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Comprehensive Biocompatibility Assessment of the STARBEAM™ OCT Imaging Catheter: In-Vivo and In-Vitro approaches

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Abstract- Biocompatibility evaluation is a critical regulatory requirement for establishing the preclinical safety of medical devices in accordance with the ISO 10993 series of standards. The present study aimed to comprehensively assess the biological safety of the OCT Imaging Catheter, in-vitro and in-vivo tests selected based on its intended use and blood-contacting nature. In-vitro cytotoxicity was evaluated using L929 mouse fibroblast cells by qualitative morphological assessment and quantitative MTT assay, followed by in-vivo assessments including skin sensitization, intracutaneous irritation, acute systemic toxicity, and material-mediated pyrogenicity. Hemocompatibility was investigated through hemolysis, platelet activation, coagulation parameters, leukocyte activation, and complement activation studies. Genotoxic potential was assessed using the bacterial reverse mutation (AMES) assay and an in-vitro mammalian chromosomal aberration test in human lymphocytes. The test item demonstrated no cytotoxic effects, with cell viability exceeding ISO acceptance criteria at all extract concentrations. In-vivo studies revealed no evidence of skin sensitization, irritation, systemic toxicity, or pyrogenic response. Hemocompatibility testing confirmed the non-hemolytic nature of the device and showed no adverse effects on platelet function, coagulation pathways, leukocyte activation, or complement system activation. Genotoxicity assessments indicated that the test item was non-mutagenic and non-clastogenic under all test conditions. Collectively, the results demonstrate that the OCT Imaging Catheter exhibits an acceptable biocompatibility profile and is biologically safe for its intended clinical application. These findings support its preclinical risk assessment and provide robust evidence for regulatory submissions in compliance with ISO 10993 requirements.

Keywords – ISO 10993, Biocompatibility evaluation, OCT imaging catheter, Blood-contacting medical devices, Hemocompatibility, Preclinical safety.

I. INTRODUCTION

Biocompatibility testing is a mandatory regulatory requirement for demonstrating the preclinical safety of medical devices in accordance with internationally recognized standards such as the ISO 10993 series. These evaluations are intended to identify potential local and systemic adverse biological responses arising from contact between the device or its constituent materials and biological systems. The overall testing strategy incorporates a combination of in-vitro and in-vivo assessments selected based on the nature, duration and type of body contact.

In-vitro cytotoxicity testing represents the primary biological screening endpoint for all medical devices and evaluates the effects of device extracts on cultured mammalian cells through qualitative morphological assessment and quantitative cell viability measurements, such as the MTT assay, which reflects mitochondrial metabolic activity. In-vivo studies including skin sensitization, intracutaneous reactivity, acute systemic toxicity and material-mediated pyrogenicity are conducted to assess immunological, inflammatory, systemic, and febrile responses following exposure to device extracts.

For blood-contacting medical devices, hemocompatibility evaluations are performed to assess interactions with circulating blood and the hemostatic system. These assessments include hemolysis, thrombogenicity, coagulation parameters (e.g., PTT), platelet and leukocyte activation, complement activation, and other relevant hematological endpoints. Genotoxic potential is evaluated using bacterial reverse mutation (AMES) assay and mammalian cell chromosomal aberration tests to identify potential mutagenic and clastogenic effects.
Collectively, these studies provide a comprehensive biological safety profile that supports risk assessment and regulatory approval of medical devices [1].

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Device Description

The STARBEAM™ Optical Coherence Tomography (OCT) Imaging Catheter is a sterile, single-use device used during coronary interventional procedures to create high-resolution, real-time images of the inside of coronary arteries. It uses fiber-optic technology and near-infrared light to clearly show the vessel lumen and wall structure. The catheter has a rapid-exchange (RX) design and is compatible with 0.014″ guidewires. It consists of an outer sheath and a rotating fiber-optic imaging core. A hydrophilic distal coating helps reduce friction and improves smooth delivery through the artery. The device provides 360° imaging and supports automated or manual pullback to scan the vessel segment continuously. It includes four radiopaque markers-one distal tip marker and three proximal markers to help with positioning and identifying the imaging area under fluoroscopy. A luer fitting (connector) allows the lumen to be flushed with contrast media before imaging. The catheter connects to the STARBEAM™ OCT+IVUS Dual Imaging System Console through the Drive-motor & Optical Controller (DOC), which controls rotation and pullback of the imaging core and displays images in real time.

Intended Use

The STARBEAM™ OCT Imaging Catheter, with the STARBEAM™ OCT+IVUS Dual Imaging System, is intended for the imaging of coronary arteries and is indicated in patients who are candidates for transluminal interventional procedures. The STARBEAM™ OCT Imaging Catheter is intended for use in vessels 2.0 to 3.5 mm in diameter. The OCT Imaging Catheter is not intended for use in the left main coronary artery or in a target vessel which has undergone a previous bypass procedure.

Table 1: Size Matrix of OCT Imaging Catheter

Sr. No.	Parameters	Dimensions
1.	The total length of the imaging catheter	1670±50 mm
2.	Distal length (effective length) of the Catheter	1350±50 mm
3.	Distal outer diameter of the catheter	0.86±0.05 mm
4.	Distal radiopaque marker distance from the outer edge of the proximal radiopaque marker	20±4 mm
5.	The distal end of the imaging catheter is separated from the outer edge of the distal radiopaque marker	5±1 mm
6.	The outer edge of the proximal radiopaque marker is away from the front end of the probe	3±1 mm
7.	Maximum probe withdrawal distance	100±10 mm
8	Coating length	1000±100 mm

The schematic diagram of the STARBEAM™ Optical Coherence Tomography (OCT) Imaging Catheter illustrating the device components and their respective nomenclature is presented below,

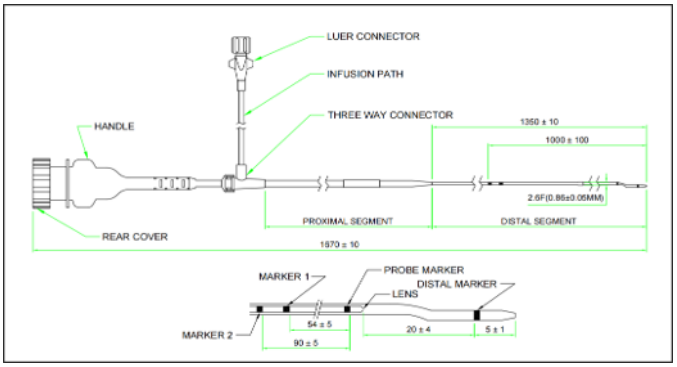


Figure 1: Schematic Diagram of OCT Imaging Catheter

OCT offers the highest spatial resolution and the most detailed visualization of vascular microstructures than IVUS system and Angiography. Its ability to accurately assess plaque characteristics, thrombus burden, vessel injury and stent-vessel interactions makes it a highly valuable tool for intravascular imaging and procedural optimization.

Representative OCT images demonstrating pre and post procedure/treatment is incorporated below for comparative evaluation and better visualization of the observed changes. The OCT images obtained are clear and predominantly golden in appearance, which enables effective interpretation of the structural observations.

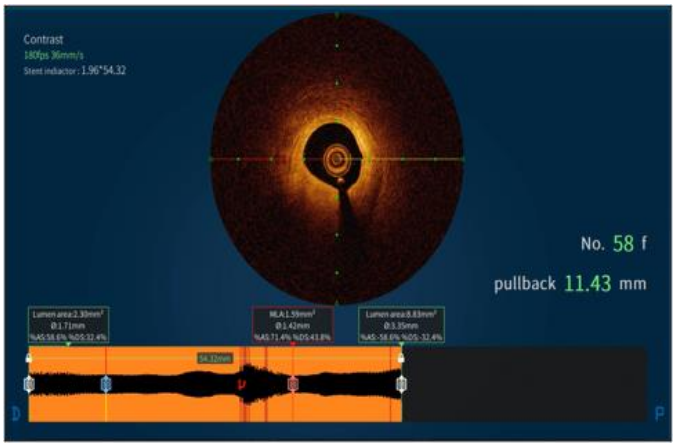


Figure 2: OCT image pre-procedure in case 1

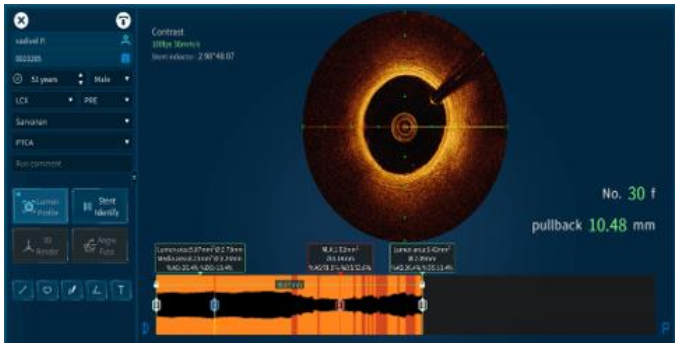


Figure 3: OCT image pre-procedure in case 2

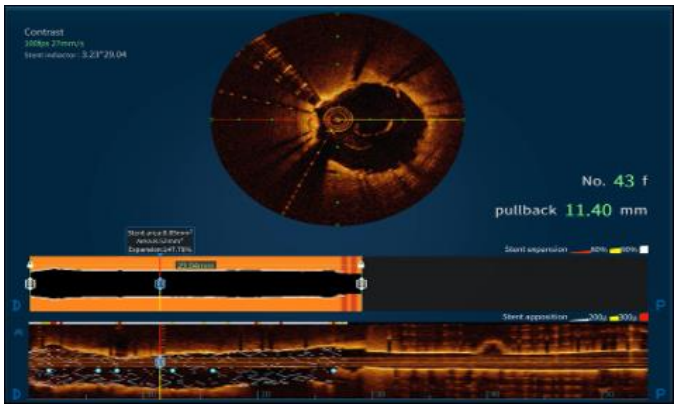


Figure 4: OCT image post procedure

Experimental Design

All biocompatibility studies were conducted under GLP-compliant conditions in accordance with relevant ISO, ASTM, and US FDA guidelines. Test selection and extraction conditions were based on the device's intended intravascular, blood-contacting use.

1. Cytotoxicity Test: Positive (ZDEC polyurethane film), negative (HDPE film), and extraction vehicle controls were prepared and extracted in complete MEM medium (MEM + 10% FBS + 1% antibiotics) under standardized conditions (37°C, ~24 h, continuous agitation) in accordance with ISO 10993-12. The L929 mouse fibroblast cell line was used as the test system. Cells were cultured, harvested using trypsin-EDTA, counted for viability, and seeded into 96-well plates at 1×10^4 cells/well. Plates were incubated for approximately 24 h to obtain a half-confluent monolayer. The test item was extracted at a ratio of 6 cm²/mL in complete MEM at 37±1°C for ~24 h with agitation. The extraction vehicle without test item served as the vehicle control. Extracts were used within 24 h without further processing. Cells were exposed in triplicate to the undiluted test extract (100%) and serial dilutions (50%, 25%, 12.5%, and 6.25%), along with positive, negative, and vehicle controls, and incubated for ~24 h. After exposure, cells were microscopically examined for morphological changes. Cytotoxicity was quantitatively assessed using the MTT assay, and absorbance was measured at 570 nm to determine cell viability as per ISO 10993-5.

2. Skin Sensitization Test: A Guinea Pig Maximization Test (GPMT) was conducted to evaluate the skin sensitization potential of the test item in accordance with ISO 10993-10:2021 / EN ISO 10993-10:2023 and US FDA guidelines. 2-Mercaptobenzothiazole was used as the positive control, supported by data from a recently completed validation study. Normal saline (0.9% w/v) and sesame oil were used as polar and non-polar extracting vehicles, respectively. Test item extracts were prepared at an exaggerated extraction ratio 6 cm²/ml and incubated at 50 ± 2°C for approximately 72 h with continuous agitation, as per ISO 10993-12. Vehicle controls were prepared similarly without the test item. Thirty male Dunkin-Hartley guinea pigs were acclimatized, randomized into four groups (polar vehicle control, polar test extract, non-polar vehicle control, and non-polar test extract), and maintained under controlled environmental conditions. Animals were prepared by clipping the dorsal and flank regions prior to dosing. Intradermal induction (day 0) was performed using three paired injections per animal, including Freund's Complete Adjuvant (FCA), vehicle control, or test item extract, depending on the group. Topical induction was carried out following mild irritation induced by 10% SDS in petroleum jelly, with occlusive patches containing vehicle or test extract applied for approximately 48 h. Challenge application (Day 21) was conducted by topical application of vehicle control and undiluted test extract on opposite flanks under occlusive dressing for 24 h. After exposure, patches were removed and sites were cleaned. All administrations and exposure routes (intra-dermal and topical) were selected in accordance with ISO 10993-10 recommendations to assess the sensitization potential of the test item.

3. Skin Irritation Test: An intracutaneous reactivity study was conducted in New Zealand White rabbits in accordance with ISO 10993-23:2021 / EN ISO 10993-23:2021 and US FDA (FR 2-291):2021 guidelines to evaluate the local tissue response of the test item. Sodium dodecyl sulfate (SDS) was used as the positive control, supported by data from a previously validated study. Normal saline (0.9% w/v) and sesame oil were used as polar and non-polar extraction vehicles, respectively, as recommended in ISO 10993-12. The finished test item was extracted at an exaggerated extraction ratio 6 cm²/ml using both vehicles and incubated at 50 ± 2°C for approximately 72 h with continuous agitation. Vehicle controls were prepared similarly without the test item. Extracts were visually examined, pH-checked, and used within 24 h without further modification. Three female rabbits were acclimatized, identified, and prepared by clipping the dorsal intrascapular region prior to dosing. Each rabbit received intracutaneous injections 200 µL/site of polar test extract, non-polar test extract, polar vehicle control, and non-polar vehicle control at five separate sites, following the prescribed site arrangement. All administrations

were performed via the intracutaneous route, which is the intended route for intracutaneous reactivity testing as per ISO 10993-23. The animals were observed for local skin reactions to assess the irritation potential of the test item.

4. Acute Systemic Toxicity Test: An acute systemic toxicity study was conducted in Swiss albino mice in accordance with ISO 10993-11:2017 / EN ISO 10993-11:2018 and US FDA guidelines. The finished medical device was extracted using polar (normal saline, 0.9% w/v) and non-polar (sesame oil) vehicles at an exaggerated extraction ratio of 6 cm²/mL, under conditions of 50 ± 2°C for ~72 h with continuous agitation, as per ISO 10993-12. A total of 20 male mice were acclimatized, randomized, and divided into four groups (n = 5/group); polar vehicle control, polar test extract, non-polar vehicle control, and non-polar test extract. Polar extracts were administered via the intravenous route, while non-polar extracts were administered via the intraperitoneal route, at a dose volume of 50 ml/kg body weight as a single administration. Animals were observed for clinical signs, morbidity, and mortality following dosing. All housing, environmental conditions, feeding, and animal management procedures were maintained under GLP-compliant conditions throughout the study.

5. Pyrogenicity Test: A rabbit pyrogen test was conducted in New Zealand White male rabbits in accordance with ISO/TR 21582:2021, USP, EP, BP, IP, and US FDA guidelines. The finished medical device was extracted using pyrogen-free normal saline (Sodium Chloride Injection IP, 0.9% w/v) as the polar extraction medium. The test item was cut into pieces and extracted at an exaggerated ratio 6 cm²/mL under conditions of 50 ± 2°C for approximately 72 h with continuous agitation, as per ISO 10993-12. The extract was cooled to room temperature, visually examined, and used within 24 h without any modification. Three rabbits were acclimatized, fasted overnight, and prepared for dosing. Baseline rectal temperatures were recorded prior to treatment. The test extract was warmed to 37°C and administered as a single intravenous dose via the marginal ear vein at a volume of 10 ml/kg body weight using pyrogen-free syringes and needles. Post-administration, animals were monitored for changes in body temperature to evaluate the pyrogenic response of the test item under GLP-compliant conditions.

II. HEMOCOMPATIBILITY TEST

In-Vitro Hemolysis test (Direct Contact):
An in-vitro hemolysis study (direct method) was conducted using pooled rabbit blood in accordance with ISO 10993-4, ASTM F756, and US FDA guidelines to evaluate the hemocompatibility of the test item. Fresh blood was collected from three male New Zealand White rabbits using sodium citrate as an anticoagulant and pooled. Plasma free hemoglobin was first determined using Drabkin's reagent to ensure suitability of blood (< 2 mg/mL). Total blood hemoglobin was measured, and the blood was diluted with calcium and magnesium-free PBS (CMF-PBS) to adjust hemoglobin concentration to 10 mg/mL. A hemoglobin standard calibration curve was established using Drabkin's reagent, and absorbance was measured at 540 nm. The test item (direct contact components), negative control (HDPE), positive control (sterile water for injection), and blank were prepared at an exposure ratio of 6 cm²/mL as per ASTM F756. Samples were incubated with diluted blood at 37°C for 3 hours with periodic mixing. After incubation, samples were centrifuged, and the supernatant was collected. The supernatant was reacted with Drabkin's reagent, incubated for 15 minutes, and absorbance was measured at 540 nm. Hemoglobin concentration was calculated from the standard curve, and blank-corrected percent hemolysis was determined. The average hemolytic index of the test item was calculated and compared against negative and positive controls to assess hemolytic potential under GLP-compliant conditions.

In-Vitro Hemolysis test (Indirect Contact): An in-vitro hemolysis study (indirect contact method) was performed in accordance with ISO 10993-4, ASTM F756, and US FDA guidelines using fresh pooled rabbit blood anticoagulated with sodium citrate. A hemoglobin standard curve was established using bovine hemoglobin powder prepared in Drabkin's reagent, with absorbance measured at 540 nm. Plasma free hemoglobin was first determined and confirmed to be < 2 mg/mL, ensuring suitability of the blood. Total blood hemoglobin was measured and subsequently diluted with calcium and magnesium-free phosphate buffered saline (CMF-PBS) to achieve a final hemoglobin concentration of 10 mg/mL. The test item, negative control (HDPE), positive control (sterile water for injection), and blank (CMF-PBS) were extracted at an exaggerated ratio of 6 cm²/mL and incubated at 50 ± 2 °C for ~72 h with continuous agitation, as per ISO 10993-12. Extracts were cooled to room temperature and used without further modification. For exposure, 1 ml of diluted blood was mixed with 7 ml of each extract and incubated at 37°C for 3 hours with periodic mixing. Following incubation, samples were centrifuged, and the supernatant was collected. Aliquots of supernatant were reacted with Drabkin's reagent, incubated for 15 minutes, and absorbance was measured at 540 nm. Hemoglobin concentration was calculated from the standard curve, and blank-corrected percent hemolysis and average hemolytic index were determined to assess the hemolytic potential of the test item under GLP-compliant conditions.

Platelet Count and PF4 Estimation: An in-vitro hematology and platelet activation study was conducted in accordance with ISO 10993-4, ASTM F2888, and US FDA guidelines using fresh human whole blood. Fresh blood was collected from three healthy male donors (25–31 years), pre-screened for normal leukocyte and platelet counts. Donors had not taken NSAIDs, antithrombotic, or anti-inflammatory drugs for at least 10 days

prior to collection. Blood was anticoagulated with 3.2% sodium citrate (9:1 v/v), pooled, and used within 8 hours of collection. The test item, positive control (glass beads), and negative reference control (HDPE) were prepared from finished products. Untreated human blood served as the negative control. Test and control materials were exposed to blood at a ratio of 12 cm² material per 1 mL of blood. Prior to exposure, blood was re-calcified with $CaCl_2$ (10 mm final concentration) and supplemented with heparin (2 U/ml). Samples were incubated for 1 hour at 37°C in an orbital shaker (60 rpm) in triplicate. Following incubation, EDTA (5 mm final concentration) was added to terminate further reactions. Blood was then separated from materials, gently mixed, and analyzed for hematological parameters, including platelet count, using a hematology analyzer. Remaining blood samples were centrifuged to obtain plasma for platelet factor 4 (PF4) estimation. PF4 levels were quantified using a human PF4 ELISA kit. Plasma samples were diluted 1:200, standards were prepared by serial dilution, and the ELISA was performed as per the manufacturer's instructions. Absorbance was measured at 450 nm, and PF4 concentrations were calculated using the standard curve. The results were used to evaluate the hematological compatibility and platelet activation potential of the test item under GLP-compliant conditions.

In-vitro Assessment of Partial Thromboplastin Time, Thrombin and Fibrin Formation: An in-vitro coagulation and thrombogenicity assessment was conducted in accordance with ISO 10993-4, ASTM F2382, and US FDA guidelines using sodium-citrated human blood plasma. The test item, positive control (glass beads), negative reference control (HDPE), and negative control (plasma only) were prepared from finished products. Test and control materials were exposed to plasma at a ratio of 6 cm² material per 1 ml plasma. All samples were incubated at 37°C for 15 minutes in a metabolic shaker bath at 60 rpm, in triplicate. Following incubation, plasma was transferred to pre-chilled tubes and used for activated partial thromboplastin time (aPTT/PTT) assay. Plasma aliquots were equilibrated at 37°C, mixed with rabbit brain cephalin, incubated, and re-calcified with 25 mm calcium chloride.

Clot formation time was measured using a coagulation analyzer. Remaining plasma samples were used for fibrin and thrombin–antithrombin complex (TAT) estimations. Fibrin concentration was quantified using a human fibrin ELISA kit after appropriate dilution, with absorbance measured at 450 nm against a standard curve. TAT levels were determined using a human TAT ELISA kit following the manufacturer's protocol, and absorbance was read at 450 nm. The results were used to evaluate the coagulation activation and thrombogenic potential of the test item in comparison with controls under GLP-compliant conditions.

Complete Blood Count by Leukocyte Activation Test of Human Blood: An in-vitro hematological compatibility and leukocyte activation study was conducted in accordance with ISO 10993-4 using fresh pooled human whole blood. Fresh blood from three healthy male donors (23–28 years) was pre-screened for normal leukocyte and platelet counts and collected in 3.2% sodium citrate (9:1 v/v). Donors had not taken NSAIDs, acetaminophen, or antithrombotic drugs for at least 10 days prior to collection. Blood was pooled and used within 8 hours. The test item, positive control (glass beads), negative reference control (HDPE), and negative control (blood only) were prepared from finished products. Materials were pre-wetted with saline and exposed to blood at a ratio of 12 cm² material per 1 ml of blood. Prior to exposure, blood was re-calcified with $CaCl_2$ (10 mm final concentration) and supplemented with heparin (2 IU/ml). Samples were incubated for 1 hour at 37°C in an orbital shaking incubator (60 rpm) in triplicate. After incubation, EDTA (5 mm final concentration) was added to terminate further reactions. Blood was then separated from materials, and aliquots were analyzed for complete blood count (CBC) using a hematology analyzer. Remaining blood samples were centrifuged to obtain plasma, which was used to evaluate leukocyte activation by measuring neutrophil elastase levels using an ELISA method. Plasma samples were diluted 1:4, standards were prepared by serial dilution, and the ELISA was performed as per the manufacturer's instructions. Absorbance was measured at 450 nm, and neutrophil elastase concentrations were calculated using the standard curve. The results were used to assess the hematological compatibility and leukocyte activation potential of the test item under GLP-compliant conditions.

Complement Activation Test by using ELISA method: An in-vitro complement activation study (SC5b-9) was performed in accordance with ISO 10993-4 using freshly fractionated human serum. The test item, positive control (cellulose acetate), negative control (polypropylene), blank control (serum only), and kit-provided SC5b-9 high and low controls were evaluated. Human serum collected in sodium citrate was stored at 2–8°C and used for testing. The test item was prepared from the finished product, aseptically cut into small pieces and extracted in human serum at a ratio of 6 cm²/ml in accordance with ISO 10993-12, based on limited exposure and thickness <0.5 mm. Extraction was carried out at 37 ± 1°C for 60 minutes, ensuring complete immersion. Serum appearance, color, turbidity, and particulate matter were assessed before and after extraction. Serum extracts and controls were diluted at 1:40 and 1:200 as required for ELISA analysis. Complement activation was quantified by measuring SC5b-9 levels using a commercial MicroVue™ Complement SC5b-9 Plus ELISA kit, following the manufacturer's instructions. Standards and controls were run in duplicate, while samples were analyzed as specified by the protocol. After incubation, washing, conjugate reaction, substrate development, and stopping of the enzymatic reaction, absorbance was measured at 450 nm using a microplate reader.

SC5b-9 concentrations were calculated from the standard curve and used to assess the complement activation potential of the test item under GLP-compliant conditions.

III. GENOTOXICITY

Bacterial Reverse Mutation Test: A Bacterial Reverse Mutation Test (AMES Test) was conducted in accordance with ISO 10993-3 and ISO 10993-12 using Salmonella typhimurium strains TA98, TA100, TA102, TA1535, and TA1537, both with and without metabolic activation (S9 mix). The test item extracts were prepared from the finished medical device using polar (water) and non-polar (DMSO) vehicles at an exaggerated extraction ratio of 6 cm^2/ml, under 50±2°C for approximately 72 hours with continuous agitation (80 rpm), as recommended for limited exposure devices (<24 h). Corresponding vehicle controls were prepared under identical conditions. Bacterial cultures were grown to the required density (~1–2 × 10^9 cells/ml) and verified for genetic integrity and sensitivity. A dose range finding (DRF) study was first performed using undiluted (100%) neat extracts to assess cytotoxicity. As no cytotoxicity was observed, the limit test was conducted using 100 µl/plate of undiluted extracts. The plate incorporation method was employed. For each strain, the test item extract, vehicle control, and concurrent strain-specific positive controls were tested in triplicate, both in the absence and presence of 5% (v/v) S9 metabolic activation system. The test mixtures containing bacterial suspension, extract or control, and overlay agar (with or without S9 mix) were poured onto minimal agar plates and allowed to solidify. Plates were incubated in an inverted position at 37°C for approximately 48 hours, after which revertant colonies were counted. The number of revertants in test item–treated plates was compared with concurrent vehicle controls to evaluate the mutagenic potential of the test item under GLP-compliant conditions.

In-Vitro Mammalian Chromosomal Aberration Test using Human Peripheral Blood Lymphocytes: An in-vitro chromosomal aberration test was conducted in accordance with ISO 10993-3 and ISO 10993-12 using human peripheral blood lymphocytes, both with and without metabolic activation (S9 mix). The test item extract was prepared from the finished medical device using RPMI 1640 medium supplemented with 10% fetal bovine serum and antibiotics at an exaggerated extraction ratio of 6 cm^2/ml, under 37±1°C for approximately 72 hours with continuous agitation, as recommended for limited exposure devices (<24 h). The extraction medium alone served as the vehicle control. Human whole blood from healthy donors was cultured in RPMI 1640 growth medium supplemented with 20% FBS, PHA-M, and antibiotics, and incubated at 37°C with 5% CO_2 to stimulate lymphocyte proliferation. A preliminary cytotoxicity (dose range finding) study was performed using 100% (neat) test item extract under short-term (3–6 h) treatment with and without S9 and long-term (≈24 h) treatment without S9. As no cytotoxicity was observed, a limit test was conducted using the neat extract. Cultures were treated with the test item extract, vehicle control, and positive controls (Mitomycin-C without S9 and Cyclophosphamide with S9). After treatment, cultures were incubated under the specified conditions, and colchicine was added prior to harvesting to arrest cells in metaphase. Cells were harvested by hypotonic KCl treatment, fixed with Carnoy's fixative, and slides were prepared and stained with 5% Giemsa. Slides were microscopically evaluated to determine the mitotic index (cytotoxicity) and to assess chromosomal aberrations in metaphase cells with acceptable chromosome numbers (46 ± 2). The results were used to evaluate the clastogenic potential of the test item under GLP-compliant conditions.

IV. OBSERVATIONS

1. Cytotoxicity test: Microscopic examination of cells exposed to the test item extracts showed normal cell morphology with no significant evidence of cell lysis, vacuolization, or detachment. The observed reactivity grades were ≤ 2, indicating no cytotoxic effect. In the MTT assay, a clear purple formazan color was observed in the test wells, comparable to the vehicle control, confirming the presence of viable and metabolically active cells. No yellowish or colorless wells were observed for the test item. Quantitative spectrophotometric analysis at 570 nm showed that cell viability for the test item extracts was ≥ 70% of the vehicle control. In addition, the 50% extract demonstrated equal or higher cell viability compared to the 100% extract. Based on these findings, the test item was considered non-cytotoxic under the conditions of the study.

% cell viability was calculated using following equation:
% cell viability =OD570e – OD570b/ OD570c – OD570b × 100

Where,

- **OD570e:** The mean value of the measured optical density of the extracts of the test sample, negative control or positive control.
- **OD570c:** The mean value of the measured optical density of the vehicle control.
- **OD570b:** The mean value of the measured optical density of the blank control.
- **Vehicle Control** : Reactivity (None) Grade 0 Positive Control (ZDEC) : Reactivity (Severe) Grade:4

Negative Control (HDPE): Reactivity (None) Grade: 0
Test Item D0 : 100% , Reactivity (None) Grade: 0

Test Item D1: 50%, Reactivity (None)
Grade: 0 Test Item D2 : 25%, Reactivity (None) Grade: 0
Test Item D3 : 12.5% , Reactivity (None) Grade: 0 Test Item D4 : 6.25% , Reactivity (None) Grade: 0

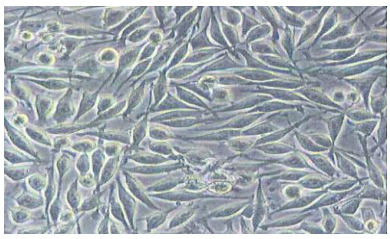
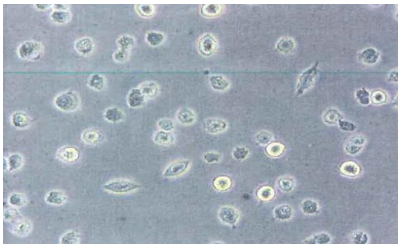
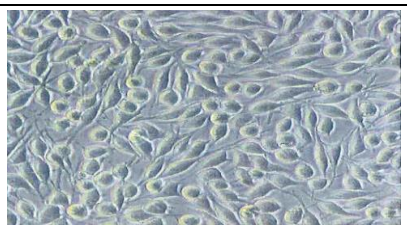
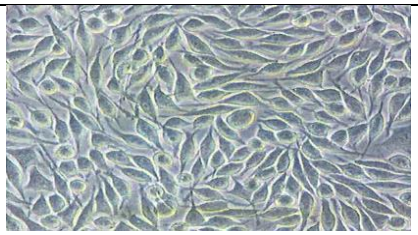
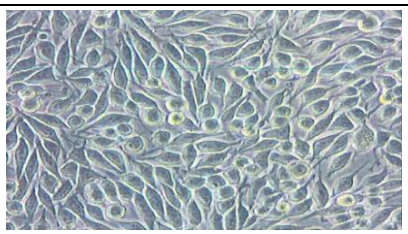
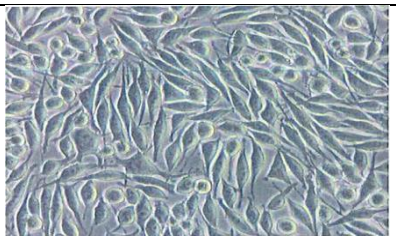
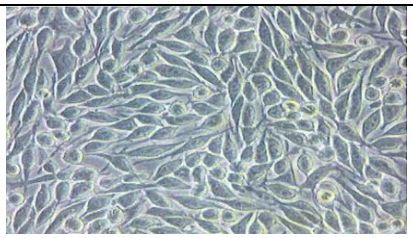
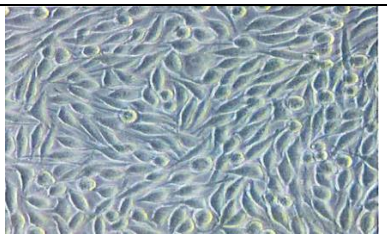
	
Vehicle Control : Reactivity (None) Grade 0	**Positive Control (ZDEC) : Reactivity (Severe) Grade:4**
	
Negative Control (HDPE): Reactivity (None) Grade: 0	**Test Item D0 : 100% , Reactivity (None) Grade: 0**
	
Test Item D1: 50%, Reactivity (None) Grade: 0	**Test Item D2 : 25%, Reactivity (None) Grade: 0**
	
Test Item D3 : 12.5% , Reactivity (None) Grade: 0	**Test Item D4 : 6.25% , Reactivity (None) Grade: 0**

Figure 2: Representative Experimental Photographs of L929 Cell Line

2. Skin Sensitization Test: No mortality or morbidity was observed in any animal throughout the study period. All animals remained clinically normal with no treatment-related adverse signs. Body weight of all animals showed normal progression from day 0 to day 24, with no abnormal losses observed. Dermal observations at induction and challenge sites did not show any treatment-related irritation or sensitization reactions in the test or vehicle control groups. The challenge sites of test animals showed scores of 0 to 1 on the Magnusson and Kligman scale, comparable to the vehicle control group. The acceptance criteria were met, as the positive control produced a clear sensitization response while no sensitization was observed in the vehicle control group. Therefore, the study was considered valid.

Sensitization Rate (%) = No. of animals with positive reaction /No. of tested animal×100.

3. Skin Irritation Test: No mortality or morbidity was observed in any animal during the study period. All animals remained clinically normal, with no treatment-related adverse signs. Body weights recorded before treatment and at the end of the observation period were within the normal range, with no abnormal changes. No significant erythema or oedema was observed at any injection site at 23, 47 or 72 hours after dosing. Dermal reaction scores for both the test item and the vehicle control were minimal and comparable. The difference between the mean irritation score of the test item and the corresponding vehicle control was zero, meeting the acceptance criteria. Based on these observations, the test item was considered non-irritant under the conditions of the study.

4. Acute Systemic Toxicity: The following observations were made during the course of the study.

- **Mortality/Morbidity:** Mortality/Morbidity was checked twice daily throughout the study period.
- **Clinical Sign**: All the animals were observed for clinical signs once daily throughout the study period.
- **Body Weight**: Individual animal body weight was recorded on day 1 (prior to treatment) and on day 2, 3, 4 and on day 7. The body weight data is expressed as Mean ± SD.
- **Clinical Pathology:** No hematology and clinical chemistry analysis were carried out because no adverse effects were observed in test item treated groups as well as vehicle control groups shown any signs of adverse effects.
- **Gross Pathology:** At the end of the observation period, all animals were euthanized by CO2 asphyxiation followed by exsanguination and subjected to gross necropsy, including examination of external surfaces, orifices and thoracic/abdominal cavities. Histopathology was not performed in any of the treated animals as no gross lesions were observed.

Pyrogenicity Test: The following observations were made during the course of the study.

- **Mortality/Morbidity:** Animals were checked for occurrence of mortality/morbidity twice in day on day 1 of treatment.
- **Clinical Sign**: Animals were checked once in a day for clinical signs on day 1 of treatment.
- **Body Weight:** Individual animal body weight was recorded on day 1 prior to treatment.
- **Recording of Animal Temperature:** Initial animal temperature was determined for each rabbit at 30 minutes before treatment was considered as control temperature reading which was compared with animal temperature observed after treatment of test extract injection. Following treatment, animal temperature was recorded at 30 minutes intervals up to 3 h subsequent to the injection of polar test item extract. After completion of test, all animals were returned to animal facility without any further investigation.

VI. HEMOCOMPATIBILITY TEST

In-Vitro Hemolysis test (Direct & Indirect Contact):
Calculation for Percent Hemolysis: The percent hemolysis was calculated for test item, positive control and negative control using following equation:
% Hemolysis Supernatant hemoglobin concentration=Total hemoglobin concentration in tube× 100
Where, Supernatant hemoglobin concentration= Abs (Sample) × Slope × 2
Total hemoglobin concentration in tube = Total Diluted Blood Hemoglobin concentration / 8

Calculation for Blank corrected % Hemolysis: The blank corrected percent hemolysis was calculated for test item, positive control and negative control using following equation:
Blank corrected % Hemolysis =Abs (Sample)-Abs (Blank)/Abs (Diluted Blood) - Abs (Blank) ×100

Calculation for % Hemolytic Index: An average hemolytic index of test item was calculated compared to negative control. A hemolytic index of less than 2% was considered as non-hemolytic.
Hemolytic Index = % Mean Blank Corrected Hemolysis of test item or positive control - % Mean Blank Corrected Hemolysis Negative control

Platelet Count and PF4 Estimation: a) Platelet Count: To account for inherent measurement variation of the hematology analyzer, two readings for each sample was measured and average reading was reported.

b) Calculation: For each replicate, the average cell counts (from two readings) of the test sample, negative reference control and positive control were normalized to the negative

control (untreated blood) values. In each case, the cell count percentage relative to the negative control was calculated as:
A/B × 100 = C
Where, A= average count (platelet) of the test item replicate
B = Average count (platelet) of negative control (untreated blood)
C = Percentage (%) of negative control

Percentage (%) of Test Item compared to Negative Control= Average Platelet Count of the Test Item / Average Platelet Count of Negative Control × 100
The platelet count of the test item and negative reference control was 115.68% and positive control value was 52.40%.
c) Visual observation for PF4: After addition of substrate, blue color was observed and after addition of stop solution, yellow color was observed which is proportional to the amount of PF4 in the sample.

d) Biochemical Analysis: The test item group exhibited PF4 activation similar to that of the negative reference control while a lower level was observed compared to the positive control. The test sample result (% negative control) for test item, negative reference control and positive control sample mean was calculated as below:

% Negative Control= Average clotting time of sample (s)/ Average clotting time of negative control (s) ×100
Mean and SD for partial thromboplastin time was calculated for test item, negative control, negative reference control and positive control. The values for the test item, negative control, negative reference control and positive control were compared to each other with Analysis of Variance (ANOVA) including Tukey test. No statistically significant difference was observed between test item and negative control.

Complete Blood Count by Leukocyte Activation Test of Human Blood: The CBC of the test item was found to be within the range of the negative control and negative reference control. No test item related changes were observed in hematology parameters. For Neutrophil Elastase estimation, mean absorbance for each set of duplicate standards and triplicate samples were determined. A graph was plotted with the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Based on the concentrations of the standard, unknown Human Neutrophil Elastase was determined. Low levels of Neutrophil Elastase formation were observed in the test item as compared to the positive control.

Complement Activation Test by using ELISA method: a) Visual observation: Blue colour was observed after addition of substrate and yellow colour was observed after addition of stop solution.

b) Evaluation and interpretation of results: The average of every duplicate reading for the standard, control and test sample was calculated. The reading of blank was subtracted from each average experimental value of all controls and test sample. The standard curve was generated for each standard (on the Y-axis) and concentration for each standard (on the X-axis). The values of vehicle control, negative control, positive control and test item were graphed manually and read directly from the best-fit line of the standard curve. Test sample evaluation was made by statistical comparison of test sample results to negative controls. The dilution factor was considered during the analysis. The experimental value of complement activation was found to be maximum for positive control as compare to other conditions whereas minimum for negative control. The percentage of activation was calculated with comparing positive control.

VII. GENOTOXICITY

Bacterial Reverse Mutation Test: The following observations were made during the DRF and limit study.
a) Plate Count Results: Revertant colonies in each plate were counted manually and background bacterial lawn was observed under microscope. The number of revertant colonies in neat extract (100%) along with concurrent controls (vehicle and positive) for DRF and Limit study has been provided.

b) Data Recording: Data has been presented as the number of revertant colonies per plate in the tabular format. Individual plate counts, background bacterial lawn, the mean number of revertant colonies per plate and the standard deviation have been presented for neat extract (100%) along with concurrent controls (vehicle and positive) for DRF and Limit study have been provided.

c) Acceptability of the Assay: The assay was considered acceptable as it meets the following criteria; Regular background growth was observed in the vehicle control and the positive control produced a significant increase in mutant colony frequencies.

In-Vitro Mammalian Chromosomal Aberration Test using Human Peripheral Blood Lymphocytes: All slides from the test item, vehicle control and positive control groups were evaluated under blinded conditions to ensure unbiased microscopic assessment. A sufficient number of cells and metaphases were analyzed for each concentration to reliably detect cytogenetic effects. The mitotic index and relative mitotic index values indicated that the test item did not cause excessive cytotoxicity or inhibit cell division. Structural chromosomal aberrations, including chromatid and chromosome-type changes, were carefully recorded and compared between the test item–treated cultures and the vehicle control. No statistically significant increase in chromosomal aberrations was observed in any of the test item concentrations across all experimental phases. In contrast, the positive control produced a clear and statistically significant

increase in aberrations, demonstrating the sensitivity and validity of the assay. All acceptability criteria were met, including appropriate performance of controls, adequate cell proliferation, and sufficient analyzable concentrations. Based on these findings, the test item did not induce chromosomal damage and was therefore concluded to be non-clastogenic under the conditions of the study.

Mitotic Index (%) = Number of mitotic cells / Total number of cells scored × 100
RMI (%) = Test Concentration MI / Solvent Control MI × 100
RESULTS

1. Cytotoxicity Test: Microscopic evaluation showed that the test item extracts at all concentrations, including the undiluted extract, produced no morphological changes in L929 cells and were graded as 0 (no reactivity), indicating absence of cytotoxicity. In contrast, the positive control showed severe cytotoxic effects, confirming the validity of the assay. The MTT assay further supported these findings, with the undiluted test item extract showing 93.56% cell viability and all diluted extracts maintaining cell viability greater than 70%, which meets the acceptance criteria of ISO 10993-5. Strong purple formazan formation was observed in all test item wells, reflecting preserved cellular metabolic activity. Based on both qualitative and quantitative assessments, the test item was found to be non-cytotoxic to L929 mouse fibroblast cells under the conditions of the study. Based on qualitative observations and quantitative evaluation of this study, test item 'OCT Imaging Catheter' was found to be Non-Cytotoxic at undiluted (100%) and all diluted concentrations (50%, 25%, 12.5% and 6.25%) of test item extract after treatment under the test conditions as described in ISO.

2. Skin Sensitization Test: No mortality, morbidity, or abnormal clinical signs were observed in any animals during the study. All animals showed normal body weight gain from day 0 to day 24. No treatment-related dermal reactions were observed at the test item injection site (Site B) during induction, before topical induction, or after challenge. Mild erythema observed at sites A and C was attributed to the local effect of Freund's Complete Adjuvant (FCA) and not to the test item. The mean sensitization score for the test group was 0, and the sensitization rate was 0%, comparable to the vehicle control. No re-challenge was required. Overall, the test item was found to be non-sensitizing under the conditions of the study. Based on the results, it was concluded that the test item 'OCT Imaging Catheter' did not induce skin sensitization while evaluated using polar (normal saline) and non-polar (sesame oil) extracts in Dunkin Hartley guinea pigs. Hence, the test item was considered a "Non-sensitizer" under the conditions and procedures followed in the present study.

3. Skin Irritation Test: No mortality, morbidity, or abnormal clinical signs were observed in any animals during the study. Body weights remained within the normal range throughout the observation period. No erythema or oedema was observed at any time point for either the test extracts or the vehicle control. Overall, the test item was non-irritant under the study conditions. Administration of polar and non-polar extracts of test item 'OCT Imaging Catheter' in New Zealand White Rabbits via intracutaneous route, did not result in any skin reactions at site of injection. Under the tested conditions, the test item was found non-irritant.

4. Acute Systemic Toxicity Test: No mortality, morbidity, or abnormal clinical signs were observed in any animals treated with the polar or non-polar extracts or the vehicle control. Body weights increased normally throughout the study, with no statistically significant differences between groups. Gross pathological examination revealed no abnormalities. Based on these findings, the test item did not produce any systemic toxic effects and was considered non-toxic under the conditions of the study. Based on the results of present study, it could be concluded that, the test item 'OCT Imaging Catheter' did not produce any systemic toxicity when polar and non-polar test item extracts were administered through intravenous and intraperitoneal route in swiss albino mice, under the conditions and procedures followed in the study.

5. Pyrogenicity Test: No mortality, morbidity, or abnormal clinical signs were observed in animals treated with the test item extract. Body weights were within the required range (1.757–1.971 kg). Individual animal temperatures showed minimal change (<0.5 °C) compared to controls. Overall, the test item was non-pyrogenic and well tolerated under the study conditions. Based on the results of the present study, it could be concluded that, the test item 'OCT Imaging Catheter' was found to be "Non-pyrogenic" when the polar extract of the test item was administered intravenously to New Zealand White Rabbits, following the conditions and procedures outlined in the study.

6. Hemocompatibility Test

In-Vitro Hemolysis test (Direct Contact): The hemolytic index of test item, as per direct contact method was found to be 0.730 % (< 2%) and the same was graded as non-hemolytic. The hemolytic index of positive control, as per direct contact method was found to be 87.020% and the same was graded as hemolytic. The test item 'OCT Imaging Catheter' was found non-hemolytic to rabbit whole blood when evaluated according to direct contact method as per ISO 10993-4.

In-Vitro Hemolysis test (Indirect Contact): The hemolytic index of test item, as per Indirect Contact Method was found to be -0.150 % and it is equivalent to 0 (< 2%) and the same was graded as non-hemolytic. The hemolytic index of positive control, as per Indirect Contact Method was found to be 89.260 % and the same was graded as Hemolytic. The test item 'OCT Imaging Catheter' was found non-Hemolytic to rabbit whole blood when evaluated according to Indirect Contact Method as per ISO 10993-4.

Platelet Count and PF4 Estimation: Based on the results, it was observed that the platelet count of test item treated with blood was within the range of the negative reference control material (that is, 80-120% of the negative control blood) and the test item was 63.28 % above that of the positive control material. The Platelet Factor 4 ELISA showed almost similar result in test group as compared to the negative reference control and negative control in human plasma. Hence, it could be concluded that the test item did not have any effect of the platelet based on the conditions and procedures followed in the present study.

In-vitro Assessment of Partial Thromboplastin Time, Thrombin and Fibrin Formation: The partial thromboplastin time of the test item 'OCT Imaging Catheter' was found to be within the acceptable range of the negative reference control. The test item group exhibited Fibrin and TAT formation similar to that of the negative control, while a lower level was observed compared to the positive control. No statistically significant difference in partial thromboplastin time was observed between the test item and either the negative control and negative reference control. Hence, it was concluded that the test item did not have any adverse effect on human plasma when evaluated according to ISO 10993-4.

Complete Blood Count by Leukocyte Activation Test of Human Blood: The CBC of the test item was found to be within the range of negative control and negative reference control. Neutrophil Elastase estimation showed low levels of Neutrophil Elastase formation in test item group as compared to positive control. Based on the results, it was observed that the CBC and Neutrophil Elastase formation of the test item treated blood was within the acceptable range of the negative control and negative reference control material. Hence, it was concluded that the test item 'OCT Imaging Catheter' did not have any adverse effect on human blood.

Complement Activation Test by using ELISA method: The test item was incubated with serum to evaluate the potential of complement activation components. The analysis was performed on the basis of the ELISA assay as the quantitative evaluation. In negative control, it was observed that the complement activation was non-reactive during exposure whereas the positive control had produced a significant effect on the complement system activation in the assay. The complement system activation was not observed for the test item. Based on the quantitative evaluation of this study, the test item was a found non- reactive to the complement system when exposed to human serum.

VIII. GENOTOXICITY

Bacterial Reverse Mutation Test: In the dose range finding (DRF) study, the 100% test item extract showed normal growth of bacterial lawns and no cytotoxic effects in any of the five S. typhimurium tester strains (TA 98, TA 100, TA 102, TA 1535, TA 1537), both in the presence and absence of metabolic activation (5% S9 mix). The number of revertant colonies for the test item was similar to the vehicle control and significantly lower than the positive control, indicating no induction of mutations. Based on these findings, the same results were used in the limit study to assess mutagenicity. No increase in revertant colonies was observed under any conditions, confirming that the test item did not cause mutagenic effects in any of the tested strains. The neat extract (100%) of the OCT Imaging Catheter was non-mutagenic, both with and without metabolic activation, under the conditions of the study, in accordance with ISO 10993-3 and relevant guidelines.

In-Vitro Mammalian Chromosomal Aberration Test using Human Peripheral Blood Lymphocytes: The vehicle control was considered acceptable as found within the range of historical control database available for the same assay whereas concurrent positive controls were considered acceptable as induced responses that are compatible with the assay requirements. The results obtained with the test item did not show any increase in the incidence of cells with aberrant chromosomes in all the tested conditions. There was no increase in numerical aberrations in the conditions tested. Under the test conditions used in this study, it is concluded that 'OCT Imaging Catheter' did not induce chromosome damage in human lymphocytes. Hence, it can be concluded that the test item was found to be non-clastogenic under the condition and procedures followed in the present study hence complying with the ISO 10993-3. [2-7]

IX. CONCLUSION

This study was conducted to evaluate the biocompatibility and safety of the OCT Imaging Catheter through a comprehensive series of in-vitro and in-vivo assessments, including cytotoxicity, skin sensitization, skin irritation, acute systemic toxicity, pyrogenicity, hemocompatibility, and genotoxicity, following ISO 10993 guidelines. Across all endpoints, the OCT Imaging Catheter demonstrated an excellent safety profile: it was non-cytotoxic, non-sensitizing, non-irritant, non-toxic systemically, non-pyrogenic, hemocompatible, and non-genotoxic. No adverse effects were observed on cell viability, platelet function, complement activation, or chromosomal integrity under the tested conditions.

Overall, the OCT Imaging Catheter demonstrated excellent biocompatibility and a favorable safety profile across all evaluated endpoints. The absence of cytotoxic, sensitization, irritant, systemic toxic, pyrogenic, hemocompatibility, and genotoxic effects further support the safety and reliability of the device under the tested conditions.

These preclinical findings provide strong supportive evidence for the biological safety of the OCT Imaging Catheter and

confirm its suitability for further clinical evaluation as a safe and reliable intravascular imaging tool.

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