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Synthesis, characterization, drug release and transdentinal delivery studies of magnetic nanocubes coated with biodegradable poly(2-(dimethyl amino) ethyl methacrylate)☆

Phranot Ajkidkarn[a], Patcharee Ritprajak[b], Wishulada Injumpa[c], Thantrira Porntaveetus[d,e], Numpon Insin[c,*]

[a] Petrochemistry and Polymer Science Program, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[b] Department of Microbiology and RU in Oral Microbiology and Immunology, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand
[c] Departmen of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[d] Department of Physiology, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330, Thailand
[e] STAR on Craniofacial and Skeletal Disorders, Chulalongkorn University, Bangkok 10330, Thailand

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ABSTRACT

Nanotechnology on magnetism and magnetic materials has been developed and studied extensively for the recent decades. Magnetic nanoparticles were applied in magnetic targeting, magnetic drug carriers, and diagnostic materials. In this work, the development of magnetic nanocomposites and their applications as drug carriers for dentistry were investigated.

Well-defined ferromagnetic magnetite nanocubes (FMNCs) with the diameter of around 60 nm were synthesized using a thermal decomposition method at 290 °C with iron-oleate complexes as starting materials resulting in nanostructure with high saturation magnetization. The FMNCs were then coated with poly(2-(dimethyl amino)ethyl methacrylate) (PDMAEMA), a water-soluble, biodegradable, and pH-responsive polymer, in order to become good drug carriers with excellent dispersity in biological buffer, low cytotoxicity, and controllable drug release. The polymer coating was performed using atom transfer radical polymerization (ATRP). By using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, FMNCs/PDMAEMA showed the high compatibility in fibroblast and macrophage cell line with the cell viability of more than 80% after incubation with the highest nanocomposites concentration of 100 μg/mL for 24 h. Furthermore, the FMNCs/PDMAEMA subsequently demonstrated the anti-inflammatory effect on macrophages by suppression of pro-inflammatory cytokines, IL-6 and TNF-α production in a dose-dependent manner. The behavior of model drug alkaline hyperchlorite released from the FMNCs/PDMAEMA indicated that the drug release could be controlled by altering pH of the environment. As a result of successfully synthesized FMCNs/PDMAEMA, dentine infiltration of FMNCs/PDMAEMA was performed. It was observed that FMNCs/PDMAEMA could significantly infiltrate the dentine within 30 min under an external magnetic field. Our findings indicated the therapeutic potential of the FMNCs/PDMAEMA as transdentinal drug carriers with its high biocompatibility and anti-inflammatory property.

1. Introduction

Currently, endodontic treatment encounters several difficulties including minimal remaining tooth structure, complicated procedure, unresolved pain or swelling, unpredictable result, high cost, and so forth [1]. On the ground of endodontic treatment, dental pulps containing nerves and blood vessels of infected teeth are removed. The pulp chambers of the teeth are then cleaned, disinfected, and filled. Novel methods for endodontic treatment such as drug delivery using electric field and ultrasonication [2] have been proposed and investigated, but experienced limitations. Magnetically-induced drug delivery using superparamagnetic nanoparticles as drug carriers is one of promising techniques for effective delivery systems [3] and is of the main focus on this study.

There has been expanding interest in the use of magnetic nanoparticles for biomedical applications including magnetic resonance

☆ In remembrance of his Majesty King Bhumibol Adulyadej (1927-2016), for his life-time dedication to Thailand.

* Corresponding author.
E-mail address: Numpon.I@chula.ac.th (N. Insin).

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imaging (MRI) [9], bacterial detection [12], hyperthermia [3,4], protein purification [12], cell separation [3], drug delivery [3,12], etc. In these applications, magnetite and maghemite iron oxide nanostructures have been extensively studied, even though they were not the highest in magnetization, due to their relatively low toxicity compared to other ferro- and ferrimagnetic materials [11]. To increase in magnetization, the iron oxides ferromagnetic magnetite nanocubes (FMNCs) have been developed, resulting in iron oxide nanostructure with 14% higher magnetization values and narrow coercivity [8].

In practical biological applications, however, the MNCs were required to be both stably dispersed and biocompatible in aqueous solution. Surface modification of the MNCs with hydrophilic and biocompatible polymers such as polyethyleneglycol (PEG), polylactic acid (PLA) and poly(lactic-*co*-glycolic acid) (PLGA), [7] was reported to achieve the purpose mentioned above. In this study, poly(2-(dimethyl amino)ethyl methacrylate) (PDMAEMA) was selected to coat onto MNCs as the polymer was reported to have additional satisfactory releasing behavior for many drugs at physiological conditions. After the FMNCs/PDMAEMA composites were successfully synthesized, drug loading and releasing behavior, cytotoxicity, and trandentinal delivery were investigated.

2. Materials and methods

2.1. General

2.1.1. Materials

Iron (III) acetylacetonate ($Fe(acac)_3$, 98%), oleic acid (OA, 90%), benzyl ether, 2,2′-bipyridine (bpy, 99%), α-Bromoisobutyric acid (BIB), [2- (dimethyl amino)-(ethy methacrylate)] (DMAEMA), and alkaline hypochlorite were purchased from Sigma-Aldrich. MTT was purchased from Life Technologies.

2.2. Synthesis of magnetic nanocubes (FMNCs)

The FMNCs were successfully synthesized as Kim *et al.* have demonstrated [10], and the preparation process were employed with slight modification. Briefly, iron acetylacetonate (0.71g) was added into a mixture of oleic acid (1.13g) and benzyl ether (10.4g). A mixture was degassed for an hour at 60 °C then thermal decomposition was further applied by heating a mixture to 290 °C at the rate of 20 °C per min under vigorous magnetic stirring. The reaction was maintained at this temperature for an hour. Cooling to room temperature was required before the product was washed with toluene and hexane. Black precipitate was attained by centrifugation and decanted by magnet separation and washed with chloroform for at least 3 times to obtain FMNCs capped with oleic acid (FMNCs-OA).

2.3. Synthesis of PDMAEMA polymer modified magnetic nanocubes (FMNCs/PDMAEMA)

Dopamine (250 mg) was added to dried precipitate of FMNCs-OA in order to functionalize the FMNCs with amino group. The mixture was dispersed in 3 mL of DMF and stirred for 45 min. Black precipitate was isolated with magnet and rinsed with ethanol for at least 3 times. α-Bromoisobutyric acid (1.67 mmol, 277.2 mg) was added, and the mixture was then stirred for 24 h at room temperature. A solution was washed with methanol and dried under vacuum at room temperature overnight to yield FMNCs with initiator groups on their surface. The PDMAEMA polymer was then favorably synthesized; briefly, FMNCs with initiator (50 mg) was dispersed in isopropanol (10 mL) using ultrasonic agitation for 2 min. DMAEMA monomer (5.0 g) was added into the mixture then degassed. A copper (I) bromide (0.1 g) and bipyridyl (0.33 g) was used as catalysts. The sealed reaction was agitated by stirring at room temperature for 24 h before further magnetic decantation. Ultimately, precipitate was washed with water, ethanol, and hexane respectively to obtain the product FMNCs/PDMAEMA.

2.4. Loading FMNCs/PDMAEMA with alkaline hypochlorite and in vitro drug release

The solution of alkaline hypochlorite, the model drug used in the initial treatment of infected teeth [15], was added dropwise with stirring to 3 mL of FMNCs/PDMAEMA with the concentration of 2.5 mg/mL in distilled water. The mixture was continuously shaken for 24 h at room temperature so as to allow the drug partition into the polymer shell. Moreover, the black particles were magnetically precipitated and subsequently isolated by centrifugation. The drug loading capacity was calculated as follows.

Loading capacity (%)=100x(initial weight of drug – weight of free drug)/(initial weight of the modified magnetic nanocubes).

The drug-loaded magnetic nanocubes were transferred into a 3.5 K molecular weight cut-off dialysis bag and immersed into three buffers of 0.01 M in concentration, including pH5 cacodylate buffer, pH7 phosphate buffer solution, and pH10 carbonate-bicarbonate at 37 °C. While stirred in the dark, aliquots (4 mL) of the buffer solutions were taken at different time intervals and then monitored by a UV–visible spectrometer at 292 nm. A 4 mL of the buffer solution was added to keep the total volume constant. The drug concentration was determined using standard curves and as the equation below.

Cumulative release (%) =100 W_t/W;

where W_t is the weight of drug released from FMNCs/PDMAEMA at time t, and W is the total weight of drug loaded into the polymer shell.

2.5. Cell culture

L929 and RAW264.7 cells (fibroblast and macrophage cell lines) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplied with 10% FBS (fetal bovine serum) and 1% antibiotics at 37 °C incubated in a humidified atmosphere composed of 5% CO_2.

2.6. Cytotoxicity measurement

L929 and RAW264.7 cells were prepared to measure the cytotoxicity of the synthetic materials. The PDMAEMA-modified magnetic nanocubes were dispersed in DMEM with different concentrations ranging from 4 to 500 μg/mL. L929 and RAW264.7 cells were seeded into 96-well plates with ca. 30,000 cells/well in 200 μL medium and incubated for 24 h at 37 °C. Then the culture was carefully removed and replaced with 200 μL of fresh medium comprising the serial concentration of FMNCs/PDMAEMA to incubate the cells. The 20 μL of 5 mg/mL MTT assays stock solution in PBS was added to each well after 48 h incubation at 37 °C and further incubated for 1.5 h at 37 °C. Lastly, the medium containing unreacted dye was removed, and 200 μL per well DMSO was added to dissolve the achieved purple formazan crystals. The absorbance was measured in a BioTek Elx80 at a wavelength of 570 nm.

2.7. Preparation of dentine discs

Fifteen extracted sound permanent molars were included in the study. In order to observe the physical responses of the FMNCs in transdentinal passages, we have prepared dentine discs according to the previous reported process [16]. The teeth were stored in 0.01 M phosphate buffered saline (PBS) at 4 °C and kept not more than 4 weeks. A cross section of dentine disc was achieved using a diamond blade. Most importantly, the dentine thickness should not be less than 0.7 mm. and the teeth were sliced as close as possible to the pulp chamber for sample utilization. Then, the dentine discs were neatly polished on an abrasive rotator. To remove smear layers, 35% of