Citotoxicity of Vulcanized Natural Rubber Latex Films by the Conventional Process with Sulphur and by the Alternative Process with Ionizing Radiation

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Introduction

Since the 1960s there has been a rapid increase in the use of biomaterials and medical devices, and an associated increase in awareness of the need for such products to be biocompatible. This is especially true for the newer synthetic elastomers and polymers where the biocompatibility problems have been extensively studied. Incidences of urethritis and urethral strictures following the use of Natural Rubber Latex (NRL) urinary catheteres in the hospitals have been reported (Graham et al., 1984). The NRL materials provide strong cytotoxicities (Wilsnack, 1976) and are used as positive controls for cytotoxicity tests (ASTM F895-84, 1981). In this paper the citotoxicity of radiation vulcanized NRL films were compared with vulcanized NRL films by convencional process with sulphur.

Experimental

NRL Films

A high ammonia type centrifuged NRL from Malaysia imported by INAL (Indústria Nacional de Artefatos de Látex LTDA) was vulcanized by both processes: conventional and alternative.

Conventional Process

S, ZnO and zinc diethyl dithiocarbamate (ZDEC) dispersion were added to the latex. The vulcanization was carried out at 70°C for 3 hours. After vulcanization a sample was diluted with 1% ammonia solution to 50% of total solids content.

Alternative Process

The latex was irradiated with γ-rays from ^{60}Co source type panoramic of Yoshizawa Kiko Co LTD, carried out at room temperature at dose rate of 0.9 kGy/h. One of samples for irradiation was diluted with 1% ammonia solution only to 50% of total solids content and in another sample, commercial n-butyl acrilate (n-BA) as sensitizer was added to the diluted latex.

NRL films were prepared by casting method followed by leaching with water at 70°C for 15 minutes and drying at 70°C for 1 hour and half. The vulcanization dose (dose at which the maximum tensile strength of dried film of irradiated latex is found) of sample without n-BA was 250 kGy and the sample with n-BA was 12 kGy.

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Citotoxicity Test

Preparation of extracts

NRL films cut were placed in screw capped glass bottles and sterilized by autoclaving at 121°C for 20 minutes. For each sample was used 0.2g of test materials / ml culture medium consisting of RPMI 1640 supplemented with 10% fetal calf serum (FCS) obtained from Cultilab Materials Cell Culture LTD and penicillin-streptomycin (100μg/ml) obtained from Sigma Chemical Company. The bottles were shaken and incubated stationary at 37°C for 72 hours. After incubation, the test materials were discarted and the extracts were diluted with culture medium serially.

Cell Culture

Chinese hamster ovary (CHO) K-1 cells from American Type Culture Collection (ATCC) were obtained from Chemistry Institute of USP and were grown in RPMI 1640-FCS. The cells grown in plastic tissue culture flask in atmosphere of 5% CO_2 and 95% air at 37°C. Then the culture medium was removed and growing cells were washed with calcium and magnesium free medium (CMF). After trypsinization, the cells were transferred to a screw capped plastic centrifuge tube and washed twice with CMF. The cells were resuspended in RPMI 1640-FCS, adjusted to give 100 cells/ml. Two milliliter of the cell suspension was seeded into a 60 mm diameter culture dish and incubated to attach for 4 hours. The medium was then removed and the cells were replaced with 5ml of fresh medium as control, undiluted or serial diluted extracts of test materials. Each test solution was tested in triplicate. Positive and negative control materials were treated in a similar manner. The positive control material was a 0.2% Phenol solution and the negative control material was High Density Polyethylene. After incubation at 37°C for 7 days, colonies formed were fixed with 10% formalin solution and stained with Giemsa. The number of colonies on each dish was counted. Relative percentage of colony number as control were plotted on semilogarithmic graph paper against the extract concentration expressed percentage.

Results and Discussion

The method described in this paper measures the cytolethality by comparing colony forming ability. The cytotoxicity potencial of 4 (four) samples of NRL films was expressed in terms of extract concentration that

suppress colony formation to 50% of the control value (IC50 %), as shown in Fig. 1 and Table 1.

The NRL films not vulcanized (sample 1) and γ-ray vulcanized (samples 2 and 3) presented IC50% values ranging from 48 to 55%. The different IC50% values in the NRL films (samples 1, 2 and 3) was not considered because the dispersion from method. Those values can be due to substances of low toxicity which should remain in the films. According to Makuuchi et al. (1994), the substances of the radiation vulcanized films could be removed by treatment with aqueous alkaline solution.

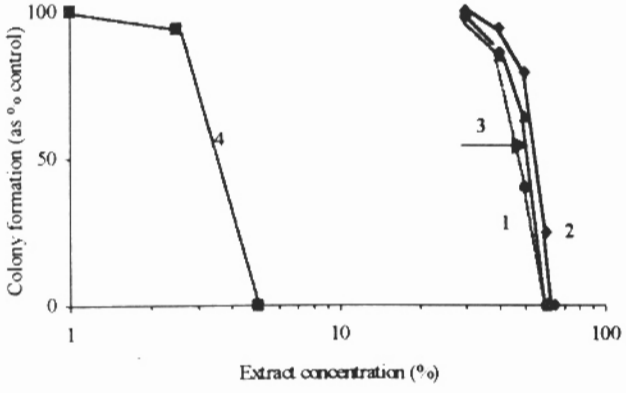

Figure 1. Colony suppression curves of NRL films.

However, the IC50% value of sample 4 from the conventional sulphur vulcanized film was 3,4%, may be due to the content of zinc diethyldithiocarbamate, which was the accelerator used in the refered film (Nakamura et al., 1989).

TABLE 1. CITOTOXICITY OF NRL FILMS

Sample type	Dose kGy	n-BA phr	S[c] phr	IC50(%) RPMI+FCS
1[a]	-	-	-	48
2[b]	250	0	-	55
3[b]	12	4	-	52
4[c]	-	-	0.8	3.4
Positive control[d]				3.7
Negative control[e]				>100

a. NRL film not vulcanized
b. Alternative process
c. Conventional process
d. 0,2% Phenol solution
e. High Density Polyethylene

Conclusion

The obtained results of citotoxicities of NRL films showed that conventional process promoted strong citotoxicity relating to NRL while alternative process not promoted meaning differences. The radiation vulcanized process promoted smaller citotoxicity than conventional process but it is not enough to use in biomaterials applications that has contact with cells. Therefore futhermore studies are necessary to remove the substances which are responsable for the citotoxicity.

Acknowledgements

We are grateful to INAL (Indústria Nacional de Artefatos de Látex LTDA) for the reagents, and to CNPq for the fellowship granted to the project.

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