International Journal of Pharmaceutics 436 (2012) 771–777

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Synthesis, characterization and *in vitro* assessment of the magnetic chitosan–carboxymethylcellulose biocomposite interactions with the prokaryotic and eukaryotic cells

Alexandru Mihai Grumezescu[a,*], Ecaterina Andronescu[a], Anton Ficai[a], Coralia Bleotu[c], Dan Eduard Mihaiescu[a], Mariana Carmen Chifiriuc[b]

[a] University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Department of Science and Engineering of Oxidic Materials and Nanomaterials, Polizu Street no 1-7, 011061 Bucharest, Romania
[b] University of Bucharest, Faculty of Biology, Microbiology Immunology Department, Aleea Portocalelor no 1-3, 060101 Bucharest, Romania
[c] S Nicolau Institute of Virology, 285 Mihai Bravu Avenue, 030304 Bucharest, Romania

ARTICLE INFO

Article history:
Received 4 June 2012
Received in revised form 25 July 2012
Accepted 27 July 2012
Available online 4 August 2012

Keywords:
Prokaryotic cells
Eukaryotic cells
Magnetic chitosan
Magnetic cellulose
Magnetic composite

ABSTRACT

Preparation and characterization of CS/Fe_3O_4/CMC composite scaffolds including the morphology, crystallinity, and the *in vitro* efficacy as antibiotic delivery vehicles as well as their influence on the eukaryotic cells are reported. The results demonstrated that the magnetic polymeric composite scaffolds are exhibiting structural and functional properties that recommend them for further applications in the biomedical field. They improve the activity of currently used antibiotics belonging to penicillins, macrolides, aminoglycosides, rifampicines and quinolones classes, representing potential macromolecular carriers for these antimicrobial substances, to achieve extracellular and intracellular targets. The obtained systems are not cytotoxic and do not influence the eukaryotic HCT8 cell cycle, representing potential tools for the delivery of drugs in a safe, effective and less expensive manner.

1. Introduction

During last decade, nanomaterials are of considerable interest due to the functionalities unavailable to bulk materials (Subhasree et al., 2012; Di Sia, 2012). Magnetite (Fe_3O_4) has been widely used as nanofluid (Saviuc et al., 2011a) or for biomedical applications, such as magnetic resonance imaging (Hu et al., 2006), bio-separation (Manzu et al., 2010), drug targeting (Grumezescu et al., 2011a; Mihaiescu et al., 2011) and hyperthermia (Andronescu et al., 2010a), due to their excellent magnetic properties, chemical stability, and biocompatibility (Saviuc et al., 2011b; Qu et al., 2010). Nano-sized magnetic carriers possess quite a good performance due to their higher specific surface area and to the lower internal diffusion resistance (Liao and Chen, 2002). Magnetic carriers are usually composed of a magnetic core to ensure a strong magnetic response and a polymeric shell (Andronescu et al., 2012b) to provide favorable functional groups and features for various applications (Huang et al., 2009; Grumezescu et al., 2011b; Ficai et al., 2011a).

Chitosan (CS), poly[b-(1-4)-linked-2-amino-2-deoxy-D-glucose] is non-toxic, hydrophilic, biocompatible, biodegradable and anti-bacterial (Dhanasingh et al., 2011; Shen et al., 2012; Papadimitriou et al., 2012). Due to the presence of both hydroxyl and amine groups in its structure, CS can be chemically modified to be used as novel separation media (Xia et al., 2006), DNA delivery (Zeng et al., 2011), drug delivery systems (Khunawattanakul et al., 2011) or used for tumor extracellular targeting (Duan et al., 2011). CS and its derivatives have great potential applications in the areas of biotechnology, biomedicine, food ingredients, and cosmetics (Chang and Chen, 2005; Paliwal et al., 2012; Duan et al., 2012). In comparison with many other polymers, CS contains a number of free amine groups, allowing its extensive use in drug delivery applications (Meng et al., 2011). In an acidic environment, the amino groups could be positively charged after protonation and consequently, CS is able to interact with negatively charged molecules (Calvo et al., 1997).

Carboxymethyl cellulose (CMC) is the most widely used cellulose ether today, a long chain, linear anionic polysaccharide, water-soluble, with ubiquitary distribution, composing the fibrous tissue of plants (Bayarri et al., 2009; Biswal and Singh, 2004) with applications in the detergent, food exploration, paper, textile, pharmaceutical and paint industries (Pushpamalar et al., 2006). It has a number of sodium carboxymethyl groups (CH_2COONa),

* Corresponding author. Tel.: +40 765349326.
E-mail address: grumezescu@yahoo.com (A.M. Grumezescu).

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2012.07.063

introduced into the cellulose molecule, which promote water solubility (Biswal and Singh, 2004). Among all the polysaccharides, CMC is easily available and it is also very cheap. It has opposite electric charge to chitosan, so CMC can react strongly with chitosan and could be used as an ionic cross-linking agent at the appropriate pH (Tiitu et al., 2006).

The intermacromolecular complexes are obtained by the interaction of macromolecules carrying ionized/ionizable groups of opposite sign. The electrostatic forces may be completed by hydrogen bonds, van der Waals forces, hydrophobic bonds. Intermacromolecular complexes represent successful candidates for controlled drug-release systems (Rosca et al., 2005). The chitosan complexation with carboxymethylated polysaccharides, such as carboxymethylcellulose has been studied (Peniche and Arguelles-Monal, 2001; Rosca et al., 2005).

The composite of CMC/CS has been investigated by some researchers for possible medical applications on the account of its excellent biocompatibility, biodegradability and hydrophilic surface (Hasan and Nurhan, 2004; Just and Majewicz, 1989).

In this paper, we report the fabrication, characterization of CS/Fe_3O_4/CMC and the *in vitro* efficacy as antibiotic delivery vehicles as well as their influence on the eukaryotic cells.

2. Materials and methods

2.1. Materials

Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), ferric chloride ($FeCl_3$), ammonia (NH_3), methanol (CH_3OH), chitosan (CS, 85% deacetylated) and carboxymethyl-cellulose (CMC, average Mw = 90,000) were purchased from Sigma–Aldrich.

2.2. CS/Fe_3O_4/CMC biocomposite

Magnetic iron oxide particles are usually prepared by wet chemical precipitation (Saviuc et al., 2011c; Ficai et al., 2011b) from aqueous iron salt solutions by means of alkaline media, like NH_3. In the present paper, magnetic material was prepared by a modified precipitation method (Grumezescu et al., 2011c). Five grams of CMC were solubilized in a known volume of distilled-deionized water, corresponding to a 1.00% (w/w) solution, under stirring at room temperature. Then, 2 ml of a basic aqueous solution consisting of 28% NH_3 were added to CMC solution. After these, 500 ml solution consist of five grams of CS, 0.6 g of $FeCl_3$ and one g of $FeSO_4 \cdot 7H_2O$ were dropped under permanent stirring up to pH = 8, leading to the formation of a black precipitate. The product was repeatedly washed with methanol and subsequently dried in oven at 60 °C until reaching a constant weight.

2.3. Scanning electron microscopy

SEM analysis was performed on a HITACHI S2600N electron microscope, at 25 keV, in primary electrons fascicle, on samples covered with a thin silver layer.

2.4. X-ray diffractometry

X-ray diffraction analysis was performed on a Shimadzu XRD 6000 diffractometer at room temperature. In all the cases, Cu Kα radiation from a Cu X-ray tube (run at 15 mA and 30 kV) was used. The samples were scanned in the Bragg angle 2θ range of 10–80.

2.5. FT-IR characterization

A Nicolet 6700 FT-IR spectrometer (Thermo Nicolet, Madison, WI) connected to software of the OMNIC operating system (Version 7.0 Thermo Nicolet) was used to obtain FT-IR spectra of hybrid materials. The samples were placed in contact with attenuated total reflectance (ATR) on a multibounce plate of ZnSe crystal at controlled ambient temperature (25 °C). FT-IR spectra were collected in the frequency range of 4000–650 cm^{-1} by co-adding 32 scans and at a resolution of 4 cm^{-1} with strong apodization. All spectra were ratioed against a background of an air spectrum. After every scan, a new reference air background spectrum was taken. The plate was carefully cleaned by wiping with hexane twice followed by acetone and dried with soft tissue before filling in with the next sample. The spectra were recorded as absorbance values at each data point in triplicate.

2.6. In vitro assay of the influence of the obtained magnetic polymeric composite scaffolds on the efficacy of antistaphylococal and antipseudomonal drugs

An adapted diffusion method was used in order to assess the potentiating effect of the polymeric composite on the antimicrobial activity of VA (vancomycin), DA (clindamycin), AZM (azithromycin), OX (oxacillin), SXT (trimethoprim/sulfamethoxazole), RA (rifampicin), OFX (ofloxacin), TE (tetracycline), P (penicillin), CIP (ciprofloxacin), GM (gentamicin), TZP (piperacillin/tazobactam), FEP (cefepime), ATM (aztreonam), CAZ (ceftazidim) and PRL (piperacillin) against *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 strains. The tested antibiotics have been chosen according to CLSI recommendations. Standardized antibiotic discs have been placed on the Mueller Hinton agar medium distributed in Petri dishes previously seeded with a bacterial inoculum with a density corresponding to the 0.5 McFarland standard. Five microliters of the stock solutions of the dispersed magnetic biocomposite were spotted over the antibiotic disks. The plates were incubated 24 h at 37 °C, and the growth inhibition zone diameters for each antibiotic, after the addition of the tested biomaterial suspensions were quantified and compared with the growth inhibition zones obtained for the respective antibiotics (Pănuş et al., 2012; Grumezescu et al., 2012d).

2.7. In vitro assay of the influence of the obtained magnetic polymeric composite scaffolds on the eukaryotic cells

2.7.1. Eukaryotic cell line

HCT8 line (ECAC90032006) was used in our experiments. HCT8 line was cultivated in RPMI 1640 (Gibco, NY, SUA) supplemented with 10% heat-inactivated bovine serum and penicillin/streptomycin at 37 °C with 5% CO_2.

2.7.2. Cytotoxicity assay

Cytotoxicity was performed using Trypan blue staining after treatment with 100 μg/ml of the tested system. Briefly, a freshly prepared solution of 50 μl Tripan blue (0.05% in distilled water) was mixed to 50 μl of each cellular suspension during 5 min, spread onto a microscope slide and covered with a coverslip. Nonviable cells appeared blue-stained. At least 200 cells were counted per treatment.

2.7.3. Cell cycle analysis

HCT8 cell line was treated with 100 μg/ml of the tested system, and maintained for 24 h at 37 °C, in 5% CO_2 and humid atmosphere. Thereafter, cells were harvested, washed in phosphate buffered saline (PBS) (pH 7.5), fixed in 70% cold ethanol and maintained overnight at −20 °C. Each sample was washed in PBS, treated with 100 μg/ml RNAse A for 15 min and colored with 10 μg/ml propidium iodide by incubation at 37 °C for 1 h. After cells' propidium iodide staining the events acquisition was done using Epics Beckman Coulter flow cytometer. The obtained data were analyzed