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Efficiency of resveratrol-loaded sericin nanoparticles: Promising bionanocarriers for drug delivery

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ABSTRACT

Sericin protein nanoparticles are a biocompatible, bio-viable class of nanocarriers gaining prominence in drug delivery system. This research aimed to investigate the suitability fabrication of silk protein (SP) nanoparticles for loading with resveratrol (RSV) via a solventless precipitation technique. The addition of 0.5% (w/v) pluronic surfactant proved optimal for SP nanoparticle fabrication, with obtained nanoparticles being spherical, monodispersed and having mean size of approximately 200–400 nm. All exhibited negative surface charges, the extent of which being dependent on the SP concentration, and were non-toxic to normal skin fibroblasts (CRL-2522). Loading of RSV, a promising which poorly soluble multi-targeted anti-oxidative and anti-inflammatory natural polyphenol, into SP nanoparticles proved feasible, with encapsulation levels of 71–75% for 0.6% and 1.0% (w/v) nanoparticle formulations, respectively. Resveratrol-loaded SP nanoparticles strongly inhibited growth of colorectal adenocarcinoma (Caco-2) cells although proved non-cytotoxic to skin fibroblasts, as indicated by cell viability assays. Cellular internalization of SP nanoparticles proved facile and dependent on incubation time; transfection of these carriers, *in vitro* results indicating sustained release of RSV (over 72 h), and drug solubility enhancements on encapsulation highlight their potential in therapeutic and pharmaceutical applications. Thus, SP nanoparticles is a promising approach to be potential bio-nanocarrier for drug delivery system.

1. Introduction

Novel nano-drug delivery systems are of considerable interest nowadays as these allow for drugs or bioactive small molecules to be introduced at specific target sites (Sahoo and Labhasetwar, 2003). Nanoparticles are ubiquitous in drug delivery systems, and while many materials can be used for their formation, polymers are one of the most important precursors. Biodegradable and biocompatible polymers are particularly attractive: high encapsulation levels and controlled drug release properties can be achieved in carrier systems derived from poly (lactic-co-glycolic acid): PLGA (Alqahtani et al., 2015; Surassmo et al., 2015), chitosan (da Silva et al., 2016; Vongchan et al., 2011), poly lactic acid (PLA) (Essa et al., 2011; Jain et al., 2013), liposomes (Saengkrit et al., 2014), sodium alginate (Rescignano et al., 2015), gelatin (Leo et al., 1997), collagen (Friess, 1998), zein (Zhong and Jin, 2009) and silk protein (Yan et al., 2008).

In recent years, protein-based nanocarriers have come to the fore due to their low cytotoxicity, biodegradability, biocompatibility, and high nutritional value. Carriers derived from these also exhibit high cellular binding affinities allowing significant uptake (Zhang, 2002). Proteins are also an abundant renewable resource, diverse in structure and are available at low cost. Silk protein (SP) from silkworms degumming is one such example, composed of two proteins, fibroin (70%) and sericin (30%). For deguuming which is a process of removing of the sericin or silk gum from silk. An alkaline solution was added into silk solution at 95 °C to degrade into sericin peptide or hydrolyzed sericin with molecular weight less than 20,000 Da. The structure and properties of these proteins can also vary, depending on the silkworm species (Craig et al., 1999; Wang et al., 1999). Pharmaceutical, biomedical and cosmeceutical applications such as drug delivery (Zhang, 2002), wound healing (Cuttle et al., 2006), and tissue engineering (Nayak et al., 2014) underpin its potential, with its non-antigenic nature driving investigations into its application as a surgical suture material (Kurosaki et al., 1999; Santin et al., 1999). Sericin is a globular protein with molecular weights ranging from 10 to 250 kDa. Low molecular weight sericin peptides (< 20 kDa) are widely used in cosmetics as skincare, haircare, health products and also as medications. High molecular weight sericin peptides (> 20 kDa) are used as medical biomaterials, degradable biomaterials, compound polymers, functional biomembranes, hydrogels and functional fibres. Silk protein has also been reported to have

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anti-oxidant, bio-adhesive and bioactive properties (Singer and Clark, 1999).

Many research suggested that selection of preparation techniques plays a major role in obtaining nano-formulations with desired properties for a particular drug delivery application. Even though, information on preparation techniques of various nano-formulations from SP protein is available in literatures. For example, self-assembled sericin nanoparticles were prepared using diafiltration technique which obtained a spherical nanoparticles with no silk protein aggregation (Gref et al., 1994). After that, various preparation methods were investigated including sol-gel technique (Yu et al., 2007), ionotropic gelation, phase separation (Wang et al., 2010), salting out (Lammel et al., 2010), capillary-microdot, electrospraying (Gholami et al., 2010; Qu et al., 2014), microemulsion (Kazemimostaghim et al., 2013), electric field (Huang et al., 2011) and desolvation tecniques. All of these methods were demonstrated the ability to carry both hydrophobic and hydrophilic drugs to address potential therapeutic applications (Mandal and Kunda, 2009). Moreover, silk fibroin and sericin was applied as bioactive layers in wound dr essings that help to promoted healing in deep wounds and greater degree of wound size reduction than traditional dressing types (Kanokpanont et al., 2012). Silk protein may also act as an active, being itself encapsulated by nanoparticles to control its release at particular target sites (Dong et al., 2015; Margetts and Sawyer, 2007; Ferreira et al., 2004).

Resveratrol (*trans*-3, 5, 4´-trihydroxy-stilbene, RES), a polyphenolic compound, has documented anti-carcinogenic (Sun et al., 2008), anti-inflammatory (Peng et al., 2016), and anti-oxidant (Jang et al., 1999) properties which fuel its interest as a bioactive in pharmaceuticals. Its anti-cancer potential is particularly noteworthy and has been extensively studied, although practical applications are limited by its poor water solubility, photo-sensitivity and rapid degradability (Baur and Sinclair, 2006; de la Lastra and Villegas, 2005).

This study aimed to develop protein nanocarriers by modified-desolvation, utilizing recovered sericin protein powder from wastewater. Nanocarrier fabrication was undertaken using a range of SP and pluronic stabilizer concentrations, enabling selection of the optimum conditions for the formation of spherical, small sized, stable nanoparticles. Resveratrol loading demonstrated the utility of SP nanoparticles as a functional delivery system, with loaded systems being extensively characterized in regards to their hydrodynamic diameter, surface charge, and morphology. Encapsulation efficiencies, release and cellular uptake profiles, and *in vitro* cytotoxicity assessments against human skin fibroblast and colorectal adenocarcinoma cells are also reported.

2. Materials and methods

2.1. Materials

Silk protein obtained from degumming was from Chul Thai Silk Co., Ltd. Dimethyl sulfoxide and Pluronic F-68 were purchased from Sigma-Aldrich (St. Louis, USA). Resveratrol powder (99% purity) was obtained from Namsiang Trading Co., Ltd. (Bangkok, Thailand). Dulbecco's Modified Eagle's Medium (DMEM), MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], L-glutamine, penicillin G sodium salt, streptomycin sulfate, and amphotericin B were obtained from GIBCO Invitrogen (NY, USA). Fetal bovine serum (FBS) was purchased from Biochrom AG (Berlin, Germany). All materials indicated were used without further purification.

2.2. Fabrication of SP nanoparticles and RES-loaded SP nanoparticles

Nanoparticles derived from SP were fabricated by desolvation, a technique employed previously (Kundu et al., 2010). In brief, for this, quantities of powdered SP were added to sterile distilled water, affording SP suspensions of different protein concentration. The SP suspensions were dropped at the rate of 1 ml/min through an atomizer (nozzle size 500 nm) into a solution of pluronic F-68 (0.5% w/v) in anhydrous DMSO under constant stirring at room temperature. SP nanoparticles were formed on contact with the DMSO, and precipitated as a suspension. After centrifugation at 10,000 rpm for 10 min at room temperature, the nanoparticles were collected and purified to remove excess DMSO by further centrifugation at 10,000 rpm (high speed micro refrigerated centrifuge 3700, KUBOTA) for 15 min in deionized water. The obtained pellets were re-dispersed in deionized water and sonicated using a probe sonicator (Q700, QSONICA) at 40% amplitude for 5 min (pulse on 59 s, pulse off 5 s) to yield a uniform SP nanoparticle suspension.

RSV-encapsulated SP nanoparticles were prepared by adding RSV (dissolved in ethanol) to the pluronic F-68 DMSO solution. The quantity of RSV used in the ethanol solution was varied, giving different loading levels. Fabrication of loaded nanoparticles was achieved as described above for native SP nanoparticles.

2.3. Physicochemical characterization of formulations

2.3.1. Particle size and surface potential

Hydrodynamic diameter, surface charge (zeta potential), and polydispersity index (PDI) of nanoparticles were obtained using dynamic light scattering (DLS) (Nano ZS-4700 nanoseries, Malvern Instruments, Malvern, UK) at 25 °C. For these measurements aqueous SP nanoparticle suspensions were diluted (1:50 with filtered deionized water) and sonicated for 1 min to ensure uniformity. All values obtained were derived from triplicate experiments, and represent the average value of the measurements ± standard deviation.

2.3.2. Morphological investigations

Evaluations of SP nanoparticle morphologies utilized Atomic Force microscopy (AFM: SPA400, SEIKO, Japan). Samples were prepared by dropping nanoparticles onto a freshly cleaved mica sheet, followed by air-drying. AFM imaging was done in the tapping mode using the NSG-10 cantilever, resonance frequency 190–325 KHz, and constant force of 5.5–22.5 N/m. Images were collected at a scan speed of 0.8 Hz as a phase image and topology.

Further morphology studies were undertaken using transmission electron microscopy (TEM, HT7700, Hitachi, Japan). Freshly prepared SP nanoparticles were diluted with ultrapure Milli-Q® water and dropped on a carbon coated copper grid (400 mesh). After staining with 1% (w/w) phosphotungstic acid samples were air-dried prior to imaging (accelerating voltage of 80–120 kV).

2.3.3. Fourier transform infrared spectroscopy (FTIR) measurements

FTIR spectra of freeze-dried SP, native SP nanoparticles and RSV-loaded SP nanoparticles were obtained using a Nicolet 6700 FTIR spectrophotometer (Thermo Fisher Scientific) equipped with a high performance diamond single-bounce ATR accessory (wave number 4000–400 cm^{-1}, resolution 4 cm^{-1} with 64 scans per spectrum) operating in reflectance mode. IR spectra in transmittance mode were obtained by accumulation of 3 scans.

2.3.4. Determination of RES encapsulation efficiency (EE)

The RSV loading efficacy of SP nanoparticles was investigated using HPLC (Waters, e2695, Singapore). This involved separation of free (surface) RSV from nanoparticles by centrifugation of RSV-loaded SP nanoparticle suspensions at 12,000 rpm (4°) for 30 min. The supernatant containing free RSV was filtered (0.45 μM membrane filter, Millipore, Germany) prior to injection and analysis (HPLC column Atlantis C-18, 4.6 × 250 mm, Thermo Fisher Scientific Inc., Water, USA). HPLC conditions were as follows: mobile phase acetonitrile: water: acetic acid (70:29.9:0.1 (w/v)), flow rate 1.5 mL/min, injection volume 20 μl, detection wavelength 280 nm. Method validation focused on the following parameters: specificity, linearity, detection and