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# Synthesis and characterization of magnesium gluconate contained poly(lactic-*co*-glycolic acid)/chitosan microspheres

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ABSTRACT

The goal of this study was to fabricate and investigate the chitosan coated poly(lactic-*co*-glycolic acid) (PLGA) microspheres for the development of controlled release magnesium delivery system. PLGA based microspheres are ideal vehicles for many controlled release drug delivery applications. Chitosan is a naturally occurring biodegradable and biocompatible polysaccharide, which can coat the surface of PLGA to alter the release of drugs. Magnesium gluconate (MgG) was encapsulated in the PLGA and PLGA/chitosan microspheres by utilizing the double emulsion solvent evaporation technique for controlled release study. The microspheres were tested with respect to several physicochemical and biological properties, including morphology, chemical structure, chitosan adsorption efficiency, magnesium encapsulation efficiency, in vitro release of magnesium ions, and cellular compatibility using both human adipose-derived stem cells (ASCs) and PC12 cells. Chitosan coated PLGA microspheres can significantly control the release of magnesium ions compared to uncoated PLGA microspheres. Both coated and uncoated microspheres showed good cellular compatibility.



## 1. Introduction

Magnesium (Mg) is the second most abundant intracellular mineral in the human body. Magnesium is associated with more than 300 biochemical reactions and is an indispensable element for energy metabolism, protein synthesis, and DNA replication. Magnesium helps to maintain normal muscle and nerve function, supports healthy immune system, and promotes normal blood pressure. Magnesium deficiency is a common problem in 7–11% of hospitalized patients [1]. Magnesium deficiency has been associated with critical health issues, including congestive heart failure, sudden cardiac death, myocardial infarction, diabetes, hypertension, coronary heart disease, and osteoporosis [2–5]. Patients with Mg deficiency are usually treated by magnesium administration [6,7]. Hypermagnesemia is commonly occurred due to excessive administration of magnesium, especially in patients with reduced renal function [1,8,9]. Hypermagnesemia is associated with a number of health issues, including hypotension, cardiac arrest, neuromuscular disorder, bradycardia, and paralytic ileus [9–12]. Furthermore, magnesium uptake by cells is slow and it requires sustained correction of Mg deficiency [9].

Controlled release drug delivery systems offer numerous advantages over traditional methods of drug delivery by eliminating the need of repetitive drug administration with predetermined release rates and increase of the efficacy of the drugs [13,14]. Polymeric microspheres are ideal vehicles for many controlled delivery applications due to their biocompatibility, high bioavailability, ability to encapsulate a variety of drugs, and sustained drug release characteristics [15].

Poly(lactic-*co*-glycolic acid) (PLGA) is a biodegradable, biocompatible and, Food and Drug Administration (FDA) approved synthetic polymer that exhibits a wide range of erosion times [16]. Poly(lactic-*co*-glycolic acid) has been extensively studied for the development of devices for controlled delivery of therapeutic

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drugs, proteins, genes, and other macromolecules. In these applications, surface of the PLGA is typically protected by poly(ethylene glycol) (PEG) for prolonged circulation and enhanced cellular uptake [17]. However, PEG can interfere with the interactions between drug carriers and target cells, and negatively influences the therapeutic outcomes [18].

Chitosan is a cationic polysaccharide produced by the exhaustive deacetylation of chitin, a structural element in the exoskeleton of crustaceans and insects. The primary amino groups of the chitosan are responsible for several important properties, such as mucoadhesion, in situ gelation, transfection, permeation enhancement, and efflux pump inhibitory properties. Chitosan has recently sparked interest in the field of surface modification due to several desirable properties: minimal foreign body reaction, mild processing conditions, chemical reactivity, and cost effectiveness [18,19].

This study was designed to investigate the feasibility of controlled release of magnesium ions from chitosan coated and uncoated PLGA microspheres. We used magnesium gluconate (MgG) as a model compound to study the release kinetics of magnesium ions from the microspheres. We hypothesized that chitosan coating on the surface of PLGA could be used to significantly control the release of magnesium ions. MgG is a therapeutic drug used to treat Mg deficiency [10]. In recent studies, MgG has exhibited the highest level of bioavailability among different magnesium salts and has been recommended as the optimal salt for treatment of Mg deficiency [20,21]. For controlled release study, MgG encapsulated PLGA and PLGA/chitosan microspheres were prepared by utilizing the double emulsion solvent evaporation technique. Toward the potential use of these microspheres for in vivo application, several physiochemical properties, such as morphology, chemical structure, chitosan adsorption efficiency, magnesium encapsulation efficiency, and release of magnesium ions were studied. Cellular compatibility of these microspheres was studied with human adipose-derived stem cells (ASCs) and PC12 cells.

## 2. Materials and methods

### 2.1. Materials

Poly(lactic-*co*-glycolic acid) (PLGA, lactic acid/glycolic acid = 50:50, with carboxylate end group, inherent viscosity 0.15–0.25) was purchased from Durect Corporation (Birmingham, AL, USA). Chitosan (Mw 2.5 kDa) was purchased from Creative PEGWorks (Winston Salem, NC, USA). Magnesium gluconate dihydrate (MgG, Mw 322.64, 98%) was purchased from Pfaltz & Bauer (Waterbury, CT, USA). Poly(vinyl alcohol) (PVA, ~ 99% hydrolyzed), ninhydrin, hydrindantin, lithium acetate dihydrate, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Aldrich (St. Louis, MO, USA). Dichloromethane (DCM, ~ 99%), glacial acetic acid, and sodium hydroxide (NaOH) were purchased from Acros Organics (Morris Plains, NJ, USA). Dimethyl sulfoxide (DMSO, ~ 95%), phosphate buffered saline (PBS), ethanol (92–93%), and hydrochloric acid (HCl, 35–38%) were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA).

### 2.2. Fabrication of microspheres

PLGA/chitosan microspheres were fabricated by utilizing the double emulsion solvent evaporation technique with some modifications [16]. PLGA (1 g) was dissolved in 4 ml of DCM. The PLGA solution was added to 2 ml of 1% PVA solution and emulsified on ice bath for 2 min with Sonic Dismembrator (Thermo Fisher Scientific, Fair Lawn, NJ, USA) at 50% amplitude and on a 10 sec on/off pulse mode. Chitosan was dissolved in 20 ml of deionized water (DI water, pH 5) and transferred to 80 ml of DI water containing 0.1% PVA. The emulsified PLGA solution was added to the chitosan solution dropwise using Single Syringe Infusion Pump (Cole-Parmer, Vernon Hills, IL, USA) and stirred overnight for DCM evaporation, particle hardening and chitosan adsorption to the surface of PLGA. Chitosan coated PLGA microspheres were collected by Sorvall Stratos Centrifuge (Thermo Fisher Scientific, Fair Lawn, NJ, USA) at 6000 rpm and 4 °C for 30 min. The microspheres were washed three times with DI water. The purified PLGA/chitosan microspheres were lyophilized for two days by using Freezone Freeze Dryer (Labconco, Kansas City, MO, USA).

For encapsulation study, MgG was dissolved in 8 ml of DI water and mixed with 1 g of PLGA dissolved in 4 ml of DCM. The combined mixture was emulsified in the same way as described above. Ultrasonic emulsification method was carried out for the entrapment of ionized magnesium into the polymer networks of PLGA. The control microspheres of PLGA and PLGA/MgG were fabricated by adding respective emulsified solutions drop wise to 80 ml of DI water containing 0.1% PVA.

### 2.3. Assessment of morphology and microsphere size distribution

The morphology of the microspheres was observed by scanning electron microscope (SEM) (Hitachi SU8000, Japan). Double sided carbon tape was used for attaching the specimens to the sample holder. Prior to imaging with the SEM, samples were gold sputtered under vacuum by using Polaron SEM Coating System (Quorum Technologies, East Sussex, UK) for 1 min. The samples were imaged at an accelerating voltage of 10 kV and current of 5 μA.

The size distribution of the microspheres was determined through SEM images with the use of ImageJ software (NIH, USA). First, length of each scale bar was measured in pixels. Seventy-five microspheres of each group ($n = 3$) were also measured in pixels. The number of pixels was converted to μm using scale factor. Finally, the average size and standard deviation were calculated based on converted ImageJ data.

### 2.4. Chemical structure analysis

Fourier transform infrared spectroscopy (FTIR) was used to analyze the chemistry between PLGA and chitosan. FTIR spectra were recorded at 200 scans using IR Prestige21 FTIR Spectrometer (Shimadzu, Kyoto, Japan). The spectra were calculated from 400 to 4000 $cm^{-1}$ with a resolution of 4 $cm^{-1}$. Prior to obtaining the FTIR spectra, each sample was ground and dispersed in KBr matrix. A pellet was then formed by compressing the sample at high pressure. The pellet was placed in the FTIR holder to perform the measurement at absorbance mode.

### 2.5. Determination of chitosan adsorption efficiency and in vitro dissolution study

Chitosan adsorption efficiency and dissolution study were carried out by using the ninhydrin assay according to the previously reported method [22]. Briefly, 10 ml of lithium acetate buffer was prepared by dissolving 4.08 g of lithium acetate dihydrate in 6 ml of DI water. The pH of the resulting solution was adjusted to 5.2 using glacial acetic acid and NaOH, and the volume adjusted to 10 ml with DI water. The ninhydrin reagent was prepared by adding 10 ml freshly prepared lithium acetate buffer to 0.8 g ninhydrin and 0.12 g hydrindantin in 30 ml of DMSO. Ninhydrin reagent (0.5 ml) was added to 0.5 ml of sample solution in a glass scintillation vial. The vial was immediately capped, briefly shaken by hand and heated in boiling water for 30 min. After cooling, 15 ml of 50% ethanol was added to the vial. The vial was then vortexed using Analog Vortex Mixer (Thermo Fisher Scientific, Fair Lawn, NJ, USA) for 30 sec in order to oxidize the excess of hydrindantin. The absorbance of each