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# Biocompatibility Study of Polymeric Biomaterials

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**Abstract:** Polymeric hydrogels are used as wound dressing material since these materials show advantages such as pain relief, exudates absorption, barrier to microorganisms, permeability, and others. This article shows the results obtained in a study aiming to know the biological performance of different polymeric materials to be used in contact with skin: PVP hydrogels and acrylate adhesive. The biocompatibility was determined by in vitro assay of cytotoxicity and in vivo assay by using the contact test of irritability in rabbits. All the tested samples presented no toxicity and no dermal irritation. **Key Words:** Polymeric biomaterial—Hydrogel—Biocompatibility—Cytotoxicity assay—Dermal irritability assay.

The utilization of polymeric materials such as hydrogels as biomaterials for wound care management has increased lately since these materials show advantages such as pain relief, exudates absorption, barrier to microorganisms, permeability to oxygen, transparency, mechanical behavior that can be adequate according to application, and general ability to deliver drugs in a controlled way.

Polymeric hydrogels are made of water-soluble molecules, connected usually by covalent bonds, forming a three-dimensional insoluble network. The space between chains is accessible for diffusion of solutes and this space is controllable by the level of cross-linked (connected) molecules. They usually show good biocompatibility in contact with blood, body fluids, and tissues. Therefore, they are very often used as biomaterials for medical purposes, for instance contact lenses, coating of catheters, etc. Moreover, different hydrogels have been proposed for use in bandages, burn wound dressings, adhesives, and other devices aiming to contact areas with skin lesions (1–3).

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Wound dressings based on polymeric hydrogels were first invented by Rosiak's group (4). This hydrogel system was based on the simultaneous cross-linking and sterilization by radiation of the mixture of medical grade poly(vinyl pirrolidone) (PVP), poly(ethylene glycol) (PEG), and agar polymers. This system is very clever since in such a process it is possible to save sterilization cost by doing this simultaneously with cross-linking. The radiation processing of medical grade polymers, in theory, can induce degradation and release of low molar mass compounds. PEG and PVP monomers are known to be very carcinogenic and mutagenic. Therefore, it is mandatory to assure the safety of this system.

Biomaterials are defined as materials that can be interfaced with biological systems in order to evaluate, treat, augment, or replace any tissue, organ, or function of the body (5).

The clinical application of a biomaterial should not cause any adverse reaction in the organism and should not endanger the life of the patient; any material to be used as part of a biomaterial device has to be biocompatible.

The definition of biocompatibility includes that the material has to be nontoxic, non-allergenic, non-carcinogenic, and non-mutagenic, and that it does not influence the fertility of a given patient (5).

Preliminary use of in vitro methods is encouraged as screening tests prior to animal testing. In order to

reduce the number of animals used, these standards use a step-wise approach with review and analysis of test results at each stage. Appropriate in vitro investigations can be used for screening prospective biomaterials for estimations of toxic effect. Cytotoxicity in vitro assay is the first test to evaluate the biocompatibility of any material for use in biomedical devices (6).

International Standard Organization (ISO) tests assess possible contact hazards from device-released chemicals that may produce skin and mucosal irritation, eye irritation, and delayed contact sensitization. Animal testing is currently an inherent component of the estimation of biocompatibility and is often used to assess the compatibility of a new biomaterial before its clinical use (6,7).

Different hydrogels have been proposed for use in bandages, adhesives, and other devices aiming to contact areas with skin and skin lesions.

This work presents a study on biocompatibility of different polymeric materials to be used in contact with skin. The biocompatibility was studied by in vitro assay of cytotoxicity and in vivo assay by using the contact test of irritability in rabbits.

## MATERIALS AND METHODS

Different polymeric materials were submitted to biocompatibility tests: three samples obtained by ionizing radiation of poly(vinyl pirrolidone) (PVP) from GAF Corporation and poly(ethylene glycol) (PEG) from Oxiteno, Mauá, São Paulo, Brazil, in different proportions and γ radiation doses, according to the procedure previously described by Lugão et al. (8) described in Table 1 and one sample prepared by casting of butyl acrylate adhesive (Rhom and Haas, Jacareí, São Paulo, Brazil). Depending on the synthesis parameters used, samples of solid hydrogels or fluid gels with different shear modulus and water swelling capability were obtained.

### Cytotoxicity test

The cytotoxicity assay was carried out with the exposure of NCTC clone 929 cells to the eluate obtained from samples, which stayed in contact for 24 h with culture medium MEM (minimum Eagle's medium, Sigma Co., São Paulo, Brazil) at 37°C. The cell line was acquired from American Type Culture Collection (ATCC) bank. The cytotoxic effect was evaluated using neutral red uptake (NRU), according to Ciapetti et al. (9) and ISO tests (10).

The cells were maintained in MEM containing 10% fetal calf serum and 1% non-essential amino acids (MEM-FCS) in a humidified incubator with 5% $CO_2$ at 37°C. The cells were detached by 0.2% trypsin (Difco, São Paulo, Brazil) and 0.2 mL of the cell suspension, about $2.5 \times 10^5$ cell/mL, were seeded in flat-bottomed 96 microplate wells (Costar, Cambridge, MA, U.S.A.). The microplate was incubated for 24 h at 37°C in a $CO_2$ humidified incubator. After this period, the medium was discarded and replaced with 0.2 mL of serially diluted extract of each sample (50, 25, 12.5, 6.25%). Control of cell culture was replaced with MEM-FCS. In the same assay with samples, a positive control (0.02% Phenol solution) and negative control (atoxic TiN stabilized polyvinyl chloride, Dacarto SA Indústria de Plásticos, Osasco, São Paulo, Brazil) were run. Samples and controls were tested in triplicate. The plate was incubated again for 24 h under the same conditions.

After the incubation period, the medium and extracts were discarded and replaced with 0.2 mL of neutral red solution (50 µg/mL) diluted 1:100 in MEM-FCS. After incubation at 37°C for 3 h, the dye medium was discarded and the microplate was washed twice with phosphate buffered saline. The cells were washed with a solution of 1% $CaCl_2$ in 0.5% formaldehyde. The rupture of cells and neutral red release was obtained by addition of 0.2 mL/well of extractant solution containing 50% ethanol in 1% acetic acid. The absorbances were read on an Organon spectrophotometer for microplates with 540 nm filter. The average of optical density units were calculated after blank subtraction.

### Dermal irritability test

Materials that are to be in contact with the skin should not cause irritation to it. The irritation is prob-

**TABLE 1.** *Different polymeric materials obtained by ionizing radiation*

| Sample | Composition (% w/w) | Irradiation dose (kGy) | Physical characteristics | Gel fraction (%) | 24 h Water absorption (%) |
|---|---|---|---|---|---|
| Visc06a | PVP—20.0<br>PEG 6000—4.0 | 5 | Transparent<br>Viscous gel | 54.0 ± 1.5 | 79.7 ± 1.5 |
| Visc05b | PVP—1.0<br>PEG 6000—1.0 | 5 | Transparent<br>Viscous gel | — | — |
| Loz36 | PVP—20.0<br>PEG 6000—4.0 | 25 | Transparent<br>Gelatinous block | 82.0 ± 0.5 | 74.6 ± 1.2 |

ably caused by substances leached from a material, so it is necessary to provide skin exposure by direct material-skin testing. The irritability contact test was performed according to the Draize's method (11,12), National Standard (13), and International Standards (8,14) using six healthy young adult male albino rabbits of New Zealand breed weighing 2 to 3 Kg, acclimatized and cared for according to ISO (7). One day before the test, four places on the back of the animal were closely clipped for application and observation of all denuded areas. Only healthy, intact skin was used. After sample application, all application sites were covered with non-occlusive dressings and wrapped with a semi-occlusive bandage. The observation of animals was done after 24 and 72 h and the grade of reactions in terms of erythema and edema was given, according to the Draize's table.

The in vivo assay was carried out only with the biomaterial that presented no toxicity in the in vitro cytotoxicity assay.

## RESULTS AND DISCUSSION

One significant advantage of in vitro investigations is the possibility of limiting the number of variables by employing an appropriate experimental design.

Toxic substances may induce membrane damage and impairment of metabolic activity effects in the cell. These events may finally lead to cell lysis and death.

Evaluation of cytotoxicity by in vitro methods can involve the use of changes ranging from cell death to very subtle alterations of certain cellular functions. The assessment of cell death can be based in the integrity of cell membrane, ascertained by the uptake of foreign molecules into the cell, for example neutral red. In this work the evaluation of cytotoxicity was performed by using neutral red uptake assay.

Positive and negative controls are necessary to confirm the adequate performance of the test procedure and/or to evaluate the results from a new material, as well as to control cell sensitivity, extraction efficiency, and other test parameters.

In the cytotoxicity assay, the viability percentage of samples was calculated with the average of optical density of each dilution of serially diluted extract of samples and negative and positive controls in relation to cell control (100%). Those data are shown in Fig. 1. In this graphic, the cytotoxicity index can be obtained, $IC_{50(\%)}$, which means, the concentration of the extract which injures or kills 50% of cell population in the assay.

In this study all the tested samples did not present toxic effects even at 100% extract concentration. They demonstrated similar behavior to negative control with no cytotoxicity index ($IC_{50(\%)}$) as shown in Fig. 1. Only the positive control presented cytotoxicity effect with $IC_{50(\%)} = 12$.

The same samples tested in the dermal irritability assay showed similar behavior in the denuded skin area of rabbits; No primary skin irritation index (I), according to Draize's table, I = 0.0. All tested areas showed the same aspects, with no redness and no edema demonstrating no leaching of hazards from tested biomaterials in this study, as shown in Fig. 2.

The agreement of in vitro and in vivo results is an important factor when the correlation within these

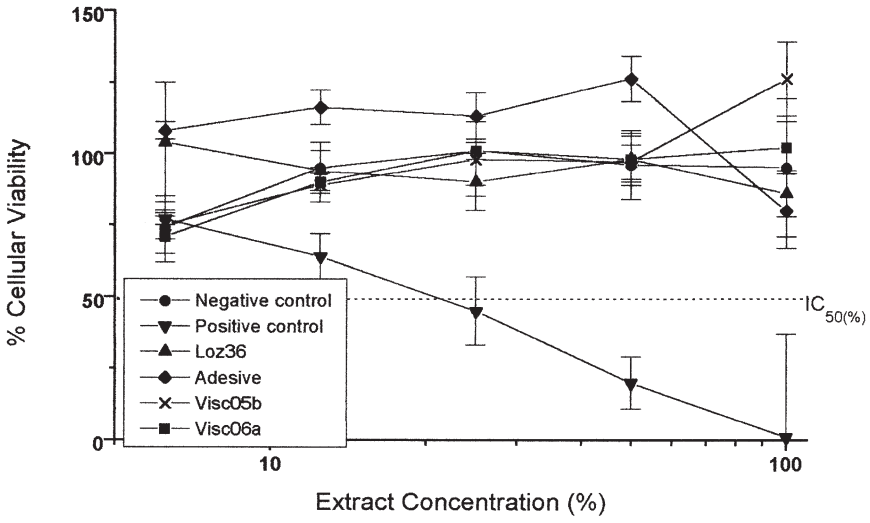


**FIG. 1.** Cytotoxicity test results are shown: cell viability curves using neutral red uptake cytotoxicity assay, with NCTC clone 929 cells for different hydrogel samples.

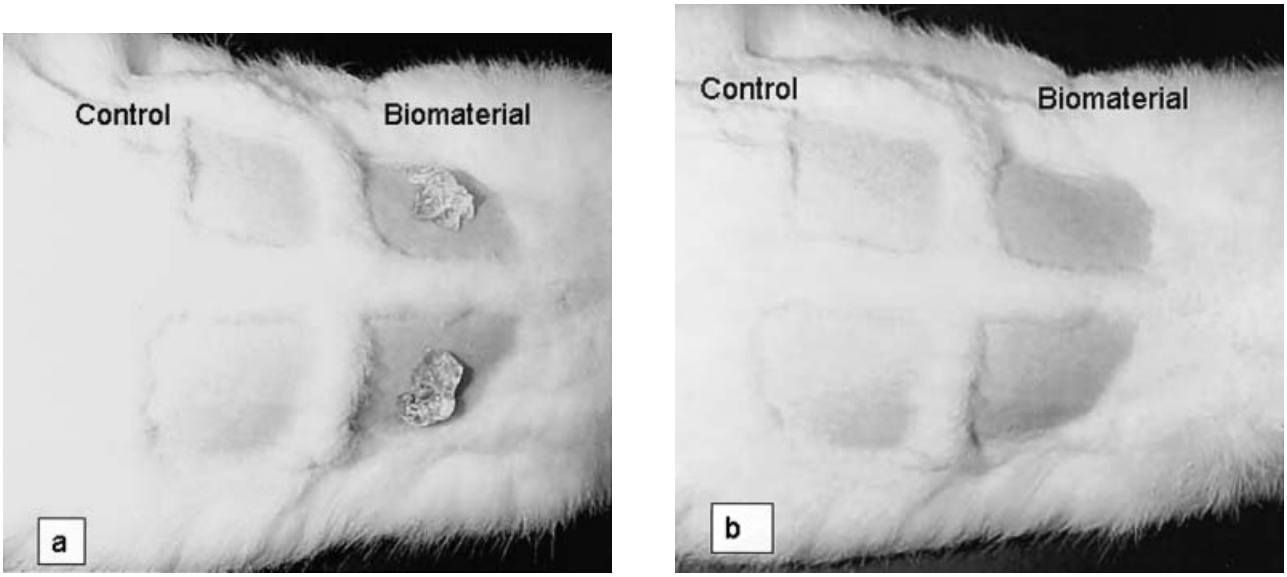


**FIG. 2.** a) The photograph shows irritability contact test in rabbit skin: sample application. b) The photograph shows irritability contact test in rabbit skin: results obtained after 72 hours of contact of samples—no irritability (no redness and no edema) was observed on the skin.

methods is the aim of the study, mainly when the proposition is to reduce or replace the use of animals due to ethical concerns.

## CONCLUSION

The radiation processing of medical grade polymers, in theory, can induce degradation and release of low molar mass compounds. It is well known that the toxicity of organic compounds increases as molar mass decreases. The solubility and diffusion of those compounds in the body tissues increases with decrease of their molar mass. The polymeric materials studied in this work did not present a toxic effect, therefore after in vitro and in vivo evaluation it can be concluded that these biomaterials are able to be used in contact with skin for wound dressing management in the form of advanced bandages or for drug immobilization systems as transdermal therapeutic systems.

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