Synthesis and Characterization of Poly(Methyl Methacrylate) PMMA and Evaluation of Cytotoxicity for Biomedical Application

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Summary: The objective this work was the synthesis, characterization and evaluation of cytotoxicity poly(methyl methacrylate), PMMA, nanoparticles (NPs) produced using miniemulsion polymerization technique adopting azobisisobutylonitrile (AIBN) as initiator and biocompatible and biodegradable surfactant, lecithin and co-stabilizer, miglyol 812 The NPs showed high stability during one year and an average diameter of 89 nm with polydispersity index of 0.11 and a zeta potential −48 mV. The PMMA NPs were tested for cytotoxicity in acute monocytic leukemia cells (THP1) and in human lung adenocarcinoma cells (A549). The PMMA NPs did not show any adverse effect on cell viability indicating their potential to encapsulate antitumor agents.

Keywords: biomaterials; miniemulsion polymerization; nanoparticles; stability

Introduction

Polymeric nanoparticles have been widely researched as drug delivery systems, and their main advantages are the protection of the drug, controlled release, the possibility of site-specific drug delivery in the target tissue and reduction of side effects.[1,2] In the last two decades, therapies based on nanoparticles (NPs) were successfully introduced for the treatment of cancer and infectious diseases. Different techniques can be employed for the production of polymer NPs and encapsulation of biomedical compounds.[3,4]

Miniemulsion polymerization allows the production of polymeric nanocapsules or nanospheres with unique characteristics and great commercial interest. In miniemulsion polymerization systems, an organic phase (which contains the monomer, co-stabilizer, the compounds to be encapsulated and, when used, the oil soluble initiator and surfactant), aqueous phase (which may contain the water soluble surfactant and initiator) and is afterwards polymerized into the polymer NPs. The original monomer droplets constitute the primary polymerization *loci* and behave as "nano-reactors". The main advantage of the miniemulsion polymerization process is the capability to produce complex nanostructures in a single reaction step.[5-9] Advances in controlled polymerization have enabled the engineering of advanced multifunctional polymeric nanoparticles with precise control over architecture, shape, size, surface charge and functionalization. Careful design and control over the targeting properties of these nanoparticles will secure their future development and versatility.[10]

Poly(methyl methacrylate), PMMA, NPs were chosen for several biomedical applications,[11] like vaccination, although these NPs are not biodegradable. Other biomedical applications of PMMA includes its use as a prosthetic material in dental and

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mandibular corrections and as a permanent implant for intraocular lens following cataract surgery.[11,12] At present, it is generally accepted that PMMA is a non-toxic polymer as it possesses a very good toxicological safety record in biomedical applications, and previous studies showed that PMMA NPs prepared with sodium dodecyl sulfate (SDS) as surfactant were not cytotoxic to K562 cells.[13] However, SDS can present some toxicity when the aim is biomedical application.[14]

In this work, the miniemulsion polymerization of methyl methacrylate using azobisisobutylonitrile as initiator and biocompatible and biodegradable surfactant, lecithin[15] and co-stabilizer, Miglyol 812, was optimized to produce PMMA NPs for encapsulation of drugs with therapeutic potential in diseases such as leukemia and solid tumors. PMMA NPs were used in cultures of acute monocytic leukemia cells (THP1) and human lung adenocarcinoma cell (A549) to investigate possible cytotoxicity of these NPs, besides the polymer latex stability was investigated aiming to explore the possibility of using this particles in long lasting formulations.

Materials and Methods

Materials

Methyl methacrylate (MMA) from ARINOS chemistry was used as monomer. Azobisisobutylonitrile (AIBN), purchased from VETEC Chemistry, was used as initiator, lecithin (Alpha Aesar) as surfactant and Miglyol 812 (Sazol) as co-stabilizer. In addition, hydroquinone (Nuclear) was used to stop the reaction after sample collection.

Methods

PMMA Nanoparticles (NPs) Synthesis

PMMA NPs were synthesized by miniemulsion polymerization. The organic phase consisted of 2.00 g of MMA (monomer), 0.09 g of lecithin (surfactant), 0.09g of Miglyol 812 (co-stabilizer) and azobisisobutylonitrile (initiator). The aqueous phase was composed of distilled water. After 30 min of magnetic stirring to prepare a coarse emulsion the miniemulsion was prepared by sonication for 5 min in an ice bath with amplitude of 70% using a Fischer Scientific Sonic Dismembrator (Model 500). The miniemulsion obtained was transferred to falcon tubes (10 mL) at 70 °C where the polymerization took place for 3 hours. Afterwards, the material was cooled, centrifuged and washed several times with distilled water. The material was dried at 60 °C for approximately 20 hours.

Polymer Characterization

Particle size and surface charge was measured by dynamic light scattering (DLS) using Zeta-sizer Nano ZS. The NPs morphology was observed using Transmission Electronic Microscopy (TEM), model JEM 2100F 100kV. Monomer consumption was followed by gravimetric analysis of samples withdrawn from the polymerization medium at different time intervals. The polymerization was stopped with the addition of a 1% of hydroquinone solution (w/w). The determination of residual monomer was performed by head-space gas chromatography (GC 2010AF Shimadzu) using a calibration curve prepared with different MMA concentrations.

Cytotoxicity in a Cell Culture (A549 and THP1)

The study of cytotoxic activity was performed using human leukemic cell lines (THP1) and human lung adenocarcinoma cell line (A549). Cells were placed at a density of 5×10^4 in 96-well plate and incubated with the NPs dissolved in DMSO at a final concentration of 1000 ng/mL.

Cells were cultured in Roswell Park Memorial Institute Medium (RPMI) or Dulbecco's Modified Eagle's Medium (DEMEM) (GIBCO, São Paulo, SP, Brazil) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 mg/ml streptomycin and 10 mM HEPES.

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The incubation of the cells was carried out for 24 hours at 37°C in a 5% CO_2 atmosphere. Cytotoxicity activity was evaluated using MTT (3-(4, 5-dimethiazolzyl)-2-5-diphenyltetrazolium bromide, Sigma Chemical Co., St. Louis, MO, USA) assay, a colorimetric method that evaluates cell viability. The MTT assay is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity. Since for most cell populations the total mitochondrial activity is related to the number of viable cells, this assay is broadly used to measure the in vitro cytotoxic effects of drugs on cell lines or primary patient cells. The viability of the control group (without treatment) of each cell line was considered as 100%.

Results and Discussions

Figure 1 shows a TEM imageof NPs prepared by miniemulsion polymerization. The TEM image shows that the size distribution of the NPs is uniform with diameters that agree with those measured by DLS, initial average droplets diameter (DLS) equal to 85 ± 2.3 nm and final average particle diameter and polydispersity index (DLS) of, respectively, 89 ± 1.9 nm and 0.11. The uniformity of the particles shown in Figure 1 and the similarity between the final particle and initial droplet average diameters indicate that it is likely that the dominant nucleation mechanism was the polymerization of monomer droplets in preference to other possible nucleation mechanisms, such as micellar or homogeneous nucleation, as would be expected when employing a hydrophobic initiator.[8] The minimization of other nucleation mechanisms, besides monomer droplet nucleation is important to improve the efficiency and homogeinity of the drug encapsulation, since particles formed by micellar or homogeneous nucleation would not contain the drug. In addition, the low polydispersity indicates that the NPs have a narrow size distribution and that, thus, coalescence and Ostwald ripening were successfully retarded.[16–19]

The PMMA NPs aqueous dispersions were stable for up to 1 year when stored at 4°C as determined by macroscopic analysis and by average particle size measurements by DLS (Figure 2). A fast average size increase indicates low system stability.[20,21] The NPs presented PdI values below 0.2 throughout the 1 year testing period, confirming the high stability of PMMA NPs prepared by miniemulsion polymerization using lecithin as surfactant. NPs stability is a crucial parameter in determining the moisturizing activity of the PMMA NPs *in vivo*.[22]

The PMMA NPs showed a zeta potential in the range of -48 ± 2.3 mV that reinforces

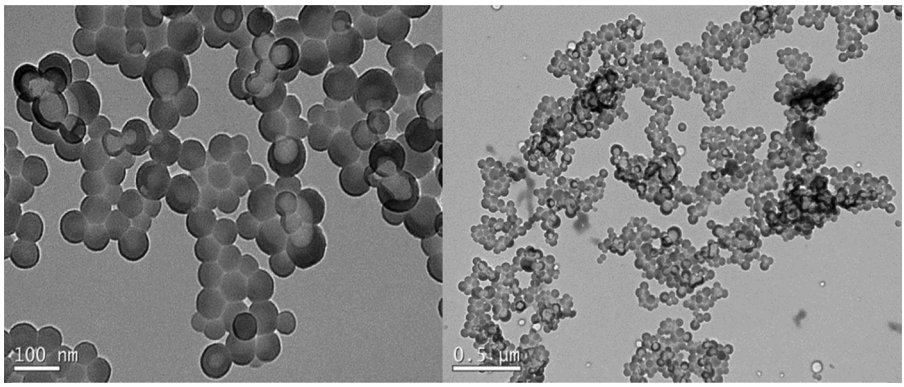

Figure 1.
Transmission Electronic Microscopy image of PMMA NPs.

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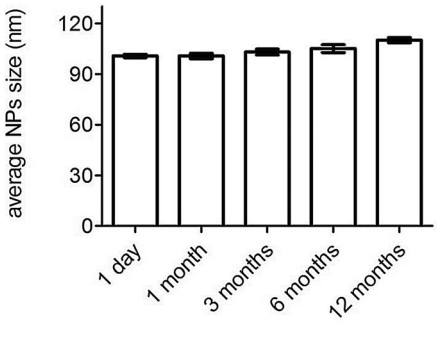

Figure 2.
Average NPs size under different storage periods, during a one year stability test.

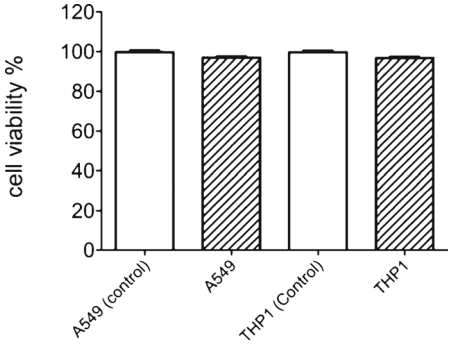

Figure 4.
Evaluation of cytotoxic of PMMA NPs in A549 and THP1 cells.

the high stability with little tendency to flocculation or coalescence.[19] The negative charge is associated with the presence of lecithin adsorbed at the NPs surface.

Reaction conversion measurements indicated that after 140 minutes of reaction the conversion reached around 100% (Figure 3).This result was confirmed by analysis of residual monomer by GC that indicated that all MMA was consumed or evaporated from the aqueous dispersion as MMA presents a high vapor pressure.

The PMMA NPs were tested for cytotoxicity in human leukemic cell lines (THP1) and human lung adenocarcinoma cell line (A549). The NPs of PMMA were suspended in a culture medium (1000 ng/ml) and incubated with THP1 and 549 cells. After incubation cytotoxity was measured at 24 hours (trypan blue and MTT). The results (Figure 4) indicated that the NPs presented no cytotoxicity to cells, with cell viability values near those obtained in the control groups without NPs.

Conclusion

PMMA nanoparticles were prepared by miniemulsion polymerization with a biocompatible and biodegradable surfactant, lecithin, and co-stabilizer, Miglyol 812. The nanoparticles had an average diameter of about 90 nm and the polymer latex showed high stability. The PMMA nanoparticles did not present any observable cytotoxicity when tested against a human leukemic cell line (THP1) and a human lung adenocarcinoma cell line (A549). These results are relevant for the development of antitumor agents encapsulated in PMMA nanoparticles.

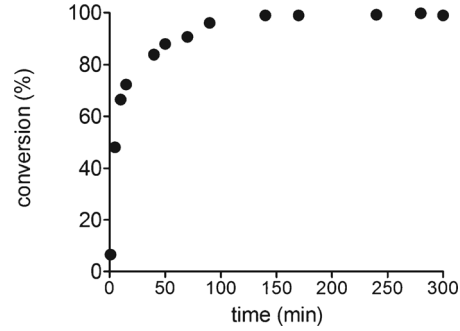

Figure 3.
Conversion of MMA during miniemulsion polymerization.

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