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Assessment of local tissue response after the subcutaneous administration of liquid embolic system

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Abstract
Medical devices may provide assistance towards the generation of healthy biological tissues as well as due to intervention as a foreign body for long term in biological system, the device can integrate into the adjacent biological tissues over time. To ensure intended functions as well as compliance of all the regulatory requirements, medical devices are subjected to robust biocompatibility testing, biological evaluation, toxicological evaluations so as to avoid any adverse health effects. Embolization procedure is the process of intentional clot formation for occluding blood vessels for therapy purpose. Host related factors like age, nutritional status, body mass index, previous interventions at the treatment site also play a vital role in response to the clinical application. In the present study, effort has been made to understand the local tissue response in the form of skin reaction as erythema and edema after administering liquid embolic system in the subcutaneous tissue. On intracutaneous injection of liquid embolic system in rabbits, no irritation reactions were observed on visual examination and on graded scores of erythema and eschar formation, as well as oedema formation observed till 72 hours of dose administration. It is concluded that the embolic system is non-irritant as it didn't elicit any signs of local toxicity.

Keywords: Biocompatibility, embolic system, embolotherapy, implantation, biomaterial, irritation

1. Introduction
Naturally derived materials are different from synthetic biomaterials due to difference in physico-chemical and biological properties and difference in the ability to discharge bioactive ligands in biological environments. Biomaterial is any substance that is not a drug but is suitable for insertion in biological system to treat or to replace the function of body tissues or organs (Tathe *et al*. 2010, Wagner *et al*. 2020, Chakrabarty 2011) [13, 15, 3] Biomaterials can be divided into three major categories, i.e., bio-inert, bioactive and bio-resorbable. Bio-inert material are those which have minimal interaction with the surrounding biological tissues e.g., stainless steel, polyethylene with ultra-high molecular weight. Bioactive materials are the materials which when places with the tissues promote regeneration of tissues e.g., bio-glass, synthetic glass ceramics. Bio-resorbable biomaterials resorb and get relaced with surrounding tissues on being placed in biological surrounding of tissues e.g., gypsum, tricalcium phosphate (Iftekhar 2004, Davis 2003) [7, 4].

The hazards posed by usage of medical devices include the proposed anatomical location, duration of exposure, the frequency of exposure and intended use by population. Such risks include toxicity due to chemical characteristics, undesired biological response to physical properties of the device, which may give rise to biological response. Considering the biological evaluation, the safety assessment of the medical devices needs to be carried out within the framework of risk management and risk evaluation of the material components. Moreover, final finished device, individual materials used in fabrication of devices and even the processing of the materials and any residuals from manufacturing process should be evaluated for risk assessment. So, assessment of potential risk arising from unacceptable host response should include not only potential of chemical toxicity, but physical characteristics as well. Therefore, risk assessment for the base material and final finished device needs to be evaluated for potential chemical interactions with living tissues. Thus, for medical devices, the determination of the acceptance of any potential adverse biological response at the cost of medical benefit is the underlying principle of biological evaluation.

Embolotherapy is a well-established endovascular clot inducing method used as the emergency treatment for many traumatic injuries (Hu *et al*. 2019, Hill *et al*. 2018) [6, 5]. It has been known as life-saving process that has potential to control bleeding in an expeditious manner with

minimal invasion and least disruption to normal tissues as compared to standard surgical treatment (Kilani *et al*. 2015) [11]. It is basically controlled occlusion of the vessels to stop hemorrhage intentionally. In modern interventional therapy, embolotherapy has become major arm (Vollherbst *et al*. 2018) [14]. The range of available embolic agents is expanded from autologous clots, Gel foam, and human tissue to polyvinyl alcohol, coil, glue, microspheres, balloons and many others. Its application is becoming core important in multi-dimensional treatment covering areas of trauma, oncology, endovascular therapy of vascular malformations and aneurysms (Lawton *et al*. 2015) [10]. To understand the complex reactions involved in implantation, characterization of cellular and secretory factors plays a vital role. In fact, it collectively defines failure or success of medical devices. Knowledge and better understanding of molecular, cellular, tissues and organ pathology in addition to the principles of healing helps in evaluating the local implants-tissue interface and potential systemic effects on the host. Selection of agents and embolization target varies with medical condition viz., in trauma, occlusion of large vessels is performed. So, trauma embolization requires either temporary or permanent occlusion of large vessels for decreasing pressure in the bleeding vessel by thrombosis (Hu *et al*. 2019) [6]. In tumor treatment, temporary or permanent embolization is manifested that affects tumor circulation at the arterial level.

The materials from devices releases chemicals called as 'leachables' which on contact with body tissues can irritate the skin, mucosa or eyes. Irritation response by local tissues is in the form of local inflammatory reactions, reddening and/or swelling and can be accompanied by burning pain with temperature rise (Anderson 1993, Anderson 1988) [1, 2]. The intracutaneous reactivity test is a standard screening assay for medical devices regardless of their tissue contact during clinical use. It is aggressive in nature as it uses extracts, which are prepared under exaggerated conditions. These extracts are then administered into the skin of the experimental rabbits by intracutaneous route (Tang and Eaton, 1993) [12]. A standard extraction procedure to obtain the test solution consists of incubating or extracting specific amounts of polymers in various polar and non-polar vehicles. The solution's injection into the skin of rabbit creates small blebs on administration, but quickly gets absorbed, leaving behind no evidence of the dose, unless the chemical has irritation potential. The study was approved by the Institutional Animal Ethics Committee, Shriram Institute for Industrial Research, New Delhi-07 and the experiments were done as per guidance provided by CPCSEA.

2. Materials and Methods

2.1 Experimental Animals: 3 clinically healthy female rabbits (*Oryctolagus cuniculus*) of New Zealand white strain weighing 2.0 - 3.0 kg were procured from animal house facility of Shriram Institute for Industrial Research, Delhi-07. The physical and clinical condition of the animals was observed throughout the experimental period and 14-d acclimatization time was provided to animals with *ad libitum* standard feed and water. They were kept individually in the stainless-steel cages.

2.2 Chemicals and Reagents

2.2.1 Test item: Liquid Embolic System

2.2.2 Chemicals: Physiological saline and cottonseed oil.

2.2.3 Reagents

a. Polar Extract: Physiological saline: 0.2 g/mL of test item were kept in a sterilized glass stoppered conical flask containing physiological saline and kept for extraction in an oven at 37 °C for 72 hours. The extractant (physiological saline) with no test item was also kept at similar temperature and time duration, to serve as control dose. Before dosing or completion of 72 hours of extraction time, both test and control dose were allowed to cool at room temperature and decanted using aseptic techniques into a dry sterile vessel to minimize any possible microbiological contamination.
b. Non-Polar Extract: Cottonseed oil: 0.2 g/mL of test item was kept in a sterilized glass stoppered conical flask containing cotton seed oil and then kept for extraction in an oven at 37 °C for 72 hours to serve as test dose. The extractant (cotton seed oil) with no test item was also kept at similar temperature and time duration to serve as control dose. Before dosing or completion of 72 hours of extraction time, both test and control dose were allowed to cool at room temperature and decanted using aseptic techniques into a dry sterile vessel to minimize any possible microbiological contamination. Test animals were clipped free of fur from the back and both sides of the spinal column 18 hours prior to treatment, to obtain a sufficient area for injection. 0.2 mL test extract of was administered by intracutaneous route at five sites on the left side of the back of each animal with gap of approximately 2 cm apart. Similarly, the control was injected on the right side of the back of each rabbit.

2.3 Guidelines: The procedure followed in the study is as per the ISO guidelines, ISO 10993 Part 10 for Irritation and Skin Sensitization tests and ISO 10993 Part 12 for Sample preparation and Reference materials for non-clinical laboratory studies (ISO 10993 Guideline 2010, ISO 10993 Guideline 2012) [8, 9]. The scoring for irritation is as per standard Draize guideline indicated in Fig 1.

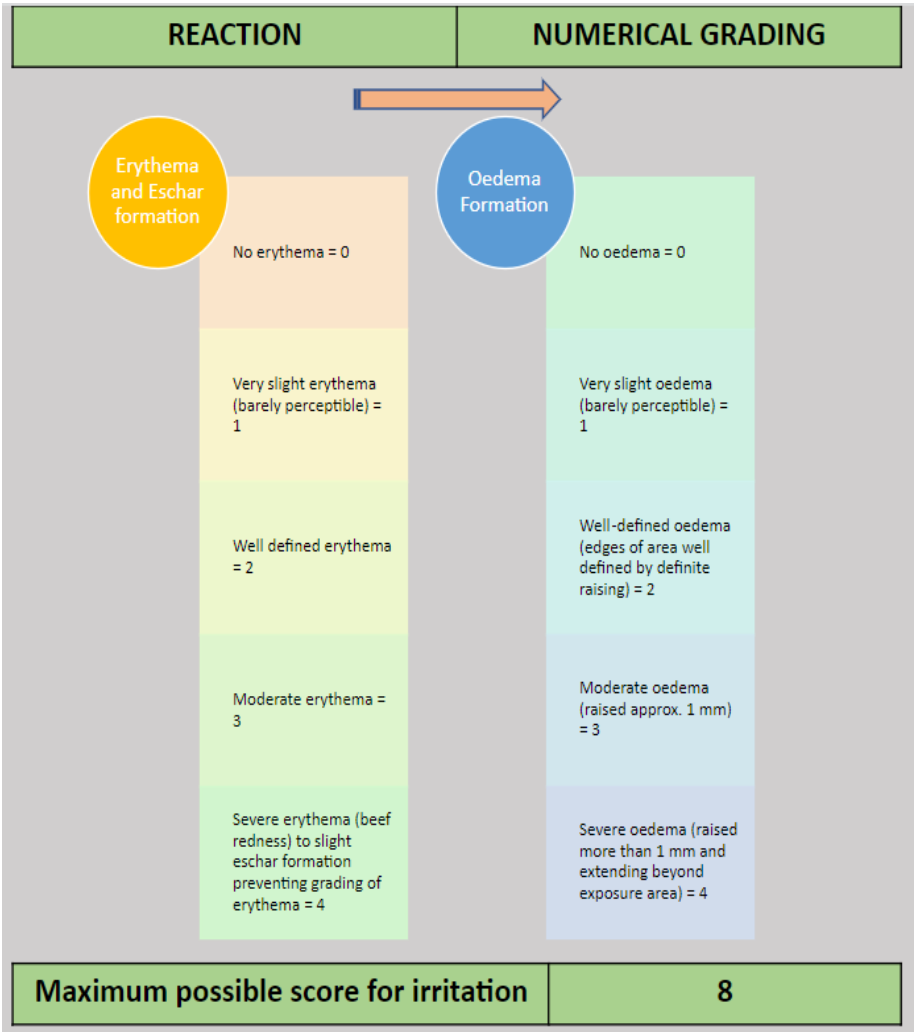

Fig 1: Grading System for Intracutaneous (Intradermal) Reaction

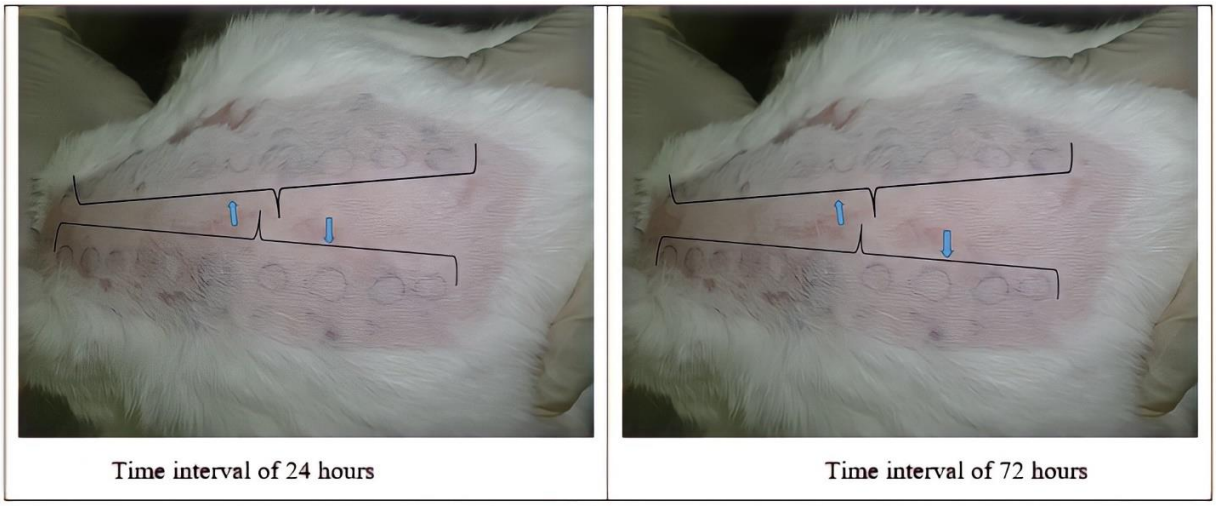

Fig 2: Sites of intracutaneous injection after a time interval of 24 and 72 hour

3. Results and Discussion

3.1 Clinical observations: Careful clinical observations of the rabbits were carried out once before and after the dose administration. All animals were observed for any toxic signs or symptoms immediately after injection at 24, 48 and 72 hours. All signs and symptoms of toxicity were noticed in the animals at 30 minutes, with special attention during first 4 hours after the injection and continued till 72 hours. All the experimental animals were observed for general status, alterations of skin and fur, mucous membrane appearance, eyes, respiration, salivation pattern, diarrhoea and autonomic activity (lacrimation, pupil size, piloerection). No signs of general toxicity were observed in any animal during observation period of 72 hours. The scores for irritation were observed as per Fig 1.

3.1.1 Mortality: Mortality was checked every day till the termination of the experiment. All the experimental animals survived the observation period.

3.1.2 Body weights: Individual body weights of test animals were noted before the dose administration and at the end of the observation period at 72 hours as indicated in Table 1. As reflected from recorded body weight shown above, there is no reduction in body weight during experiment.

3.1.3 Skin reaction and evaluation criteria: Reaction of each injection site, immediately after injection and at 24, 48 and 72 hours were subjectively assessed and scored as shown in Fig 2. Evaluation of skin reactions of each animal using the grading system as per Fig 1 was graded in terms of erythema and eschar formation, and oedema formation as described in Table 2. As clear from the above images and the scoring records, no erythema or oedema was observed in any of injected sites of the three treated rabbits.

Table 1: Mean Body Weight Data of Test Group Rabbits

Animal No.	Sex	Weight in Kilogram Day 1st	Weight in kilogram End of study
1	F	2.4	2.4
2	F	2.1	2.1
3	F	2.3	2.3
Mean ± S.D.		2.27 ± 0.15	2.27 ± 0.15

Table 2: Evaluation of Skin Reactions of Animal No 1, 2 and 3 [Values are mean of 24, 48 and 72 hours]

Animal No.	Observation time (after the administration of injection) (in hours)	Skin Reaction	Left Flank (Test site) Injected with extract of test item in physiological saline Scores of Injection sites 1 2 3 4 5	Right Flank (Control site) Injected with physiological saline Scores of Injection sites 1 2 3 4 5
1, 2 and 3	24	Erythema	0/4 0/4 0/4 0/4 0/4	0/4 0/4 0/4 0/4 0/4
		Oedema	0/4 0/4 0/4 0/4 0/4	0/4 0/4 0/4 0/4 0/4
	48	Erythema	0/4 0/4 0/4 0/4 0/4	0/4 0/4 0/4 0/4 0/4
		Oedema	0/4 0/4 0/4 0/4 0/4	0/4 0/4 0/4 0/4 0/4
	72	Erythema	0/4 0/4 0/4 0/4 0/4	0/4 0/4 0/4 0/4 0/4
		Oedema	0/4 0/4 0/4 0/4 0/4	0/4 0/4 0/4 0/4 0/4
	Observation time (after the administration of injection) (in hours)	Skin Reaction	Left Flank (Test site) Injected with extract of test item in cotton seed oil Scores of Injection sites 6 7 8 9 10	Right Flank (Control site) Injected with cottonseed oil Scores of Injection sites 6 7 8 9 10
	24	Erythema	0/4 0/4 0/4 0/4 0/4	0/4 0/4 0/4 0/4 0/4
		Oedema	0/4 0/4 0/4 0/4 0/4	0/4 0/4 0/4 0/4 0/4
	48	Erythema	0/4 0/4 0/4 0/4 0/4	0/4 0/4 0/4 0/4 0/4
		Oedema	0/4 0/4 0/4 0/4 0/4	0/4 0/4 0/4 0/4 0/4
	72	Erythema	0/4 0/4 0/4 0/4 0/4	0/4 0/4 0/4 0/4 0/4
		Oedema	0/4 0/4 0/4 0/4 0/4	0/4 0/4 0/4 0/4 0/4

4. Conclusion

The study was carried out in order to determine the localized reaction of leachable inherent or extraneous items present in Liquid Embolic System on the intracutaneous tissue of rabbit. As evident from the visual confirmation of images and the graded response, no erythema or oedema was observed in any of injected sites of the three treated rabbits. Under the condition of the study, the test item the liquid embolic system did not elicit local irritation response after getting injected on the intracutaneous tissue of rabbit.

5. Acknowledgement

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