Materials Science and Engineering C 65 (2016) 331–337

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Curcumin loaded gum arabic aldehyde-gelatin nanogels for breast cancer therapy

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ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form 16 March 2016
Accepted 11 April 2016
Available online 13 April 2016

Keywords:
Gum arabic
Gelatin
Curcumin
Nanogels
Drug delivery

ABSTRACT

Curcumin, a widely studied hydrophobic polyphenol with anticancer potential is loaded in gum arabic aldehyde-gelatin (GA Ald-Gel) nanogels to improve its bioavailability and therapeutic efficacy towards cancer cells. Physicochemical properties of the curcumin loaded GA Ald-Gel nanogels are investigated by different techniques including dynamic light scattering (DLS), NMR spectroscopy and scanning electron microscopy (SEM). These nanogels exhibit hydrodynamic diameter of 452 ± 8 nm with a zeta potential of -27 mV. The nanogels possess an encapsulation efficiency of $65 \pm 3\%$. Potential of the nanogels for controlled release of curcumin is illustrated by *in vitro* drug release studies. Hemocompatibility and cytocompatibility of the drug loaded nanogels are evaluated. *In vitro* cytotoxicity of the bare and curcumin loaded nanogels are analyzed by MTT assay towards MCF-7 cells. The results manifest that curcumin loaded nanogels induce toxicity in MCF-7 cells. Confocal laser scanning microscopy (CLSM) studies indicate *in vitro* cellular uptake of the nanogels in MCF-7 cells. All these results prove the suitability of the curcumin loaded GA Ald-Gel nanogels for cancer therapy.

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1. Introduction

Research on the development of nanoparticles for gene and drug delivery has been increased considerably in the last decades. Nano sized carriers were developed for the distribution of drugs to specific sites such as brain, skin, lungs and eyes to combat the issues such as drug toxicity, drug distribution to other tissues and inadequate drug concentration due to poor absorption or rapid metabolism and elimination [1]. Nanogels are an important class of drug delivery systems which have gained great research attention due to specific properties offered by its small size [2]. The properties of the nanogels that make them superior drug delivery systems are their tunable size and surface characteristics to avoid rapid clearance, high drug loading capacity, controlled and sustained drug release at target site and their ability to penetrate the small capillary vessels [3]. Nanogels derived from natural polysaccharides are of great interest for drug delivery and controlled release because of their biodegradability, abundance and biocompatibility.

Curcumin is a well-known spice and possesses potent medicinal properties like antioxidant, anti-inflammatory and anticancer efficacy [4,5]. Curcumin has the ability to induce apoptosis in different cancer cells such as colon, breast, prostate and lungs without any cytotoxic effects on the healthy ones. Although curcumin has a collection of promising characteristics, it's very low solubility in aqueous medium limits bioavailability and hence clinical efficacy. Different types of nanocarriers have been developed to improve the solubility and stability of curcumin in aqueous medium [6–8]. Of the nanocarriers, nanogels have gained much attraction since they can easily pass through the cell membrane of solid tumors by enhanced permeation and retention (EPR) effect owing to the small size [9]. The encapsulation of hydrophobic curcumin in nanogels makes it readily soluble in aqueous solution and improves its bioavailability towards cancer cells.

In the present study, nanogels are developed from gum arabic and gelatin for the delivery of curcumin. Gum arabic (GA) is a highly branched complex polysaccharide, which contains rhamnose, galactose and glucuronic acid residues in its structure [10]. GA based nanoparticles were used for the oral delivery of insulin [11]. Oxidized GA has been conjugated with different drugs for application in drug delivery [12–14].

Gelatin is a protein obtained by the partial hydrolysis of collagen. Biocompatibility and biodegradability of gelatin make it a favourite candidate in pharmaceutical industry as drug carrier and in biomedical field as tissue engineering matrix [15,16]. In addition to that, abundant functional groups offer the possibility for chemical modification. Cross-linking of gelatin with oxidized polysaccharides has already been reported [17,18].

In a recently published work, we reported the preparation and detailed characterization of stable nanogels from gum arabic and gelatin [19]. Gum arabic was oxidized to gum arabic aldehyde (GA Ald) which was cross-linked with gelatin (Gel) to obtain nanogels. Gum arabic and gelatin were chosen for the preparation of nanogels due to their biocompatibility and extremely high hydrophilicity. The present work describes the application of the GA Ald-Gel nanogels for the

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http://dx.doi.org/10.1016/j.msec.2016.04.044
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encapsulation and controlled release of curcumin towards breast cancer cells. Encapsulation of curcumin in GA Ald-Gel nanogel protects it from degradation and may increase its bioavailability and toxicity towards cancer cells. To assess the potential of the curcumin loaded nanogels for cancer therapy, it's physicochemical and biological properties are evaluated by various characterization techniques. The curcumin loaded GA Ald-Gel nanogels can be a potential candidate for curcumin delivery to breast cancer cells. To the best of our knowledge this is the first report on GA based nanogels for drug delivery applications.

2. Materials and methods

2.1. Materials

Gum arabic (from acacia tree) of approximate molecular weight 250 kDa, glutamine, 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), sodium pyruvate, sodium bicarbonate, Trypsin-EDTA and gelatin (Type A) were obtained from Sigma Aldrich, Saint Louis, USA. Sodium metaperiodate, sodium tetra borate (borax), Span 20, sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium carbonate, minimum essential medium (MEM), isopropanol, sodium hydroxide, cyclohexane and acetone were obtained from Merck (Mumbai, India). Curcumin was obtained as a gift from Synthite Industries Ltd., Kolenchery, Kerala. Dialysis tubing (3500 MWCO) was procured from Spectrum Laboratories Inc., CA, USA.

2.2. Preparation of gum arabic aldehyde-gelatin (GA Ald-Gel) nanogels

Recently we reported the preparation and characterization of GA Ald-Gel nanogels [19]. Gelatin miniemulsion and GA Ald miniemulsion were prepared separately by ultrasonication and later these two emulsions were mixed and sonicated again to form cross-linked GA Ald-Gel nanogels. Gum arabic aldehyde (GA Ald) solution (250 μl, 10%, *w*/*v*, prepared in 0.1 M borax) was added to cyclohexane (5 ml) containing Span 20 (6 mg) and sonicated for five minutes. The gelatin miniemulsion was also prepared in a similar fashion. Later, these two emulsions were mixed and sonicated again for 5 min to obtain GA Ald-Gel nanogels.

2.3. Preparation of curcumin loaded gum arabic aldehyde-gelatin (GA Ald-Gel) nanogels

Curcumin dissolved in acetone (2 mg/ml) was added to GA Ald-Gel nanogel inverse minemulsions (10 ml) and allowed to stir for two days. After this, curcumin loaded nanogels were separated by centrifugation at 5000 rpm for 10 min and washed thrice with distilled water. The nanogels were then vacuum dried.

2.4. Characterization of curcumin loaded GA Ald-Gel nanogels

^{1}H NMR spectra of curcumin (DMSO-d_6), bare nanogel (D_2O) and curcumin loaded GA Ald-Gel nanogel (DMSO-d_6) were recorded in 500 MHz spectrometer (Bruker Avance DRX 500). X-ray diffraction patterns (XRD) of curcumin, GA Ald-Gel nanogel and curcumin loaded GA Ald-Gel nanogel were recorded on X-ray Diffractometer (X'pert Pro, Philips, USA using copper K-a radiation). Morphology of the nanogels was analyzed by scanning electron microscopy (FEI Quanta FEG 200 HR Scanning Electron Microscope). Curcumin loaded GA Ald-Gel nanogel powder was redispersed in water (1 mg/ml) by sonication and the dispersion was drop cast on a glass slide and dried. It was sputter coated and analyzed by SEM by applying an acceleration voltage of 10 kV. FT-IR spectra of bare and curcumin loaded nanogels were recorded using Perkin Elmer FT-IR spectrometer in the range of 4000 to 400 cm^{-1} with 32 scans per sample.

2.5. Encapsulation efficiency

The encapsulation efficiency of curcumin loaded GA Ald-Gel nanogels were determined by measuring the amount of curcumin loaded in the nanogel. A known quantity of the dried curcumin loaded nanogel was dispersed in DMSO-water mixture (1:1, *v*/v, 4 ml) by sonication using a probe sonicator (Sonics Vibra-cell, Modal-VCX 750) at 25% of amplitude for 5 min. The dispersion was centrifuged at 10,000 rpm for 20 min and the supernatant was collected. The supernatant was diluted with DMSO and the amount of curcumin was estimated spectrophotometrically (Carry100 UV–Visible spectrophotometer, Melbourne, Australia) from the standard curve by measuring the absorption intensity at 430 nm. For preparing the standard curve, a stock solution of curcumin was prepared in DMSO-water mixture (1:1, *v*/v). From this stock solution (1 mg/1 ml), different concentrations of curcumin, ranging from 0.002 mg/ml to 1 mg/ml were prepared and their absorbance was measured at 430 nm. Standard curve was prepared by plotting the absorbance *versus* concentration. Encapsulation efficiency (EE) was calculated by the following equation (Eq. (1)):

$$EE(\%) = \frac{\text{Total amount of curcumin within nanogel}}{\text{Amount of curcumin taken for loading}} \times 100. \quad (1)$$

2.6. In vitro drug release studies

In vitro curcumin release from GA Ald-Gel nanogels was carried out at two different pH (7.4 and 5) for a period of 48 h [20]. A known amount of the sample was dispersed in 3 ml of buffer of pH 5 or pH 7.4 and transferred to dialysis membrane. The membranes were immersed in the corresponding buffer (10 ml) release medium and incubated at 37 °C. At definite time intervals, 4 ml of the release medium was taken and was replenished with fresh buffer. The release medium was diluted with DMSO and was quantified spectrophotometrically (Carry100 UV–Visible spectrophotometer, Melbourne, Australia) from the standard curcumin curve at 430 nm.

2.7. Hemocompatibility studies of curcumin loaded GA Ald-Gel nanogels

Blood compatibility of curcumin loaded nanogels were evaluated by hemolysis assay [21]. The total hemoglobin in the blood samples was measured using Automatic hematology analyzer (Sysmex-K 4500). Briefly, anticoagulated fresh human blood (0.9 ml) was added to nanogels dispersed in PBS (0.1 ml) and was incubated at 37 °C for 90 min. Different concentrations of the nanogels were prepared containing equivalent curcumin concentration ranging from 3.125 to 50 μg/ml. PBS and 0.1% of Na_2CO_3 were used as negative (0% hemolysis) and positive controls (100% hemolysis) respectively. Samples were centrifuged at 4500 rpm for 15 min to obtain the plasma after incubation. Optical density of the hemoglobin in plasma was analyzed spectrophotometrically and it was calculated by the following equation [20,22] (Eq. (2)):

$$\text{Plasma Hb} = \frac{[(2A_{415})-(A_{380}+A_{450})] \times 1000 \times \text{dilution factor}}{E \times 1.655}. \quad (2)$$

A_{415} denotes the Soret band based absorption of hemoglobin. A_{380} and A_{450} stand for correction factors applied for uroporphyrin absorption falling in the same wavelength range. E represents the molar absorptivity value of oxyhemoglobin at 415 nm and is 79.46. The correction factor for accounting the turbidity of plasma sample is 1.655. Hemolysis % was calculated by Eq. (3):

$$\text{Hemolysis} = \frac{\text{Plasma Hb value of the sample}}{\text{Total Hb value of blood}} \times 100. \quad (3)$$

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