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Surface modification of PLGA nanoparticles by carbopol to enhance mucoadhesion and cell internalization

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ABSTRACT

Mucoadhesive poly (lactic-co-glycolic acid) (PLGA) nanoparticles having a modified shell-matrix derived from polyvinyl alcohol (PVA) and Carbopol (CP), a biodegradable polymer coating, to improve the adhesion and cell transfection properties were developed. The optimum formulations utilized a CP concentration in the range of 0.05–0.2% w/v, and were formed using modified emulsion-solvent evaporation technique. The resulting CP-PLGA nanoparticles were characterized in terms of their physical and chemical properties. The absorbed CP on the PLGA shell-matrix was found to affect the particle size and surface charge, with 0.05% CP giving rise to smooth spherical particles (0.05CP-PLGA) with the smallest size (285.90 nm), and strong negative surface charge (−25.70 mV). The introduction of CP results in an enhancement of the mucoadhesion between CP-PLGA nanoparticles and mucin particles. *In vitro* cell internalization studies highlighted the potential of 0.05CP-PLGA nanoparticles for transfection into SiHa cells, with uptake being time dependent. Additionally, cytotoxicity studies of CP-PLGA nanoparticles against SiHa cancer cells indicated that low concentrations of the nanoparticles were non-toxic to cells (cell viability >80%). From the various formulations studied, 0.05CP-PLGA nanoparticles proved to be the optimum model carrier having the required mucoadhesive profile and could be an alternative therapeutic efficacy carrier for targeted mucosal drug delivery systems with biodegradable polymer.

1. Introduction

Research in drug delivery systems is an important endeavor paving the way toward next generation therapeutics. These studies result in an increased level of understanding regarding the conditions and different methods required for the development and application of safe, effective new drug delivery therapies. Polymers, in particular synthetic biodegradable systems, have been increasingly employed as key materials in new approaches to therapeutics, most notably in drug delivery systems. Amongst the polymers employed for this purpose, poly (lactic-co-glycolic acid) (PLGA) has shown great potential as the basis for biopolymeric nanocapsular drug carriers [1,2] due to its excellent biocompatibility and biodegradability profile. In addition, it has been reported that the burst release of drugs can be controlled in PLGA nanoparticles, which is significant as burst release can result in the majority of the drug being ineffective, with decreased cytotoxicity levels [3–5]. In these cases, the initial burst rate was reduced, allowing a stable release rate to be reached resulting in a prolonged and more controlled release profile. Moreover, PLGA has been approved by the U.S. Food and Drug Administration (FDA) for use in drug delivery systems [6], and PLGA nanoparticles have been employed for the therapeutic delivery of a wide range of bioactives, for example in ocular applications to control bacterial conjunctivitis (*Staphylococcus aureus* and *Pseudomonas aeruginosa*). In this instance, ciprofloxacin was loaded into PLGA nanoparticles with

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an entrapment efficiency of up to 87%, and controlled release after 6.5 h under optimum conditions was observed [7]. Ungaro et al. (2011) [8] reported the potential of PLGA nanoparticles for delivering antibiotics in inhalation formulations, with high drug loading efficiency and antimicrobial activity. PLGA has, however, limited use in mucosal tissue due to its poor mucoadhesiveness, and this in combination with agglomeration behavior [9–12] limits its effectiveness as a targeted drug delivery system for certain applications (*e.g.* ocular, nasal, vaginal).

Drug delivery systems having high specificity for mucosal tissue are therefore of great interest as the basis for new mucosal (buccal, vaginal and ocular) therapeutics [13–15]. A key strategy underpinning such systems is the application of mucoadhesive polymers in drug delivery formulations. Mucoadhesive polymers are macromolecules that are capable of adhering to the mucosal surface, and these often contain hydrogen bond forming functional groups [16,17]. Additionally, interaction of the mucoadhesive polymer with the absorption membrane provides the potential for prolonging the residual time of the drug in its dosage form and maintaining a sustained drug release to the target site. Numerous types of polymer have been proposed thus far as mucoadhesive agents. Chitosan has been widely used in drug delivery systems to modify the surface of carriers and improve their mucoadhesive properties. Due to the chitosan structure having many reactive groups ($-NH_2$), functional site targeting has improved the biological properties of the particles [10,18]. However, the drawback of parent chitosan is on its poor water solubility. Therefore the use of acidic condition is needed for medium and high molecular weight chitosan, to dissolve the chitosan particles. This harsh condition majorly affected the toxicity of chitosan particles on the cell culture application depending on incubation time [19,20]. To overcome this limitation, some water-soluble chitosan derivatives have been synthesized such as N,N,N-trimethylammonium chitosan chloride (TMChC), thiolated quaternary ammonium–chitosan or methylated N-(4-N,N-dimethylaminocinnamyl [21,22]. Nevertheless, water-soluble chitosan derivatives still need the complicated chemical reaction during the synthesis process, therefore researchers are currently striving to develop alternatives approaches to overcome these disadvantages and optimize performance of the delivery system.

Carbopol (hereafter referred to as CP) is the trade name for a series of biodegradable polyacrylic acid (PAA) polymers whose chains are cross-linked with polyalkenyl ethers, or divinyl glycol. The nature of the cross-linking agent can be varied, with its length, critical molecular weight (Mc), functionality and rigidity giving rise to different grades exhibiting a range of distinct properties. CP 934 consists of chains of polyacrylic acid cross-linked with allyl ethers of sucrose. The general benefit of CP have been used as an emulsifying and gelling agent for the topical formulations. Moreover, this polymer has shown prior usefulness as mucoadhesive agents exhibiting controlled drug release profiles [23–25] and showing bioadhesion in buccal [26], nasal [27], intestinal [28] and rectal applications [29]. Moreover, this mucoadhesive polymer is capable to attach on the mucosal membrane, which offers to prolong the drug mechanism [30,31]. However, the low toxicity and good biodegradability of CP allow it to be used in potential therapeutic applications [25,32], with its mucoadhesive properties being of key relevance in this study. In this study, CP was selected to enhance mucoadhesive property of PLGA nanoparticles as well as to protect drug or active compounds in nanoparticles.

The current research intends to enhance the mucoadhesive and cell internalization properties of PLGA nanoparticles through integration of bioadhesive polymers and polymeric stabilizer on their surfaces for targeted drug delivery. CP was selected to serve as a model bioadhesive agent due to its low toxicity, biodegradability, biocompatibility and its ability to adhere to mucus membranes. Fabrication of PLGA and CP-PLGA nanoparticles was achieved using emulsion-solvent evaporation techniques with polyvinyl alcohol (PVA) as a stabilizer. The nanoparticles were characterized in terms of their particle size by dynamic light scattering (DLS), and physical morphology using SEM and TEM. Interactions between CP and PLGA were investigated using ATR-FTIR spectroscopy. Mucoadhesion between CP-PLGA nanoparticles and mucin particles related to hydrogen bonding was probed using ^{1}H NMR spectroscopy as this was shown to be an effective methodology for determining interactions of this type [25,33]. To assess the potential of CP-PLGA nanoparticles to act as potential templates for loading of drug molecules, the transfection efficiency in an *in vitro* model was carried out, and the cytotoxicity of CP-PLGA nanoparticles was also evaluated to gain insights into their suitability and can be used as promising drug delivery systems especially for efficient gene therapy.

2. Materials and methods

2.1. Materials

Poly (lactic-coglycolic acid) (PLGA) having a lactide to glycolide ratio of 50:50 (MW 40–75 kDa), poly (vinyl alcohol) (PVA) (MW 13–23 kDa, 87–89% hydrolyzed), mucin (type III) from porcine and Rhodamine 6G (Dye content ~95%) were purchased from Sigma–Aldrich Co., Ltd. (St Louis, MO, USA). Carbopol 934 polymer (CP: MW 100,000) was provided by Hong Huat Co., Ltd. (Pathumthani, Thailand). Dichloromethane (DCM) was obtained from Carlo Erba (Milano, Italy). Deuterium oxide (D_2O) was purchased from Sigma Aldrich (St Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

2.2. Preparation of PLGA and CP-PLGA nanoparticles

PLGA nanoparticles were fabricated using a modified emulsion-solvent evaporation method from that described by Fessi et al. (1989) [30], and Mainardes and Evangelista (2005) [34]. Briefly, PLGA 5% (w/v) was dissolved in dichloromethane (DCM), and the aqueous phase was prepared by dissolving 2% PVA (w/v) and Rhodamine (to give a final concentration of 20 μg/mL) in deionized water. In the case of CP-PLGA particle formation, CP (0.05%, 0.1% and 0.2%, w/v) was added to the aqueous phase. Then, the organic phase was added drop-wise into the aqueous phase with sonication for 3 min (Amplitude 40%, pulse on 30 s, pulse off 5 s) using an ultrasonic processor (Sonic, Vibra cell, SON-1 VCX130, probe number 422-17, U.S.A.) while cooling in an ice bath. The resultant oil-in-water emulsion was mixed with 20 mL of an aqueous solution of emulsifier (0.5% wt PVA) and stirred with a magnetic stirrer at 300 rpm for 3 h to remove excess DCM from the internal phase. The resultant nanoparticles were collected using a high speed refrigerated micro-centrifuge (Tomy Centrifuge MX-305, US) at 10,000 rpm for 30 min, and then washed three times with deionized water. Finally, the sample was freeze-dried for 48 h at −80 °C (Freeze-dryer, Martin Christ 102152) to obtain the powdered nanoparticles.

2.3. Characterization of PLGA and CP-PLGA nanoparticles

2.3.1. Size analysis and zeta potential

The mean particle size and zeta potential of nanoparticles were determined using a Zetasizer nano ZS90 instrument (Malvern Instruments, Malvern, UK). The particle sizes were determined at 25 °C using dynamic light scattering (DLS) under photo correlation spectroscopy (PCS), which was observed from the Brownian motion of suspended particles. In the interim, the zeta potential was determined based on the electrophoretic mobility of nanoparticles in an aqueous solution using laser doppler velocimetry and phase