Biocompatibility studies of electron beam cured pressure sensitive adhesive tape for medical application

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Polyurethane (PU)-based pressure-sensitive adhesive (PSA) tapes are commonly used during surgery. Such devices made for biomedical applications must be biocompatible and biologically safe while in use in the human body as their ingredients may leach off of a device into an adjacent tissue and can harm the body during or after application. In the present study, various methods required for biocompatibility establishment, e.g. cytotoxicity, irritation and sensitization for a device, have been analysed and presented following suitable specifications. The study also emphasizes on the developmental and curing mechanism of biomedical adhesive tape by electron beam (e-beam) irradiation.

Keywords: Biocompatibility, electron beam, medical adhesive, polyurethane, pressure-sensitive adhesive tape.

APPROVAL of devices to be used for medical applications by regulatory agencies requires that biocompatibility assessment be conducted to establish the safety of devices, which can be obtained by testing according to recommended guidelines of International Organization for Standardization (ISO)[1–5]. Surgical PSA tapes and drapes are being used for a long time for different bio-medical applications. The early PSAs were based on natural or synthetic rubber, polyacrylates and silicones, but they suffered from certain limitations[6]. The latest trend in PSAs for bio-medical applications suggests that PU-based materials may meet the essential requirements and have been reported to offer unique properties, not found in other adhesive systems[7–9]. To ensure that curing of these PSAs is done without any adverse effects on the performance of adhesives an environment-friendly processing technique can be used instead of thermal process, e.g., e-beam processing is a better option over other curing techniques[10,11].

For different biomedical applications, especially in delicate parts of human body that come in contact directly with the device used, it has become essential that such devices should be non-cytotoxic, non-irritant or non-sensitizing, i.e. biocompatible[12]. A cytotoxicity test determines whether a product or compound will have any toxic effect due to leachables on living cells and is generally used as a tool for screening component products before being put into the design of a medical device. At the same time, skin irritation and sensitization studies are important and can be defined as notable changes in the living beings, usually in the immunochemical system, by exposure to certain substances recognized by the living organisms leading to response that is marked by a reaction at lower doses than what would be observed in non-irritated organisms[13,14].

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Material and methods

PSA composition made using a combination of base resin with other functional ingredients[7–11], was investigated for equal dispersion with the help of Transmission Electron Microscopic (TEM) analysis at the All India Institute of Medical Sciences (AIIMS), New Delhi, India, (Morgagni 268D Fei Electron Optics). The developed homogenized composition was coated (0.2 mm thick and 30 g/m^2 adhesive layer) on a non-woven fabric at room temperature and e-beam irradiated in air at the Bhabha Atomic Research Centre, Mumbai, India, by an e-beam accelerator ILU-6 (Budker Institute of Nuclear Physics, Russia)[7–11]. The flow diagram and underlying chemistry for making a PSA tape are represented in Figures 1 and 2 respectively, and the materials used in the study are listed in Table 1.

Cytotoxicity study

L 929 mouse fibroblasts (American Type Culture Collection CCL-1) were used for the study maintained in continuous culture in minimum essential medium (MEM) with 100 units/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine and 5% fetal bovine serum at 37 ± 1°C, in air containing 5% CO_2. Cells were passaged on reaching 70–80% confluency by treating with 0.5 g/L trypsin and 0.2 g/L ethylene diamine tetraacetic acid (EDTA) in Earl's balanced salt solution, GibcoBRL, UK. Cell viability was determined with the trypan blue exclusion test[15].

Cells were arranged in 96-well culture clusters (Costar, USA) at a density of 15,000 cells/well in 100 µl and incubated for 24 h to allow attachment. The medium was changed with 100 µl of test or control medium in air containing 5% CO_2 at 37 ± 1°C for 30 min. After 24 h incubation, cytotoxicity was assessed for the above study.

Preparation of extracts

Pieces were cut from the PSA tape materials and placed in glass vials having cell culture medium, 6 cm^2 of PSA tape material/ml medium as per specification[5]. It was then agitated for 24 h in a water bath at 37 ± 1°C and filtered using a Millex-GS sterile filter (Millipore, France).

Preparation of cell monolayer

The confluent cell monolayer of L-929 cell line was used for qualitative morphological evaluation of cytotoxicity in the study and the cells were rinsed twice with phosphate buffer saline (PBS) and decanted. The culture medium was prepared using serum, antibiotics and filter sterilized and aspirated from cell culture flask containing a near confluent cell monolayer. Trypsin solution (5 ml) was added into the flask and incubated for 2 min at room temperature to suspend the cells. Subsequently, 10 ml media were added for neutralizing the effect of trypsin and cell suspension and centrifuged for 5 min at 700 rpm. The supernatant was discarded and the cell pellet was resuspended in 10 ml of fresh media and mixed thoroughly. Finally, a cell suspension of freshly suspended cells was obtained.

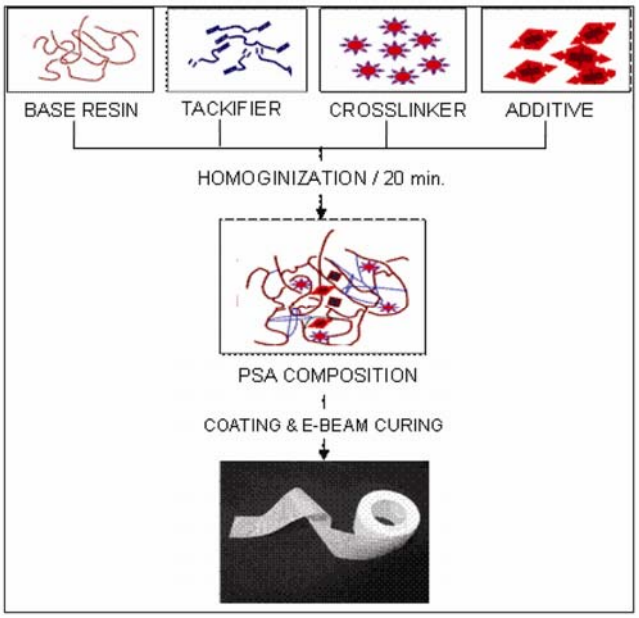

Figure 1. Scheme of PSA tape preparation.

One cell suspension was transferred into triplicate culture wells for each test item, positive control (Latex rubber) and negative control (United States Pharmacopeia Negative Bioreaction Reference Standard) (high density polyethylene). The triplicate culture wells were incubated at 37 ± 1°C in an incubator containing 5% of CO_2 until a near 70–80% confluent cell monolayer was formed as observed by microscopic examination. After 70–80% confluency was attained in each of the triplicate culture wells, the growth medium was replaced with 2 ml extract of e-beam cured PU-PSA tape negative and positive control separately.

All the triplicate culture wells were incubated at 37 ± 1°C for 24 h in a 5% CO_2 atmosphere. After 24 h, 2 ml of 0.01% Neutral Red (Sigma Aldrich, USA) solution was added to each dish (Falcon, USA) and incubated for 1 h. The neutral red solution was poured off and the triplicate culture wells examined microscopically. The presence of cytotoxic leachates is indicated by loss of cell viability. To test cytotoxicity by direct contact, agar diffusion or overlay assay was conducted by placing the test device or a representative portion directly on a mammalian cell layer, protected from mechanical damage by a layer of agar. Cytotoxic leachates diffuse into the cell layer via the agar, and toxicity is indicated by the loss of viable cells around the test device. Qualitative morphological grading of cytotoxicity of extracts and the confluency of the monolayer were recorded as (+) if present and (–) if absent.

MTT assay

Quantitative cytotoxicity of PSA tape material was assessed using the dimethylthiazol diphenyltetrazolium (MTT) assay[16,17]. Optical density (OD) was noted at 570 nm, using a UV-2600 spectrophotometer (Shimadzu, Japan). Mean test results were calculated and presented as percentage of control cells. Each value represents the mean of 2 experiments, using at least 3 replicates of each extract per experiment. Cytotoxicity was rated based on cell viability relative to controls as severe, moderate and slight or note cytotoxic[18,19]. The materials were rated based on their cytotoxicity from the highest to the lowest.

Agar overlay test

In the agar overlay test[20], the cells were observed under a microscope and the cytotoxic effect of PSA tape material was noted based on the lysis of the cells subjacent to the PSA tape material and decolourization of the stained cells. The level of cytotoxicity of the PSA tape materials was based on the diffusion strength of the toxic ingredients in the agar and the toxicity of the test material to cell membranes.

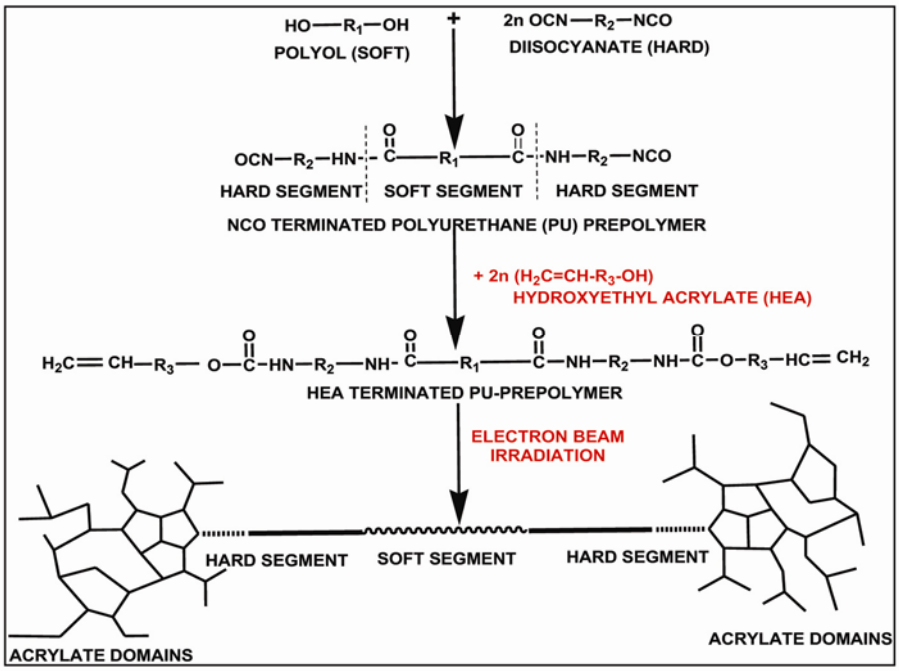

Figure 2. Chemistry and e-beam curing of PSA.

Table 1. Raw materials used for development of PSA tape

Material	Properties	Function	Source
Genomer 4269 (aliphatic urethane polyester acrylate in combination with 2-[{(butylamino)carbonyl}oxy]ethyl ester and 2-propenoic acid)	Tg –15°C, viscosity 21,000 cPs, density 1.1 g/ml, water solubility <1 g/l	Base resin	Rahn corp., USA
Genomer 6043 (inert modified saturated polyester resin with a combination of 2-[{(butylamino)carbonyl}oxy] ethyl ester and 2-propenoic acid)	Tg –18°C, flash point >100°C, viscosity 19,000 cPs, density 1.13 g/ml, water solubility <1 g/l	Tackifier	Rahn corp., USA
Fumed silica (Cab-O-Sil)	Particle size 5–50 nm, surface area 5–600 m^2/g	Filler	Cabot, USA
Triallyl cyanurate (TAC)	Density 1.105 g/cc, MP 26–28°C, BP 360.4°C, functionality 3	Crosslinker	Sigma-Aldrich, USA
Triallyl isocynurate (TAIC)	Molar mass 249.27 g/mol, density 1.11 g/cc, MP 26°C, BP 120°C	Crosslinker	Acros Organics, Belgium
Polymeric methylene diphenyl diisocyanate (PMDI)	MDI content 43%, NCO content 32%, viscosity 50 cPs, mol wt 340, density 1.234 g/cc, functionality 2.7	Crosslinker	Dow Chemical, USA
Carbodiimide modified methylene diphenyl diisocyanate (CMDI)	MDI content 73%, NCO content 29.2%, viscosity 33 cPs, functionality 2.1	Crosslinker	Dow Chemical, USA
Methylene diphenyl di-isocynate (MDI)	Molar mass 250.25 g/mol, density 1.23 g/cc, MP 40°C, BP 314.4°C, flash point 212°C	Crosslinker	Sigma-Aldrich, USA
Isophorone di-isocynate (IPDI)	Molar mass 222.3 g/mol, density 1.06 g/cc, MP –60°C, BP 158°C, flash point 155°C	Crosslinker	Sigma-Aldrich, USA

Filter diffusion test

In this study[21], the filters were observed macroscopically and the stain intensity of each test specimen contact area was evaluated with the background stain[15,22]. Each test was carried out twice using at least three replicates for each experiment. Cytotoxicity of the PSA tape material was established based on a scoring system that takes into account the staining intensity of the zone and extension of the affected region. The median values of observed results were calculated and transformed to a relative degree of cytotoxicity.

Irritation test

Three young adult males of *Oryctolagus cuniculus* (New Zealand White, 2–3 kg body weight) were randomly selected as a test system with topical route of administration

as it is a suitable model for assessing the skin irritation potential. Each rabbit cage was attached with a tag marked with experimental details. All the animals were housed individually in metal cages with perforated floors. The room temperature was maintained at 20 ± 3°C with 50–60% relative humidity (RH). Light was controlled to give 12 h artificial light (8 a.m.–8 p.m.) each day. Animals were acclimatized for 7 days before commencement of the study. Water and standard pelleted feed were freely available to the experimental animals. There were no known contaminants in the feed and water at the levels that would have interfered with experimental results obtained. Twenty four hours before the test, the hair on the back and flanks of each rabbit was closely clipped. Animals without any blemishes on skin were selected for the test.

This study was done to evaluate the dermal irritation potential of e-beam curable PSA tape for different medical applications in *Oryctolagus cuniculus* as per specification[1–5]. PSA tape (2.5 × 2.5 cm^2) was applied evenly on the clipped skin area of all the three male albino rabbits and covered with gauze patch, which was kept in contact with skin by semi-occlusive dressing. After the 24 h exposure period, the bandages/gauze were removed and treatment sites cleaned with wet cotton to remove any residual material.

Sensitization study

This study was done as per Buehler's Method[4]. One day before the test, fur on the back of each animal was clipped on both sides of the spinal column over a sufficiently large test area. The pilot study was conducted to determine the suitable dose for the main study. Three doses of test item were applied on the clipped backs of the animals. Three animals per dose were used. The site of application was covered with the help of gauze patch and semi-occlusive dressing for the 6 h exposure period. Patches were removed after 6 h and the skin of animals was observed. As no skin reactions/irritations were observed in any dose, the highest dose was selected for the main study.

Induction phase

The hair was clipped from the back of 20 guinea pigs, designated as test group and the test item was applied topically to each of them. The test item was uniformly applied on clipped skin and left for 6 h covered with gauze patch of 6 cm^2 and held in contact with the semi-occlusive dressing. After the exposure period of 6 h, the patches were removed and the applied site was cleared with cotton soaked in distilled water to remove the residue of the applied test item. Skin of each animal was observed to detect erythema and oedema. The test item was applied on day 0 and days 6–8 and 13–15. A total of 7 applications were made at the same flank (test area) during the induction phase.

Challenge phase

Two weeks after the last induction exposure, challenge phase was conducted. Twenty four hours before the challenge phase, the hairs of all animals were removed from the untreated flank. The test item was uniformly applied on clipped skin for 6 h and covered with gauze patch of 6 cm^2 and held in contact with semi-occlusive dressing. After the 6 h exposure period, patches were removed and the applied site was cleared with cotton soaked in distilled water to remove the residue of applied test item. Skin of each animal was observed for the presence of erythema and oedema. Observations for the presence of skin reactions were noted after 24 and 48 h of patch removal. The resulting reaction values were evaluated on the basis of Magnusson and Kligman scale[4].

Results and discussion

Composition developed for making e-beam curable PSA tape for different medical applications was homogenized by rotatory homogenizer and investigated for equal dispersion with the help of TEM analysis, presented in Figure 3. No distribution spots were observed as revealed by TEM images, while, in case of crosslinker systems it was established that ingredients added in the composition, were equally distributed in the form of tiny spots in adhesive matrix, required for the uniform film structure without any aggregation or localized accumulation (Figure 3)[23,24].

Cytotoxicity test

Cytotoxicity of e-beam cured PSA tape materials made for different biomedical applications was established by qualitative and quantitative methods. Also, the study specified the incubation of cultured cells in contact with extracts of device and determined the biological responses of mammalian cells *in vitro* using appropriate parameters. In the study, cell culture assays were used to assess the cytotoxicity of tape material using isolated cells *in vitro* because these techniques are useful in evaluating the cytotoxicity potential of materials and provide an excellent way to screen materials prior to *in vivo* tests. The results are summarized in form of qualitative morphological values (Table 2). It was established that there as no cell lysis or morphological change in the triplicate culture wells of the extract of e-beam cured PSA tape as compared with the positive (+ve) and negative (–ve) controls. It was based on the fact that, during

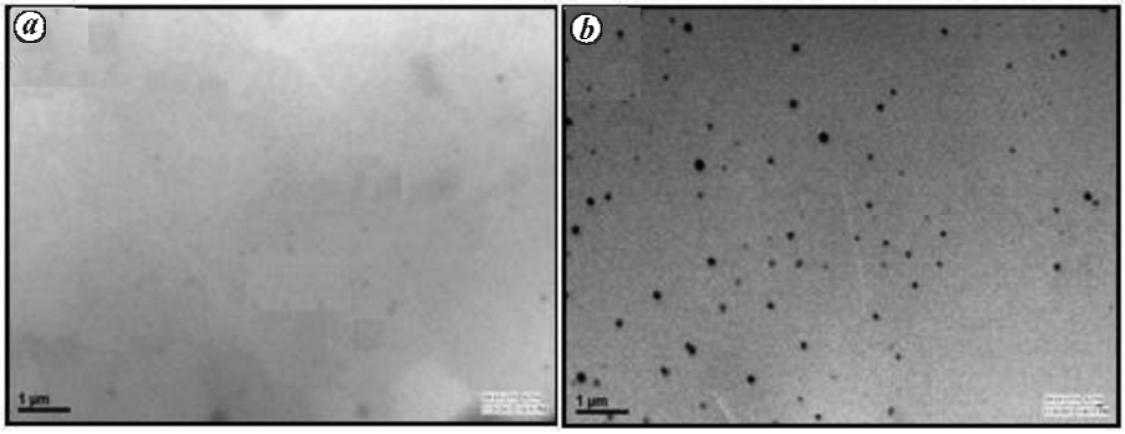

Figure 3. TEM micrograph of PSA composition for control (***a***) and with crosslinker (***b***).

Table 2. Qualitative morphological results of test item (PSA tape), negative control and positive control

Well	Confluent monolayer	Lysis (%)	Grade	Reactivity
PSA tape (test item)				
Test-A	(+)	0	0	None
Test-B	(+)	0	0	None
Test-C	(+)	0	0	None
USP negative bioreaction RS (negative control)				
Negative control-A	(+)	0	0	None
Negative control-B	(+)	0	0	None
Negative control-C	(+)	0	0	None
Latex rubber (positive control)				
Positive control-A	(–)	65	3	Moderate
Positive control-B	(–)	65	3	Moderate
Positive control-C	(–)	65	3	Moderate

Table 3. Results for cytotoxicity study

Particular of samples	MTT (Cell viability, %)	Agar overlay (Lysis index)	Filter diffusion (Score)
PSA tape (test item)			
Test-A	>90	0.2	0.2
Test-B	>90	0.1	0.0
Test-C	>90	0.2	0.1
USP negative bioreaction RS (negative control)			
Negative control-A	>90	0.0	0.1
Negative control-B	>90	0.2	0.1
Negative control-C	>90	0.2	0.2
Latex rubber (positive control)			
Positive control-A	30–60	2.5	1.8
Positive control-B	30–60	2.4	1.9
Positive control-C	30–60	2.2	1.8

incubation, toxic leachables in the test material could diffuse into the culture medium and contact the cell layer, leading to cell lysis or morphological changes. Thus, the reported reactivity of the test sample is indicated by malformation, degeneration and lysis of cells around the test material. Hence, on the basis of qualitative morphological test results, the extract of the test item was found to be non-cytotoxic[25–28]. Similarly, quantitative relative values of cytotoxicity studies (MTT % cell viability, agar overlay and filter diffusion scores) were summarized (Table 3). From the results, it was established that MTT test cell viabilities (%) in test and negative control samples were greater than 90, i.e. the relative value of toxicity in both samples was nil; but in case of positive control it was between 30 and 60, a moderately cytotoxic value[21,29]. It was due to the ability of cells to reduce the tetrazolium salt to a formazan product, indicating mitochondrial activity, seen only in living cells[15]. Determination of formazan production in cells exposed to tests (compared to controls) enabled the assessment of relative toxicity of test material. Also, the extraction procedure using cell culture media limited the extractants to water soluble substances. Toxic response in the MTT test indicated toxic water–soluble substances released from the material. However, long extraction time (24 h) used in study might not have influenced the response in the MTT assay because leaching time is important; but the complete effect of leaching was reached within an hour and a longer leaching time did not reduce cytotoxicity[30–32].

In the agar overlay study, the median values of results for test and negative control samples were determined and transformed to a relative degree of cytotoxicity (Table 3). The lysis index ranges from 0–0.2, i.e. the relative value of toxicity in both the samples were nil, but in positive control it was found to be between 2.2 and 2.5 which comes in the category of moderately cytotoxic[33]. It was due to the fact that the agar overlay assay demonstrates the ability of a test substance diffused through the agar layer to damage plasma or lysosomal membranes of the cells, resulting in a release of the preloaded neutral red dye. Viable cells take up and retain the dye compound[18,22]. Determination of lysis subjacent to test material, and loss of colour of the stained cells, enable relative toxicity to be assessed.

The results for filter diffusion test (Table 3) indicate that the filter diffusion score for the test and negative control samples were between 0 and 0.2, hence the relative degree of toxicity was nil; but for positive control it was between 1.8 and 1.9, which is moderately toxic in nature. It was based on the fact that all positive control materials released substances with ability to penetrate through a filter and exert a toxic effect on the cell functions as shown in the study, while the remaining materials (test and negative control) were non-cytotoxic in nature[21]. Also, filter diffusion test provides the ability of cells to transform a yellow succinate solution to a blue furate product indicating mitochondrial activity, which is seen only in living cells. Determination of succinate dehydrogenase in cells exposed to test substances compared to controls, enables assessment of relative toxicity[21]. In the test, the cells were in contact with PSA tape materials and separated only by a filter. The close contact between cells and material enhances the possibility that all leachables, not only water soluble substances, will reach the cells[19].

In the case of skin irritation test, PSA tape was applied on the clipped area of skin of albino rabbits and covered with gauze patch, which was held in contact with a semi-occlusive dressing for 24 h. The patch was removed, the exposed area cleaned with wet gauze patch and the degree of irritation was observed and scored at 24, 48 and 72 h after patch removal. Skin reaction at the site of application was subjectively assessed and scored after patch removal at 24, 48 and 72 h (post treatment) according to numerical system[34]. The irritation score was compared to the categories of irritation responses and reported accordingly. Combined scores for erythema and oedema were calculated as per specification[4] and the results summarized in Table 4. Hence, irritation test presented the local irritation potential of devices, materials or extracts, using sites such as skin. The route of exposure (skin) and duration of contact was analogous to the anticipated clinical use of the device, but it was often careful to overstate exposure conditions to establish a margin of safety for patients.

Table 4. Evaluation of skin sensitization reactions for PU-PSA tape

		Score		
Skin responses	Time (h)	Rabbit 1	Rabbit 2	Rabbit 3
Erythema	24	0	0	0
	48	0	1	0
	72	1	0	0
Oedema	24	0	0	0
	48	0	0	0
	72	0	0	0
PII		1/6 = 0.17	1/6 = 0.17	0/6 = 0

Average PII = 0.17 + 0.17 + 0.0/3 = 0.11.

In the study, only 24, 48 and 72 h single exposure test observations were used for estimating the primary irritation index (PII). Observations made prior to dosing or after 72 h to monitor recovery were not used for determination. After 72 h grading, all erythema plus oedema grades at different time intervals (24, 48 and 72 h) were added separately for each of the test samples and blank for each animal. The primary irritation score for an animal is calculated by dividing the sum of all scores by 6 (two test/observation sites, three time points). In case of blank, we calculated the primary irritation score for the controls and subtracted that score from the test materials' score. To obtain the PII for the PSA tape, we added all the primary irritation scores of the individual animals and divided it by the total number of animals. This value is the cumulative irritation index. The cumulative irritation index was compared with the categories of irritation responses[4] and the appropriate response category was recorded for the study (Table 4). From the table, the combined skin irritation scores of erythema and oedema were 0.11 at 24, 48 and 72 h after patch removal[35,36]. Hence, the test item was found to be a negligible irritant to the rabbit skin.

Sensitization study was done on certain groups of guinea pigs for different observations, e.g. clinical sign, mortality, body weight, skin reaction and total evaluation. At the end of the study, it was established that e-beam cured PSA tape meets the criteria of guidelines and does not deviate in terms of the above mentioned observations when compared with the control group of animals. Hence, this study helped to determine whether e-beam cured PSA tape contained any material that caused adverse effects after repeated or prolonged exposure. These allergic or hypersensitivity reactions involve immunologic response mechanisms[37–40]. Thus, sensitization study for tape was established to determine sensitization potential using tape as a test material.

Conclusion

Medical devices and their component materials may leach toxic compounds that may cause adverse effects when used clinically on the body surface. The selection and evaluation of these devices and materials projected for use in humans require a structured programme of assessment to establish the biocompatibility and safety within the body. It simply refers to the properties of materials being biologically compatible by not eliciting local or systemic responses from a living system. From a regulatory stance, it is a series of tests that are used to determine the potential toxicity resulting from contact of the components of medical devices with the body. The present study used different methods to assess the biocompatibility of the e-beam cured PU-PSA tape made for various medical applications with different modes of

exposure and exposure time. In case of MTT assay, cells were exposed to extracts of materials for 4 h. In the filter diffusion test, cells were exposed for 2 h to substances from the materials whereas, in the agar overlay test, substances released from the material for 24 h had to diffuse through an agar layer before reaching the cells. When the results from the three tests were compared, the three tests exhibited a similar degree of toxicity for all positive control, negative control and PSA tape materials. Skin irritation was studied to understand the response of e-beam cured PSA tape on the rabbit skin. The combined score came out to be 0.11 at different time intervals on the basis of reactions observed after 24, 48 and 72 h after patch removal. Similarly, the sensitization study of e-beam cured PSA tape on Dunken Hartley Guinea Pigs did not show any negative deviation from the guidelines and met all the requirements of the study. Hence, the developed product is biocompatible and can be used for further study.

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