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Preparation, characterization and biological evaluation of curcumin loaded alginate aldehyde–gelatin nanogels

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ABSTRACT

Curcumin, a natural polyphenol exhibits chemopreventive and chemotherapeutic activities towards cancer. In order to improve the bioavailability and therapeutic efficacy, curcumin is encapsulated in alginate aldehyde–gelatin (Alg Ald-Gel) nanogels. Alginate aldehyde–gelatin nanogels are prepared by inverse miniemulsion technique. Physicochemical properties of the curcumin loaded nanogels are evaluated by, Dynamic light scattering (DLS), NMR spectroscopy and Scanning electron microscopy (SEM). Curcumin loaded nanogels show hydrodynamic diameter of 431 ± 8 nm and a zeta potential of −36 ± 4 mV. The prepared nanogels exhibit an encapsulation efficiency of 72 ± 2%. *In vitro* drug release studies show a controlled release of curcumin from nanogels over a period of 48 h. Hemocompatibility and cytocompatibility of the nanogels are evaluated. Bare nanogels are cytocompatible and curcumin loaded nanogels induce anticancer activity towards MCF-7 cells. *In vitro* cellular uptake of the curcumin loaded nanogels using confocal laser scanning microscopy (CLSM) confirms the uptake of nanogels in MCF-7 cells. Hence, the developed nanogel system can be a suitable candidate for curcumin delivery to cancer cells.

1. Introduction

Curcumin is a hydrophobic natural polyphenol present in the perennial herb *Curcuma longa*. It has been used as spice, coloring agent and flavoring agent. Medicinal properties of curcumin have been listed in Ayurveda centuries ago [1]. Therapeutic properties of curcumin include anticancer, anti-inflammatory, antibacterial and antioxidant [2] activities. In recent years, considerable research has been carried out on the anticancer potential of curcumin [3,4]. These reports describe the potential of curcumin in overcoming multidrug resistant cancer [5], suppress colon cancer [6], prostate cancer [7,8] ovarian cancer [9] and breast cancer [10].

Though, pharmacology of curcumin attracts great attention, shortcomings restrict its use as therapeutic agent. Curative efficacy of curcumin is limited due to low systemic bioavailability and poor pharmacokinetics which has been attributed to its poor aqueous solubility and fast metabolism [11,12]. To improve aqueous solubility of curcumin, formulations such as curcumin-cyclodextrin complex [13], PEG-curcumin conjugate [14] and liposomal curcumin [15] have been developed. Delivery systems using nanoparticles for improving the bioavailability of curcumin also have been investigated. Nanogels are an important class of nanomaterials for the delivery of hydrophobic therapeutic agents [16]. They are nanoscale sized hydrogels formed from hydrophilic or amphiphilic polymer networks by physical or chemical interactions. Nanogels protect the encapsulated drugs from degradation and provide controlled release over extended periods of time [17]and hence improve the therapeutic index of the loaded drug. Nanogels prepared from biodegradable polymers are excellent candidates for drug delivery applications. Biopolymer based nanogels are superior to synthetic ones in terms of biodegradability, biological origin, abundance in nature and nontoxicity. The presence of large number of functional groups for further conjugation with biomolecules [18] is yet another advantage.

In the present work, an anionic polysaccharide, namely alginate and a protein, namely gelatin are used for preparing nanogels. Alginate is composed of β-D-mannuronic acid and α-L-guluronic acid units and form gels in the presence of divalent ions in aqueous medium. These gels are extensively used in drug delivery applications [19,20]. Gelatin, a protein obtained from the hydrolysis of collagen has been used for nanoparticle preparation for drug delivery applications [21–23].

Recently, we reported the development and characterization of alginate aldehyde–gelatin bare nanogels by an inverse miniemulsion technique [24]. The present work concerns with the application of the prepared alginate aldehyde–gelatin nanogels in drug delivery. Curcumin, a hydrophobic drug is encapsulated in Alg Ald-Gel nanogels for improving the therapeutic efficacy of curcumin in cancer cells. The

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properties of curcumin loaded nanogels are analyzed by DLS, SEM, TEM, XRD and NMR. Anticancer potential of the curcumin loaded nanogels is examined in MCF-7 cells. The results prove the suitability of the curcumin loaded alginate aldehyde–gelatin nanogels for delivery of curcumin to breast cancer cells.

2. Materials and methods

2.1. Materials

Sodium alginate (medium viscosity grade, viscosity of 2% solution: 2000 cps at 25 °C, average molecular weight of alginate obtained by GPC analysis: Mn = 494,134 Da, Mw = 904,473 Da M/G ratio: 0.89), gelatin (Type A, contains 78–80 mmol of free carboxyl groups per 100 g of protein and has a pI of 7.0–9.0. The pH of 1.5% solution of gelatin at 25 °C = 3.8–5.5, bloom strength = 300 g, Molecular weight of gelatin obtained by MALDI-TOF analysis is found to be 424,126 Da), sodium bicarbonate, glutamine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), sodium pyruvate and trypsin-EDTA were obtained from Sigma Aldrich, Saint Louis, USA. Span 20, sodium metaperiodate, sodium tetra borate (borax), sodium chloride, disodium hydrogen phosphate, sodium dihydrogen phosphate, minimum essential medium (MEM), cyclohexane, isopropanol and acetone were obtained from Merk (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. Methods

2.2.1. Preparation of alginate aldehyde–gelatin (Alg Ald-Gel) nanogel

Alg Ald-Gel nanogels were prepared by inverse miniemulsion technique [24]. Initially, sodium alginate was oxidized to alginate aldehyde (Alg Ald) by periodate oxidation. Alginate aldehyde solution (10%) was prepared in 0.1 M borax. Later, 250 μl of Alg Ald was mixed with 250 μl of gelatin (10% solution in water) and added to Span 20 (0.2 g) dissolved in cyclohexane (10 ml).The mixture was sonicated for five minutes to obtain inverse miniemulsion. To obtain the nanogel powder, the prepared emulsion (10 ml) was poured drop wise into acetone (50 ml) and the precipitate was separated, washed with distilled water and vacuum dried. Details of preparation and characterization of Alg Ald-Gel nanogels can be found elsewhere [24].

2.2.2. Preparation of curcumin loaded alginate aldehyde–gelatin (Alg Ald-Gel) nanogel

Curcumin dissolved in acetone (2 mg/ml) was added (how much) to the Alg Ald-Gel nanogel inverse minemulsion (10 ml) and was stirred for two days. The drug loaded nanogels were separated by centrifugation at 5000 rpm for 10 min and washed thrice with distilled water. The nanogels were then dried under reduced pressure.

2.2.3. Characterization

2.2.3.1. Size distribution and zeta potential measurement. Dried curcumin loaded nanogels were redispersed in water by sonication for 10 min. The hydrodynamic radius and zeta potential of the nanogels were measured by dynamic light scattering (DLS) using a Malvern Zetasizer (Nano ZS, UK) equipped with a 4 mW He/Ne laser beam operating at λ = 633 nm. All the measurements were performed at 25 °C and each value reported is the average of three series of 10 measurements.

2.2.3.2. Morphology analysis of nanogels. The morphology of curcumin loaded nanogels was analyzed by scanning electron microscopy (FEI Quanta FEG 200 HR Scanning Electron Microscope). Dried nanogel powder was redispersed in water (1 mg/ml) by sonication for 10 min and a drop of dispersion was cast on a glass slide and dried. It was sputter coated and visualized by SEM by applying an acceleration voltage of 10 kV. Transmission electron microscopy analysis also was (FEI, TECNAI S twin microscope with accelerating voltage 300 kV) performed. Sample preparation for TEM was carried out in a similar fashion as that for SEM with concentration of 1 mg/10 ml. The dispersion was placed on a copper grid and air dried for 2 days before imaging.

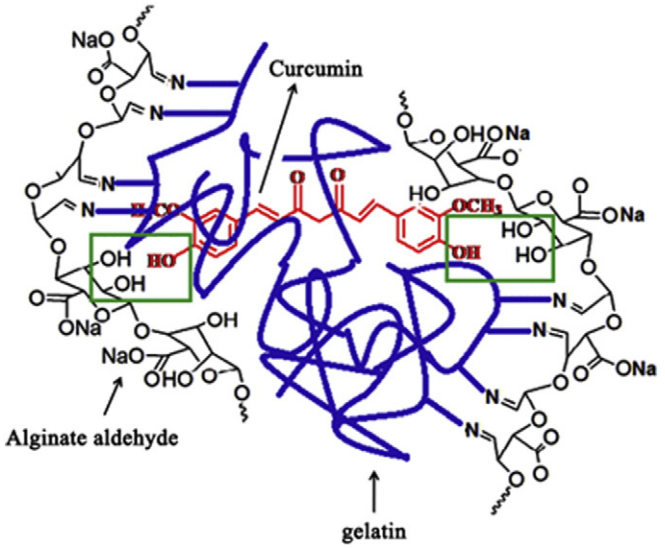
2.2.3.3. Thermal studies. Thermal stability of the nanogels, curcumin, Alg Ald and gelatin were analyzed by thermogravimetric analysis using Thermal Analysis System (Universal V4 7A, TA Instrument). Analysis was performed in N_2(g) atmosphere with a flow of 20 ml/min and a scanning rate of 10 °C min^{-1} from RT to 800 °C. Mass change of the sample was recorded continuously as a function of temperature with time. Differential Scanning Calorimetry (DSC, Fig. S1) was performed with scanning up to 400 °C at a heating range of 10 °C/min (Q20, TA Instruments, USA).

2.2.3.4. XRD and FT-IR studies. X-ray diffraction patterns (XRD) of curcumin, GA Ald-Gel nanogel and curcumin loaded GA Ald-Gel nanogel were recorded by X-ray Diffractometer (X'pert Pro, Philips, USA using copper K-a radiation). FT-IR spectra of curcumin, 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.

2.2.3.5. NMR spectroscopy. ^{1}H NMR spectra of curcumin loaded Alg Ald-Gel nanogel and curcumin were recorded in DMSO-d_6 using 500 MHz spectrometer (Bruker Avance DRX 500).

2.2.4. Encapsulation efficiency

The encapsulation efficiency of curcumin loaded Alg Ald-Gel nanogels were determined by quantifying the curcumin loaded in the nanogel [25]. A known quantity of the lyophilized 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 amount of curcumin in the supernatant 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



Scheme 1. Representation of the possible interaction mechanism between curcumin and Alg Ald-Gel nanogel.