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Design of pH-responsive antimicrobial nanocomposite as dual drug delivery system for tumor therapy

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ABSTRACT

A novel antibacterial clay/polymer nanocomposite with average particle size of 20–40 nm and two cationic compartments in polymer was synthesized via ion exchange. The structure of the nanocomposites was characterized by XRD, FT-IR, TG-DTA, and SEM. This multifunctional nanocomposite was used for dual drug delivery of anticancer drug methotrexate (MTX) and an antibacterial agent ciprofloxacin (CIP) with encapsulation efficiency of >90% for both drugs. The in vitro antimicrobial activity of the clay/polymer nanocomposites was studied against *Escherichia coli* and *Pseudomonas aeruginosa* bacteria by a well diffusion method. The nanocomposite showed good or moderate antimicrobial activities. However, CIP loaded nanocomposites showed enhanced antimicrobial activity in comparison to free CIP. The potential antitumoral activity of this clay/polymer nanocomposite system was evaluated against MCF7 cell lines by MTT assay and cell cycle studies. The cytotoxicity studies demonstrated enhanced cytotoxicity of developed MTX loaded nanocomposite in comparison to free MTX. Cell cycle study showed that MTX-loaded nanocomposite caused S-phased arrest in MCF-7 cells compared to control non-treated cells ($P < 0.001$). Therefore, dual drug-loaded antibacterial nanocomposite has the potential to be used for cancer therapy.

1. Introduction

One of the most important health threats is multi-drug resistance of human cancer cells and pathogens (Housman et al., 2014; Fair and Tor, 2014; Tanwar et al., 2014). The pathogenic bacteria have evolved mechanisms of resistance to most commercially produced antibiotics (Tanwar et al., 2014; Alekshun and Levy, 2007). It is necessary to develop novel methods of antibacterial treatment which do not use traditional therapeutic systems. Latest progresses in nanotechnology have offered the basis for using metallic nanoparticles in the fight against MDR bacteria (Miller et al., 2015; Ray et al., 2012). Other researches have proved the antibacterial potential of some polymers like polyhexamethylene guanide (PHMB) (Chindera et al., 2016) or cationic polymers (Carmona-Ribeiro and de Melo Carrasco, 2013; Xue et al., 2015; Salehi et al., 2015a). Chemotherapy antibiotics are prescribed to patients due to susceptibility to infectious diseases (Salehi et al., 2015a).

Cancer cells can enlarge resistance to chemotherapy drugs, but are less likely to develop resistance while chemotherapy drugs are applied in nanoformulation (Ma et al., 2012; Shen et al., 2013). Nanoparticle drug delivery has added more progress in the efficacy of cancer therapeutics and diminishes toxic side effects, therefore revealing a major guarantee in cancer therapy (Shen et al., 2013; Salehi et al., 2015c, 2014b, 2014a).

Laponite is a 2:1 synthetic clay mineral, consisting of one magnesium octahedral layer between two silicon tetrahedral layers. It is a biocompatible non-toxic synthetic disk-shaped clay mineral with a thickness of approximately 1 nm and a diameter of 25 nm (Thomas et al., 2011) which has attracted attention in drug delivery and biomedical fields because of its high specific surface area (370 $m^2 \cdot g^{-1}$) (Mustafa et al., 2015). High loading capacity of Laponite for anticancer drug DOX (98.3%) and pH-dependent sustain release profile indicated superior therapeutic efficiency of LAP/DOX systems as compared to free DOX (Wang et al., 2013). In aqueous dispersion, the Laponite RD particles have negative charges on the faces and a weakly positive charge on the edges. Laponite RD potential in pharmaceutical applications is due to cationic exchange capacity as well as adsorption properties (Thomas et al., 2011). Sodium ion in Lapnoite can be exchanged with organic cations and the degree of intercalation of organic cationic parts related to sodium ion concentration.

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Clay nanocomposites (NC) are used as scaffolds in tissue engineering (Haroun et al., 2009), wound healing (Sandri et al., 2014), and as drug delivery systems (Salcedo et al., 2012)and their positive effect on cancer cell death protection have been proven by several studies (Abbes et al., 2008; Maisanaba et al., 2014). Cervini-Silva et al. (2016) reported the effect of clay on cell growth of cancer cells, cell growth inhibition and/or cell growth increase for different cancer cell types (Cervini-Silva et al., 2016).

Despite the beneficial effect of Laponite RD clay, its application in drug delivery is limited due to some inherent drawbacks such as instability and its tendency to flocculate and precipitate under physiological conditions. The ion exchange of inorganic cations with organic cations, especially with quaternary ammonium compounds, and synthesis of polymer-clay NCs not only would alleviate this disadvantage, but also introduce antibacterial property in polymer-clay NCs (Nigmatullin et al., 2008). Clay polymer have been used as drug delivery systems (Suresh et al., 2010; Jafarbeglou et al., 2016), antimicrobial agents (Nigmatullin et al., 2008), excipients and active agents in pharmaceutical applications (Khurana et al., 2015; Aguzzi et al., 2007). Hydrogels are known as three-dimensional polymeric networks with extraordinary capacities to absorb and keep water, with potential applications in biomedical fields. Due to the restricted molecular motion of polymer chains caused by the large amount of cross-links arranged randomly, the majority of developed hydrogels are soft, weak, and fragile, which seriously restrict their applications. Therefore, polymeric hydrogel NCs would ease this drawback. NCs hydrogels (first produced by Haraguchi et al.) (Haraguchi and Takehisa, 2002) are class of nanoclays that have attracted considerable attention in biomedical fields because of their superiority to traditional hydrogels. Incorporating Laponite particles into polymers and hydrogels improves mechanical strength, elasticity, hardness, swelling, and biological behaviors of the NC compared to the polymer alone (Ghadiri et al., 2013; Schexnailder et al., 2010; Yang et al., 2011). MTX is a known competitive inhibitor of dihydrofolate reductase enzyme (Spurlock et al., 2012). In lower doses MTX is used as synchronization agent, but in higher doses, exhibits cytotoxic effects on proliferating cells at S-phase by inhibiting DNA synthesis (Sen et al., 1990). Driven by this need, a nanotechnology-based approach was used to prepare a novel nanocarrier with potent antibacterial property and ability to be used as dual drug delivery system for cancer therapy. The article is divided into two parts. In the first part, two kinds of cationic vinyl monomers were synthesized and intercalated in Laponite particles by cationic exchange with sodium ions. The modified NCs were dispersed in water and used as seeds by in situ emulsion polymerization to synthesize Laponite/polymer NC with potent antibacterial property. The obtained NC was used as smart carrier for dual delivery of MTX as chemotherapy and ciprofloxacin (CIP) as antibacterial agents.

2. Materials & methods

2.1. Synthesis of 2-chloroethyl acrylate (CEA)

A novel monomer of 2-chloroethyl acrylate was synthesized from chloroethanol and acryl chloride as follows: Firstly 0.06 mol chloroethanol and an equimolar of acryl chloride (0.06 mol) were reacted in 17 mL dichloromethane followed by dropwise addition of triethylamine (0.06 mol) which was used as HCl scavenger at room temperature for 12 h. the produced salt (three ethyl ammonium chloride) was filtered and organic phase was washed with deionized water several time, after separation of two phase (water and organic phase) the solvents removed by rotary evaporation.

2.2. Synthesis of cationic 3-methyl 1-[2-(acryloxy)-ethyl] imidazolium chloride ionic liquid monomer (AcImIL)

A novel imidazolium based ionic liquid monomer was synthesized by mixing 0.041 mol CEA and 0.048 mol methyl imidazole in a 50 mL two-necked round bottom flask under stirring and refluxed for 72 h at 45 °C under argon flow. At the end the product was extracted with acetonitrile and the solvent was removed by rotary evaporation The product was dried under vacuum, and stored at 4 °C.

2.3. Synthesis of 2-(methacryloyloxy) ethyl trimethyl ammonium chloride (MADQUAT)

Cationic quaternary ammonium alkyl halide monomer (MADQUAT) was prepared as described in previous work (Salehi et al., 2015c). Firstly, 19.1 mmol of MADQUAT was added to 5 mL of dry THF and stirred for 5 min, then 22 mmol of CH_3I was added dropwise to the solution. The reaction was continued for 12 h at room temperature during stirring. The final products were filtered, washed with 20 mL hexane and put in vacuum dry oven overnight to obtain the MADQUAT product (white solid powder, yield: 95%).

2.4. Synthesis of organo-modified Laponite RD (MADQ-AcImIL&LP)

The organo modified Laponite-RD (MADQ-AcImIL&LP) was prepared by adding two kind of cationic ionic liquids, 1.2 g of AcImIL and 0.36 g MADQUAT in 1 g of Laponite-RD dispersion in 1000 mL of deionized water at 50 °C and ultrasonically dispersed for 5 min with sonication (400 W) by using probe-type ultrasonic generator. Then the resultant dispersion was vigorously stirred for 72 h. The precipitate was repeatedly filtered and washed with hot deionized water until all of chloride ion was removed (detected with 0.1 N $AgNO_3$ solutions). It was dried at 50 °C for 24 h.

2.5. Synthesis of multifunctional stimuli-responsive nanocomposite with organo-modified Laponite RD

0.3 g organo modified Laponite-RD (MADQ-AcImIL&LP) was added into 20 mL dioxane after 1 h dispersion at 70–80 °C, 0.14 g *N*-isopropyl acryl amide (NIPAAm) and 0.1 g methacrylic acid (MAA) were added to the mixture. The obtained mixture was placed in a clean polymerization tube, and the reaction mixture was degassed for 10 min with argon. Then 0.016 g AIBN was added to the mixture as an initiator in argon gas condition. Then, the tubes were sealed under argon atmosphere and the mixture polymerized for 96 h at 70–72 °C. After completion of reaction, the product P(NIPAAm-MAA)&MADQ-AcImIL&LP was rinsed and completely milled in a mortar to get fine powder.

2.6. Instrumentation

2.6.1. Hydrogen nuclear magnetic resonance (^{1}H NMR) spectroscopy

^{1}H NMR spectroscopy AcImIL was recorded in d6–DMSO solvent on a Bruker DRX-400 spectrometer with tetramethylsilane as internal reference.

2.6.2. Fourier transforms infrared (FTIR) spectroscopy

The chemical structures of the Laponite-RD, AcImIL IL and P(NIPAAm-MAA)&MADQ-AcImIL&LP nanocomposites were studied by FTIR spectroscopy (mix with KBr and press to disk) (Equinox 55 LS 101, Bruker, Germany).

2.6.3. X-ray diffraction (XRD)

Powder X-ray diffraction patterns of the Laponite-RD and P(NIPAAm-MAA)&MADQ-AcImIL&LP nanocomposite were recorded on a Bruker AXS model D8 Advance diffractometer using CuKα radiation ($\lambda = 1.542$ Å), with the Bragg angle ranging from 2 to 70 °C.

2.6.4. Scanning electron microscopy studies

The surface morphology, average diameter, particle size and pore volume of organo modified Laponite (MADQ-AcImIL&LP) and