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Fabrication, characterization and *in vitro* profile based interaction with eukaryotic and prokaryotic cells of alginate–chitosan–silica biocomposite

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ABSTRACT

This work is focused on the fabrication of a new drug delivery system based on polyanionic matrix (*e.g.* sodium alginate), polycationic matrix (*e.g.* chitosan) and silica network. The FT-IR, SEM, DTA-TG, eukaryotic cell cycle and viability, and *in vitro* assay of the influence of the biocomposite on the efficacy of antibiotic drugs were investigated. The obtained results demonstrated the biocompatibility and the ability of the fabricated biocomposite to maintain or improve the efficacy of the following antibiotics: piperacillin-tazobactam, cefepime, piperacillin, imipenem, gentamicin, ceftazidime against *Pseudomonas aeruginosa* ATCC 27853 and cefazolin, cefaclor, cefuroxime, ceftriaxone, cefoxitin, trimethoprim/sulfamethoxazole against *Escherichia coli* ATCC 25922 reference strains.

1. Introduction

In the last years, protection of organs from toxic drugs has been widely designed (Tang et al., 2011). Targeting specific sites in the body simplifies drug administration procedures, reduces the quantity of drug required to reach therapeutic levels and increases the concentration of the drug at target sites (Arias et al., 2001; Grumezescu et al., 2011a). In this respect the use of polymers play an important role in the development of such systems, because they can provide tailored properties of biocompatibility, stability, size, structure, and functionality (Liu et al., 2009a; Huang et al., 2011b). Alginates are naturally occurring polysaccharides obtained from marine brown algae, consisting of two monomeric units, *i.e.* β-D-mannuronic acid and α-L-guluronic acid (Chen et al., 2011a; Mata et al., 2011). In mild conditions, in the presence of divalent ions, such as Ca^{2+} solution forms a gel matrix. Numerous efforts have done to control the erosion of alginate microspheres and extend drug release. The most commonly investigated approach consists in coating alginate microspheres with polycationic polymers such as chitosan (Silva et al., 2006).

Chitosan molecule is a copolymer composed of *N*-acetyl-D-glucosamine and D-glucosamine units available in different grades depending upon the degree of acetylated moieties (Sinha et al., 2004; Meng et al., 2011). The positive facets of excellent biocompatibility and admirable biodegradability with ecological safety and low toxicity with versatile biological activities such as antimicrobial activity and low immunogenicity have provided ample opportunities for further development (Dash et al., 2011; Yang et al., 2009, in press). However, chitosan has limited ability for controlling release in acid medium (George and Abraham, 2006). One approach to overcome this and other obstacles is by coating an acid-resistant polymer, such as sodium alginate, onto the surface of chitosan microparticles (Li et al., 2008; Suksamran et al., 2011). These polysaccharides has drawn increasing attention within pharmaceutical and biomedical applications, owing to its abundant availability, unique mucoadhesivity, inherent pharmacological properties, and other beneficial biological properties (Kumar et al., 2004).

In addition to being capable of swelling with water absorption, ionic groups of polymers allow interactions with oppositely

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charged molecules through formation of a complex for different biomedical applications (Liu et al., 2007). In the field of pharmaceutics, a spontaneous loading technique for encapsulating positively charged molecules in negatively charged polymers (*e.g.* carboxymethyl-cellulose (Butun et al., 2011), sodium alginate (Chen et al., 2011b), was developed for drug delivery applications. Also, negatively charged drugs are entrapped in the positively charged polymers (*e.g.* chitosan) (Realdon et al., 1998; Thein-Han and Stevens, 2004).

Inorganic micro- and nanomaterials become an important target for biomedical fields due to their completely new properties as compared to the bulk materials (Subhasree et al., 2012; Baier et al., 2012; Mihaiescu et al., 2012). Especially, silica has attracted great interest due to its surface modification by various functional groups and leading to the decreasing release rate of the drug from the matrix (Halamová et al., 2010). Silica surface can influence its biocompatibility (Wang et al., 2009). Due to their interesting properties (monodispersity, high specific surface area, tunable pore size and diameter, versatile functionalization), silica are designed for diagnostic and for therapy (Gary-Bobo et al., 2012). Silica has low toxicity and biocompatibility, high chemical and mechanical stability, and hydrophilic character and porous structure that can be tailored to control the diffusion rate of an adsorbed or encapsulated drug (Yague et al., 2008). For biomedical applications, sol–gel derived silica with nano-sized pores in diameter have been reported to be biodegradable and eventually dissolve (Livage et al., 2001).

Here, we present the fabrication and bio-evaluation of a new antibiotic microcarrier system based on sodium alginate, chitosan and silica network possessing all of the following advantages: (i) biocompatibility; (ii) ecofriendly; (iii) an efficient carrier with the capacity to improve the antimicrobial therapy. The facile synthesis of biocomposite could be used for potential applications, such as the multi-functional agents for drug delivery and release control.

2. Materials and methods

2.1. Materials

Sodium alginate, chitosan (M_v 60,000–120,000), Na_2SiO_3, HCl (ACS reagent, 37%) were purchased from Sigma–Aldrich, and they used without any further purification.

2.2. Preparation of biocomposite

Sodium alginate was dissolved in 100 mL distilled water (1%, w/v) and chitosan (1%, w/v) was dissolved in 100 mL CH_3COOH solution 2 N. The sodium alginate solution was then immersed in 100 mL of Na_2SiO_3 solution (1%, w/v) under vigorous stirring. After these, chitosan solution was dropped under permanent stirring up to pH 7, leading to the formation of white hydrogels. The products were filtered and repeatedly washed with ultrapure water and subsequently dried at room temperature.

2.3. Characterization of the prepared biocomposite

2.3.1. FT-IR

A Nicolet 6700 FT-IR spectrometer (Thermo Nicolet, Madison, WI) connected to the 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 rationed 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.

2.3.2. SEM

SEM analysis was performed on a HITACHI S2600N electron microscope, at 15 keV, in primary electrons fascicle, on samples covered with a thin silver layer.

2.3.3. DTA-TG

The thermogravimetric (TG) analysis of the biocomposite was followed with a Netzsch TG 449C STA Jupiter instrument. Samples were screened to 200 mesh prior to analysis then was placed in alumina crucible and heated with 10 K min^{-1} from room temperature to 800 °C, under the flow of 20 mL min^{-1} dried synthetic air (80% N_2 and 20% O_2).

2.4. Biological assay

2.4.1. Antimicrobial susceptibility

An adapted diffusion method was used in order to assess the potentiating effect of the biocomposite on the antimicrobial activity of piperacillin-tazobactam (TZP), cefepime (FEP), piperacillin (PIP), imipenem (IPM), gentamicin (CN), ceftazidime (CAZ) against *Pseudomonas aeruginosa* ATCC 27853 and cefazolin (KZ), cefaclor (CEC), cefuroxime (CXM), ceftriaxone (CRO), cefoxitin (FOX), trimethoprim/sulfamethoxazole (SXT) against *Escherichia coli* ATCC 25922 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 μL of the stock solutions of the dispersed 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 alone (Grumezescu et al., 2011b, 2012c; Marutescu et al., 2011; Kumar et al., 2011).

2.4.2. Eukaryotic cell cycle assessment

In order to obtain a fine powder each compound was mortared and was weighed to obtain 100 mg/mL stock concentration. 3.5×10^5 HEp-2 cells were seeded for 24 h in 3.5 cm diameter Petri dishes, and thereafter treated with 1 mg/mL compound (final concentration). After 24 h, the cells were harvested, washed in phosphate saline buffer (pH 7.5), fixed in 70% cold ethanol and maintained at −20 °C, overnight. Each sample was washed in phosphate buffer saline (PBS), treated with 100 μg/mL RNAse A for 15 min and colored with 10 μg/mL propidium iodide (PI) by incubation at 37 °C, for 1 h. The acquisition of events was done using the Epics Beckman coulter flow cytometer. Data were analyzed using FlowJo software and expressed as fractions of cells in the different cell cycle phases.

2.5. Assessment of cell viability

3.5×10^5 HEp-2 cells were seeded in each well of 24 well plate. After 24 h, the cells were treated with 1 mg/mL compound. The effect of compounds was evaluated after 24 h by adding 100 μL PI (0.1 mg/mL) and 100 μL fluorescein diacetate (FdA). In order to evaluate the dead cells (red) and the viable ones (green),