severe erythema (beef redness) to eschar formation (preventing grading of erythema)	**4**
Oedema formation	
No oedema	**0**
Very slight oedema (barely perceptible)	**1**
Slight oedema (edges of area well defined by definite raising)	**2**
Moderate oedema (raised approximately 1 mm)	**3**
Severe oedema (raised more than 1 mm extending (beyond the area of exposure)	**4**
Maximal possible score for irritation	**8**
Other adverse changes at the skin sites shall be recorded and reported	

2.5.2. Test Item Extraction

The process of extraction procedure and conditions followed the guideline 10993-12:2021(E), "Biological Evaluation of Medical Devices," Part 12 [29]. The extractions have been prepared with polar (physiological saline) and non-polar (sesame oil) solvents separately (as discussed in previous section 2.3.1.1.).

2.5.3. Dose selection and Route of administration

The undiluted extracts were tested in accordance with ISO 10993 Part 23 guidelines (2021). The test item extraction has been administered intracutaneously (intradermal) in accordance with the ISO 10993 Part 23 guideline (2021).

2.5.4. Animal Preparation

Fur had been removed by trimming the backside of each animal within 18 hours of administering the test item extract, leaving enough space on both sides of the spine for injection.

2.5.5. Administration of the Test item extracts

On the day of injection, 0.2 mL of the test item extraction produced from both polar and non-polar solvents was administered intracutaneously at five different sites on the left side of each rabbit's spine. Similarly, 0.2 mL of polar and non-polar solvent controls was administered at five different sites on each rabbit's contralateral side (right). Figure 2 depicts the site of injection locations on the animal.

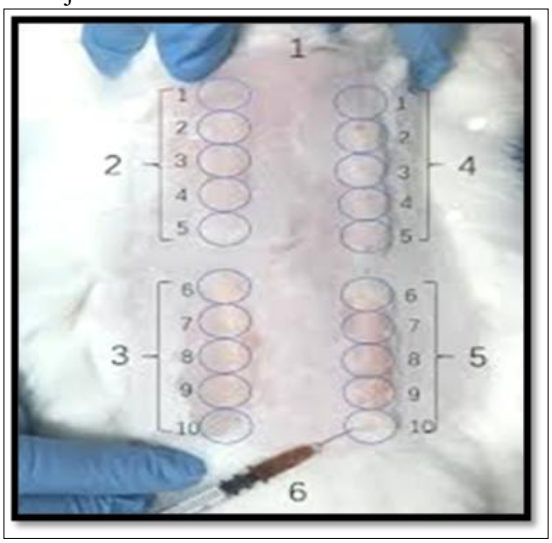

Figure 2. Injection sites on the animal.

Key: 1- Cranial end of the clipped area; 2- 0.2 mL injections of polar test item extract; 3-0.2 mL injections of non polar test item extract; 4- 0.2 mL injections of the polar solvent control; 5-0.2 mL injections of non polar solvent control; 6- Caudal end of the clipped area.

2.5.6. Observations

During the course of the study, mortality and morbidity were examined twice per day, in the mornings and evenings. Body weights were measured when the animals were received, on Day 0 before the test items were administered, as well as after at the conclusion of the experiment. Routine clinical assessments were made once daily during the entire acclimatization process and from Day 0 to the end of the examination. Tissue responses, especially erythema and oedema, were measured right after administration and again after 24, 48, and 72 hours, utilizing the scoring criteria shown in Table 5. No notable responses were observed for up to 72 hours; consequently, intravenous injection of essential dyes such as Trypan blue or Evans blue was not attempted.

2.5.7. Pathology

2.5.7.1. Necropsy

The animals that were treated were mercifully put to death at the end of the investigation by administering an intravenous overdose of thiopentone sodium. The rabbits were administered into their auricular veins. Gross pathology has been carried out with cautious focus to the treated site.

2.5.7.2. Histopathology

Histopathological evaluation of the treated and control cutaneous specimens was not done since no responses of erythema or oedema were detected up to 72 hours after injection.

2.5.8. Evaluation of results

After the 72-hour evaluation, each animal's erythema and oedema grades at 24, 48, and 72 hours have been added up individually for each test item extract and the solvent controls, then divided by 15 (3 scoring time points × 5 test or blank sample injection sites). To determine the overall result for every test item, extract and control, the results of three animals were summed up and divided by 3. The final test item score has been calculated by subtracting the score of the corresponding solvent control from the test sample/item score.

III. Results and Discussion

3.1. In vitro cytotoxicity

The in vitro cytotoxicity evaluation investigated the cytotoxicity potential of the test item. Prior to the procedure, the confluence of the cells throughout all wells was roughly 80%. After extraction, the final test item extraction was transparent, the test item's physical appearance remained the same, and no particles or variations in colour were apparent in the test item extraction medium as compared to the blank. Therefore, test item extractions were applied without any modifications (e.g., filtering, centrifuging, diluting, or pH adjustment) before evaluation.

3.1.1. Qualitative Evaluation

Microscopically, the cells incubated with the blank and negative control displayed no cytotoxic effect or reactivity (grade 0). The cells treated with the positive control showed total breakdown of cell layers at 100% concentration and were rated as extremely cytotoxic (grade 4). The cells incubated with the test item did not exhibit any cytotoxic reaction with no reactivity (grade 0).

3.1.2. Quantitative Evaluation

The negative control item extract-treated cells exhibited a vitality of 103.2% when compared with the blank control. The cells incubated with test item extract with different concentrations (0.1%, 1%, 10%, and 100%) displayed viability more than 100% (106.0%, 107.9%, 110.7%, and 91.9%, correspondingly). Lower vitality, ranging from 2.7% to 65.4%, has been found in the cells administered with the positive control item extraction at different concentrations (0.1%, 1%, 10%, and 100%).

3.1.3. Test Validity

The experiment has been considered acceptable because the findings met the acceptance requirements. Before treatment, the cells exhibited a confluency of about 80%. There were no significant variations noticed in the results of experiments throughout replicates. In terms of qualitative and quantitative evaluation, the positive control responded as expected.

There was not any indication that the MEM test extract was toxic under the experimental conditions. For L929 cells, the test item's MEM extract did not appear cytotoxic. The test's standards have been fulfilled by the product. The results for the positive, negative and blank control items were as expected.

3.2. Skin Sensitization

3.2.1. Mortality and Clinical Observations

None of the animals in the test or control groups experienced any mortality (Table 6). Each one of the animals remained normal through the evaluation duration (Table 7).

Table 6: Mortality and Mobility Data

Group	Sex	Animal Numbers	Mortality/Morbidity
G1 (Control)	Female	11486–11490	No mortality/morbidity observed
G2 (Test)	Female	11491–11500	No mortality/morbidity observed
G3 (Control)	Female	11501–11505	No mortality/morbidity observed
G4 (Test)	Female	11506–11515	No mortality/morbidity observed

Table 7: Individual Clinical Observations

Group	Animal Numbers	Observation Period (Days)	Observation
G1 (Control)	11486–11490	Day 0–24	All animals remained normal/alive throughout the observation period
G2 (Test)	11491–11500	Day 0–24	All animals remained normal/alive throughout the observation period
G3 (Control)	11501–11505	Day 0–24	All animals remained normal/alive throughout the observation period
G4 (Test)	11506–11515	Day 0–24	All animals remained normal/alive throughout the observation period

3.2.2. Skin Reactions Observations

The grading of skin response after the challenge phase was carried out at 24 and 48 hours after patch removal. None of the animals experienced any skin reactions throughout both of the observations (Table 8).

Table 8: Skin Reactions Observations of Challenge Phase

Group	Sex	Animal Numbers	24 h Score (Control / Test Item Extract)	48 h Score (Control / Test Item Extract)	Observation
G1 (Control)	Female	11486–11490	0 / 0	0 / 0	No dermal reaction observed
G2 (Test)	Female	11491–11500	0 / 0	0 / 0	No dermal reaction observed
G3 (Control)	Female	11501–11505	0 / 0	0 / 0	No dermal reaction observed
G4 (Test)	Female	11506–11515	0 / 0	0 / 0	No dermal reaction observed

3.2.3. Body Weight

Animals in the control and test groups showed ordinary body weight gain on Day 24 compared to Day 0.

3.2.4. Gross Pathology

At the completion of the testing period, all animals were euthanized with CO_2 exposure. External and internal thorough pathology assessments revealed no abnormalities in either of the test or control group animals.

Under the experimental conditions, both the saline and sesame oil extraction of the test item demonstrated no indication of creating delayed cutaneous contact reactivity in the guinea pig tested, which contributed to the conclusion that the product was non-sensitizing.

3.3. Skin Irritation

Each of the item extracts and its corresponding blank was assessed individually, along with the average mean result for all animals. The final test item outcome for each of the solvent vehicles was computed by integrating the total mean score of the blank and the test item extract together. The evaluation conditions had been fulfilled if the final test item extract/sample grade was less than 1.0. If, during any observation time, the average reaction to the test item extract/sample exceeds the average reaction to the blank, the test is carried out with three more rabbits. The test objectives were accepted if the final sample score was < 1.0.

In this investigation, the mean score for the polar test item extract and the polar control vehicle was 0.0. Similarly, the non-polar test item extracts and control vehicle had mean scores of 0.7. For both polar and non-polar extracts, the final test item score that is, the difference between the total test item score and the control vehicle score was found to be 0.0, which is less than 1.0. Hence, no further animal testing was conducted, as the test item meets the specifications of the test requirements.

During the period of the investigation, there were no instances of mortality or symptoms of morbidity in the treated groups (Table 9).

Table 9: Morbidity /mortality observations

Animal Number	Sex	Morbidity	Mortality
10674	Female	No	No
10675		No	No
10676		No	No

All treated animals grew in body weight as compared to day 0, which happens to be in line with predicted growth rates for this strain and age group (Table 10).

Table 10: Individual animal body weight

Animal Number	Sex	Body weight (in gram)	
		Day 0	Day 3
10674	Female	2285.8	2299.5
10675		2320.1	2379.9
10676		2309.5	2387.4

Erythema and oedema tissue responses have been monitored and scored immediately following injection, as well as 24, 48, and 72 hours later. In the injection sites of the polar test item extract and solvent control, there were neither any erythema nor oedema tissue responses. After 24 and 48 hours of observation, there was slight (barely noticeable) oedema and no erythema in the non-polar test item extract and control regions; at 72 hours, the oedema disappeared and there were no skin responses. Gross pathological examination showed no abnormalities in any of the treated animals, based on both external and internal assessments (Table 11 and Table 12).

Table 11: Intracutaneous observation of test item (left side)

Animal Numbers	Injection Site	Erythema/Eschar Score (0–72 h)	Oedema Score (0–72 h)	Total Score	Mean Score	Observation
10674–10676	Polar solvent control	0 at all observation periods	0 at all observation periods	0	0.0	No irritation observed
10674–10676	Non-polar solvent control	0 at all observation periods	Mild oedema (score 1) at 24 h and 48 h; 0 at immediate and 72 h	10	0.7	Slight transient oedema observed

Table 12: Intracutaneous observation of test item (Right side)

Animal Nos.	Injection Site	Erythema/Eschar (0–72 h)	Oedema (0–72 h)	Mean Score	Observation
10674–10676	Polar solvent control	0 at all time points	0 at all time points	0.0	No irritation observed
10674–10676	Non-polar solvent control	0 at all time points	Score 1 at 24 h and 48 h; 0 at immediate and 72 h	0.7	Slight transient oedema observed

The results showed no erythema or oedema noticed among rabbits after administering intracutaneous injections of polar test item extracts. Oedema and no erythema had been observed at 24 and 48 hours of examination of non-polar specimen extraction and control sites, respectively, while no erythema or oedema had been observed at 72 hours. The test item's final score had been determined as 0.0 for both the polar and non-polar extracts. As a result, the test item Device fulfilled the testing criteria, as the final test item scoring was less than 1.0, and it was determined that it was non-irritant.

IV. Conclusion

A systematic series of in vitro and in vivo biological investigations designed in compliance with the standards listed in ISO 10993-1 to evaluate a limited-duration, blood-contacting medical device was utilized for evaluating the biocompatibility of the pulmonary clot retrieval device. This evaluation procedure was designed according to the type of patient interaction, the period of exposure, and the desired practical application of the medicinal device, and it has been integrated into the biological risk assessment approach. Device extractions have been generated utilizing both polar and non-polar extraction solvents under exaggerated circumstances designed to improve the possible release of leachable chemicals. Under the defined test situations, in vitro cytotoxicity tests performed on L929 cell cultures exhibited no indication of cell death, growth inhibition, or morphological modifications associated with device extracts as in contrast to negative controls. Furthermore, in vivo investigations for evaluating cutaneous irritation and delayed skin hypersensitivity revealed no physiologically significant adverse reactions. Where erythema or oedema was observed, they were equivalent to control reactions and were below accepted standards for irritating or sensitizing categorization.

These findings demonstrate that, within the confines and limitations of the investigations, the construction materials, fabrication residuals, or possibly extracted substances corresponding with the pulmonary clot retrieval device did not trigger adverse biological responses such as cytotoxicity, skin irritation, or skin sensitization. The results noted above provide convincing proof for the device's biological safety for its purpose of intravascular clinical application when examined as part of a wider biologic assessment and risk management approach. Since biocompatibility evaluation is considered to be an iterative procedure, further biological evaluations may be evaluated according to necessity in light of extended clinical exposure concerns, manufacturing innovations, or alterations to the architecture of the device.

COI (Conflict of Interest)

All the authors are an employee of Meril Medical Innovations Private Limited, Vapi, Gujarat – India and declare no competing interests related to this study.

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