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Paclitaxel molecularly imprinted polymer-PEG-folate nanoparticles for targeting anticancer delivery: Characterization and cellular cytotoxicity

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ABSTRACT

The aim of this work was to synthesize molecularly imprinted polymer-poly ethylene glycol-folic acid (MIP-PEG-FA) nanoparticles for use as a controlled release carrier for targeting delivery of paclitaxel (PTX) to cancer cells. MIP nanoparticles were synthesized by a mini-emulsion polymerization technique and then PEG-FA was conjugated to the surface of nanoparticles. Nanoparticles showed high drug loading and encapsulation efficiency, 15.6 ± 0.8 and 100%, respectively. The imprinting efficiency of MIPs was evaluated by binding experiments in human serum. Good selective binding and recognition were found in MIP nanoparticles. In vitro drug release studies showed that MIP-PEG-FA have a controlled release of PTX, because of the presence of imprinted sites in the polymeric structure, which makes it is suitable for sustained drug delivery. The drug release from polymeric nanoparticles was indeed higher at acidic pH. The molecular structure of MIP-PEG-FA was confirmed by Hydrogen-Nuclear Magnetic Resonance (H NMR), Fourier Transform InfraRed (FT-IR), and Attenuated Total Reflection (ATR) spectroscopy, and their thermal behaviors by Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA). Scanning Electron Microscopy (SEM) and Photon Correlation Spectroscopy (PCS) results showed that nanoparticles have a smooth surface and spherical shape with an average size of 181 nm. MIP-PEG-FA nanoparticles showed a greater amount of intracellular uptake in folate receptor-positive cancer cells (MDA-MB-231 cells) in comparison with the non-folate nanoparticles and free PTX, with half maximal inhibitory concentrations (IC_{50}) of 4.9 ± 0.9, 7.4 ± 0.5 and 32.8 ± 3.8 nM, respectively. These results suggest that MIP-PEG-FA nanoparticles could be a potentially useful drug carrier for targeting drug delivery to cancer cells.

1. Introduction

Molecular imprinting technology is a new and rapidly developing technique which offers the ability to prepare polymers having specific molecular recognition properties for a specific compound, its analogs or for a single enantiomer [1]. Molecularly imprinted polymers (MIPs), the polymeric matrices provided by imprinting technology, are powerful recognizing elements which have the ability to mimic natural recognition entities like antibodies and biological receptors which can be programed to specifically separate complicated samples like biological fluids and environmental samples [2]. Different uses and potential applications of the imprinted polymers demand morphology properties. MIPs are generally insoluble polymers whose subsequent use depends on their morphology in terms of the shape and particle size [3]. MIPs have broad application in different fields of science but perhaps their most attractive application is therapeutics and medical therapy such as drug-delivery systems [4]. For example, using MIPs as targeted drug delivery in which the drug, bounded either covalently or non-covalently to the MIP, would be released when the MIP binds to its target on the surface of a cell [5,6]. When a drug has a narrow therapeutic index it is important to keep the concentration at a constant level. Imprinted materials can potentially do this work by reducing the rate of drug release. In this case the MIP delivery devices keep the concentration of drug below the concentration in which adverse side effects become dominant [2,7,8].

Being used as drug delivery systems, MIPs must be biocompatible or biodegradable and have the ability to be transformed into non-toxic fragments that can be eliminated harmlessly from the body [9]. Therefore, it might be more appropriate to try to adapt the imprinting technique to already tested materials instead of creating a completely new polymeric system [10]. Monomers such as methacrylic acid (MAA), methyl methacrylate (MMA) and ethylene glycol dimethacrylate

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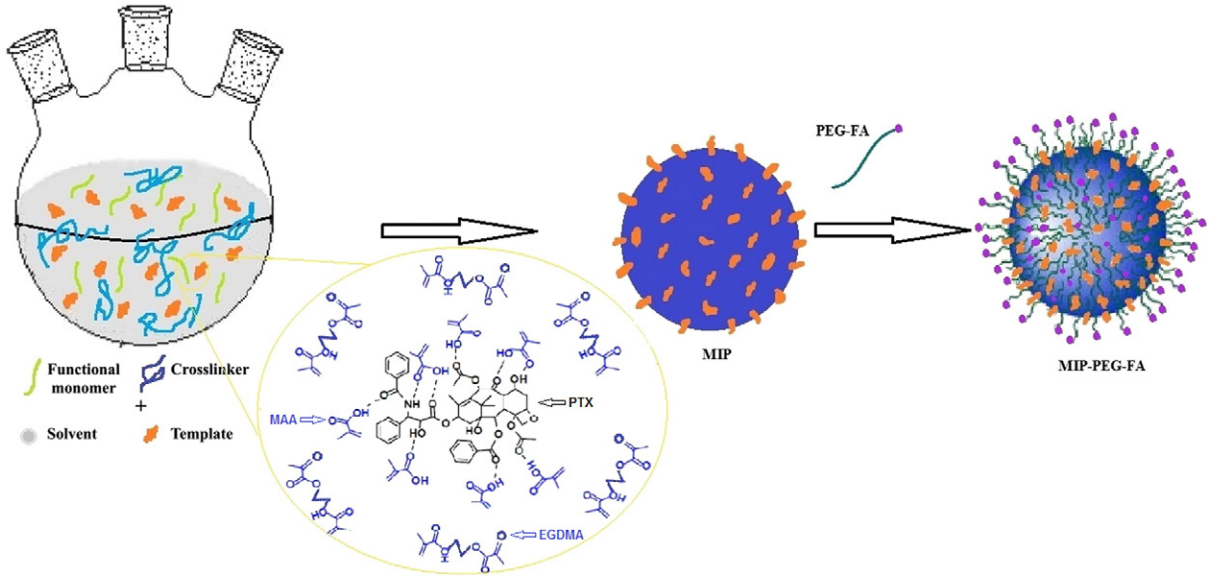

Fig. 1. Scheme of preparation of MIP-PEG-FA nanoparticles. First step is the synthesize of MIP nanoparticles using the miniemulsion polymerization method; second step is the conjugation of PEG-FA to the surface of MIP nanoparticles.

(EGDMA) are biocompatible and non-toxic [11,12]. N-vinylpyrrolidinone and MAA cross-linked by EGDMA are broadly used in enteric coated drug delivery systems, have shown to be biocompatible and have the ability to excite the fibroblast viability of skin cells [13]. Also Peppas et al. [14,15] have shown that MAA-based nanoparticles don't have any cytotoxic effects on the Caco-2 cell cultures.

Nanotechnology has also been used to produce polymeric nanoparticles which have greater penetration ability across the biological membranes and controlled release of the adsorbed drug [16,17]. In addition these nanoparticles extravasate through the tumor vasculature, and deliver their contents into the cells by the enhanced permeability and retention (EPR) effect, thereby increasing their therapeutic effect [18].

Vitamin folic acid (FA) is one of the well-studied ligands to be exploited for targeting tumor cells. FA has an important role in human growth and development, cell division and DNA synthesize [19,20]. FA uptake into cells is mediated by the folate receptor (FR). Binding of FA to FR initiates receptor-mediated endocytosis and internalization of FA. Expression of FR on normal cells is low, however the demand for FA increases by increasing cellular activity and proliferation [12].

Paclitaxel (PTX) is an anti-neoplastic drug extracted from the bark of *Taxus brevifolia* which has anti-cancer activity against different types of solid tumors. PTX is one of the best anticancers, which has excellent chemotherapy effects against ovarian and breast cancers. The mechanism of anti-cancer activity of PTX is disruption in the equilibrium inside the microtubule system and blockage of cells in the late G2 phase and M phase of the cell cycle [21].

In this work, we developed MIP-PEG-FA nanoparticles for targeted delivery of PTX to cancer cells. MIPs were initially prepared using mini-emulsion method. Then FA was attached to the PEG bis-amine and finally the PEG-FA was conjugated to the imprinted nanoparticles. The morphology and size distribution of nanoparticles were investigated by photon correlation spectroscopy (PCS) and scanning electron microscopy (SEM). The molecular structure of MIP-PEG-FA was characterized by H NMR, FT-IR and ATR. Thermal properties of nanoparticles were analyzed by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Finally in vitro release and anticancer properties on MDA-MB-231 (folate positive) and A549 (folate negative) cells were studied. MDA-MB-231 is a human breast adenocarcinoma cell line and A549 is a human lung carcinoma cell line. And also the imprinting efficiency of blank MIPs was evaluated by binding experiments in human serum.

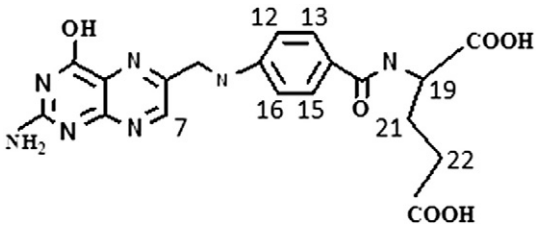

Fig. 2. Chemical structure of folic acid.

2. Experimental

2.1. Materials

Methacrylic acid (MAA) and methyl methacrylate (MMA) were obtained from Merck (Darmstadt, Germany), they were distilled under reduced pressure at 50–55 °C prior to use. Poly ethylene glycol bis amine, ethylene glycol dimethacrylate (EGDMA), folic acid (FA), (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT, and azobisisobutyronitrile (AIBN) were purchased from Sigma-Aldrich (Steinheim, Germany). AIBN was re-crystallized from methanol prior to use. Dicyclohexylcarbodiimide (DCC), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), sodium dodecyl sulfate (SDS), and hexadecane were purchased from Merck (Hohenbrunn, Germany). Roswell Park Memorial Institute-1640 Medium (RPMI-1640), fetal bovine serum (FBS), and Trypsin–EDTA 0.25% solution were obtained from Gibco, Burlington, Canada. Paclitaxel was purchased from Cipla, India. Dialysis tubes (Sigma dialyses tubes molecular weight cutoff 12 kDa) were heated in an aqueous solution of 2 wt.% sodium hydrogen carbonate and 0.05 wt.% ethylene diamine tetraacetic acid (EDTA) at 100 °C for 10 min, and then kept under refrigeration in an aqueous solution of 0.05 wt.% sodium azide until use. Human plasma was obtained from the Iranian Blood Transfusion Service (Tehran, Iran). All other reagents and solvents were analytical grade.