Rapid cytotoxicity characterization of high melt strength polypropylene films by neutral red uptake method.

Harumi Otaguro[1*], Sizue Ota Rogero[1], Luis Filipe. C. P. de Lima[1], Duclerc F. Parra[1], Beatriz H. W. Artel[2] and Ademar B. Lugão[1].

Insert here the institution and e-mail of each author and the complete address of author to whom correspondence should be addressed. (Times New Roman, 10, italic, centered paragraph, single space). Example:
[1]Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN), Brazil – hotaguro@ipen.br; sorogero@ipen.br; lfilipe@ipen.br; dfparra@ipen.br; ablugao@ipen.br.
[2] Empresa Brasileira de Radiações Ltda – Embrarad, Brazil – rhutzler@embrarad.com.br.

High melt strength polypropylene (HMSPP) was developed by gamma irradiation (20 kGy) of polypropylene (PP) in the presence of different multifunctional monomers for the improvement of film and foam production as well as thermopressed products. HMSPP is designed to improve the processability of linear polypropylene (iPP) mainly for process such as film blowing or blow molding and probably oriented polypropylene film (OPP).
PP with 1.5 of melt flow index, with and without antioxidant, were used. Four monomers have been utilized to modify these iPP samples such as, *Ethyleneglycol dimethacrylate (EGDMA), Triallycianurate (TAC), Triallyl isocianurate (TAIC)* and *Trimethilolpropane trimethacrylate (TMPTMA)*, in a wide concentration range from 0- 5 mmol/100g of pure resin. This process can produce synthetic exogenous substance. To toxicity evaluation of HMSPP films was done in samples processed at high temperature in extruder and thermopressing in sequence to 300 μm thickness. A rapid cytotoxicity characterization of HMSPP films were evaluated. In the cytotoxicity test, extract of HMSPP samples have been exposed in microplate cell culture with mouse connective tissue NCTC L929 cell line from ATCC and evaluated by neutral red uptake methodology. The results showed no toxic effect in all analyzed samples, whereas the methodology showed very efficient in this analyze. Therefore, as the HMSPP samples present good properties under elongational flow for the production of biaxially oriented polypropylene film, these non-toxic samples can be adequate to manufacture packing plastic.

Introduction

The researches and interest if the new products likes medicine drugs, chemical substance, new materials etc, puts into market can be dangerous to the human life has grew up recently.
In this context, in the last time is being reported a new area of knowledge related to the endocrinology toxicology. The aim of this area is study of the strange or exógena chemical substance deriving by plants, synthetic products, ambient pollutants, etc that finish interfering in the production, release and transport, metabolism and elimination of natural hormones. These strange substances can react with components of the endocrine system [1-3].
It has already been reported that composite of bisphenol A-based has shown their estrogenic activity [4-6]. Darmani et al shown which mousse submitted to 25 to 100 μg Kg^{-1} daily doses of bisphenol A Glycidyl methacrylate or Bis-GMA and triethylene glycol dimethacryalate TEG-DMA during 28 day displayed to be toxic in the mouse reproduction [7]. Little quantity of this substance was found in the fish a specific region of Asian. In this sense, studies in people that live near the regions are being reported by analyzes of this substance and was founded a small percentage in human body. However the among found it was not toxic to the human life.
Then, looking forward to application of high melt strength polypropylene (HMSPP) as biomaterial in the manufacturing products in the medicine field, packing materials for food industry etc.
During the process to obtain the HMSPP product by incorporation of acrylate and methacrylates monomers are formed substances by thermal degradation and residual monomers. In the present work the cytotoxicity profile of different HMSPP samples was evaluated in vitro test as rapid and previous test.
The ability of a material to be resorbed over time is an important property in many biomedical applications.

Experimental

Material and sample preparation

The isotactic polypropylene (iPP) homopolymer used in this study was a reactor grade from Braskem in the sphere, with a melt flow index (MFI) of 1.5 g 10 min^{-1} at 230°C.

Ethylene glycol dimethacrylate (EGDMA), Tri-allyl-cianurate (TAC), Tri-allyl-isocianurate (TAIC) and Trimethylol-propane trimethacrylate (TMPTMA), at a concentration of 0.5 to 5.0 mmol/100g of iPP were mixed at room temperature. After mixed the samples were extruded in a twin-screw extruder Haake with a die diameter of 2mm. The extruder polymer strand was cooled at room temperature and was being cut. After that, all samples were irradiated with gamma radiation (^{60}Co) at a dose of 20kGy under nitrogen gas atmosphere, using the source from Embrarad.
Samples to cytotoxicity have been obtained by thermopressing at 190°C from extruded material and then cooled at room temperature. After that dried square-samples were cut with dimensions of 20x10x03 mm.

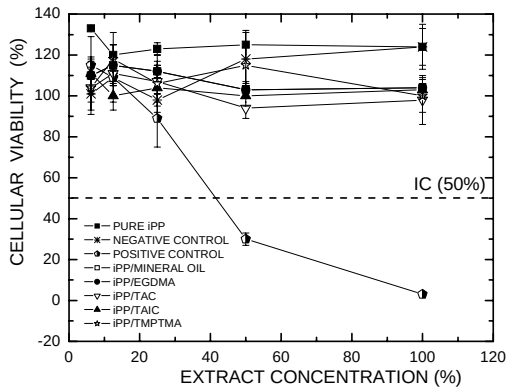
Cytotoxicity test

All samples of iPP modified with different concentrations of EGDMA, TAIC, TAC and TMPTMA were sterilized and submitted in cell culture medium, MEM (minimum Eagle's medium, Sigma Co., São Paulo, Brazil), for 24 h at 37°C.
Dilutions in the range of 6.25 up to 100% from extract were put on to cell cultured of NCTC L929 in a 96 wells microplate. The cytotoxic effect was evaluated by neural red uptake (NRU) methodology, according to Ciapetti et al [8], preview work [9] and International.
The cytotoxicity assay was carried out with to the extract obtained by the immersion of samples. The cell line was acquired from American Type Culture Collection (ATCC) bank.
The optical densities in these solutions are being analyzed by Spectrophotometer Sunrise from Tecan at 540 nm.
Negative control used was non-toxic PVC pellets and positive control used was 0.02% phenol solution. Positive and negative controls are necessary to confirm the performance of the assay.

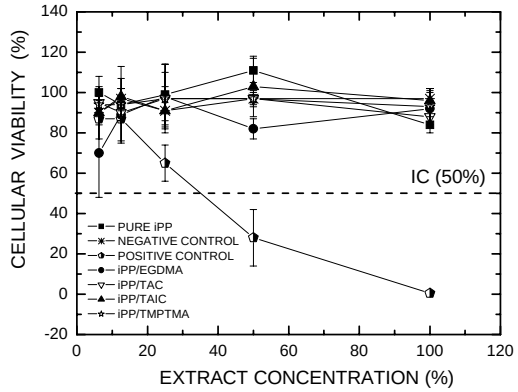
Results and Discussion

The selection and evaluation of any material or device intended to be used in humans requires a structure program of assessment such as a biological evaluation. The International Standard ISO 10993 gives guidance for biological evaluation of medical devices. The first assay for initial evaluation tests is the vitro cytotoxicity, which is used as a screening test for materials to be employed in the production of biomedical devices.
The evaluation of cytoxicity test can be by qualitative or quantitative method. Qualitative evaluation examines microscopically any changes in morphology, vacuolization, detachment, cell membranes lyses. Quantitative evaluation measures cell death, inhibition of cell growth, cell proliferation or colony formation as well as the number of cells, amount of protein, enzymes, release of vital dye, etc.
In this work the evaluation of toxicity was performed by the release of vital dye, neural red and measuring optical density (DO) in an Elisa reading Spectrophotometer Sunrise from Tecan at 540 nm.
The results were used to calculate cell viability percentage in relation to cell control, considering 100% viability. Cell viability curves were obtained in a graphic traced with these cell viabilities percentages against extract concentration.
The cytotoxicity index $IC_{50\%}$ of the cell population in the assy. The sample with cell viability curve above $IC_{50\%}$ line is considered non-cytotoxic and if under $IC_{50\%}$ is being considered toxic. The samples can be considered cytotoxic if their sequences of results cross the level of $IC_{50\%}$ line and the cytotoxicity index are obtained by projecting a line from the intersection to extract concentration axes.

(a)



(b)



(c)

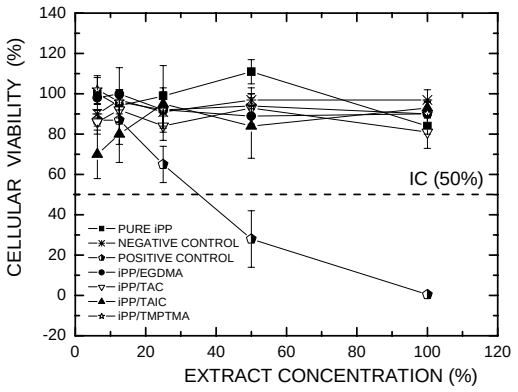


Figure 1 – Cell viability curves of cytotoxicity test by neural red uptake methodology. (a) Samples with 0.5, (b) 1.5 and (c) 5.0 monomers concentrations, respectively.

Figure 1 shows the viability curves of the tested samples for all monomers. The all curves showed a similar behavior in comparison to the negative control, which means that all samples presented non-cytotoxic effect.

The variation observed in the case of low concentration Fig 1(a) can be interpreted through the cells growth. That means during the test substance was releases and increase the cell viability. On the other hand, in this test variation around 20% up can be considered normal.

The cytotoxicity assay results demonstrated that all monomers analyzed and high processing temperature doesn't affect the toxicity of the HMSPP produced by process.

The same behavior showed by curves in Fig. 1 has been achieved to 1.0 and 3.0 mmol of monomer concentration.

Conclusions

The HMSPP films obtained with different concentrations of EGDMA, TAIC, TAC and TMPTMA process a high temperature showed no evidence of toxic effect.

The red neutral uptake method has been presented a good and quickly test to evaluate the toxicity in vitro.

Therefore after in vitro evaluation of cytotoxicity one could come to a conclusion that these materials may be used as biomaterials. Nonetheless, studies of biocompatibility should be still carried out using other methods.

Acknowledgements

The authors wish do thank to FAPESP for financial support, process number 02/13070-2 and 04/00383-8.

References

1. C. Sonnenschein, A. M. Soto. *J. Steroid Biochem. Mol. Biol.*1998, *65*, 143.
2. F. Brucker-Davis. *Thyroid*. 1998, *8*, 827.
3. T. M. Crisp, E. D. Clegg, R. L. Cooper, W. P. Wood, D. G. Anderson, K. P. Baetcke, K. P. Hoffmann, M. S. Morrow, D. J. Rodier, J. E. Schaeffer, L. W. Touart, M. G. Zeeman, Y. M. Patel. *Environ Health Perspect*. 1998, *106*, 11.
4. K. J. Söderholm, M. Phill, A. Mariotti. *J. of the American Dental Association*. 1999, *130*, 201.
5. D. Nathanson, P. Lertpitayakun, M. S. Lamkin, M. Edalatpour, L. L. Chou. *J. of the American Dental Association*. 1997, *128*, 1997.
6. J. C. Atkinson, F. Diamond, F. Eichmiller, R. Selwitz, G. Jones. *Dental Mater*. 2002, *18*, 128.
7. H. Darmani, A. S. Al-Hiyasat. *Dental Mater*, 2006, *22*, 353.
8. G. Ciapetti, D. Granchi, E. Verri, L. Savarino. A. Pizzoferrato. *Biomaterials*. 1996, *17*, 1259.
9. S. O. Rogero, S. M. Malmonge, A. B. Lugão, T. I. Ikeda, L. Miyamaru, A. S. Cruz. *Artif. Organs*. 2003, *27*, 424.
10. International Organization for Standardization, Biological Evaluation of Medical Devices. Part 5 – Test for Cytotoxicity: In Vitro Methods, ISO 10993, 1992.