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Ganoderma lucidum polysaccharide loaded sodium alginate micro-particles prepared *via* electrospraying in controlled deposition environments

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ABSTRACT

Ganoderma lucidum polysaccharide (GLP) is a functional food source deployed in preventative medicine. However, applications utilizing GLP are limited due to oxidative and acidic environmental damage. Advances in preserving GLP structure (and therefore function), *in situ,* will diversify their applications within biomedical fields (drug and antibacterial active delivery *via* the enteral route). In this study, GLP loaded sodium alginate (NaAlg) micro-particles (size range 225–355 μm) were generated using the electrospray (ES) process. The loading capacity and encapsulation efficiency of GLP for composite particles (collected at different temperatures) were ~23% and 71%, respectively. The collection substrate ($CaCl_2$, 1–20 w/v%) concentration was explored and preliminary findings indicated a 10 w/v% solution to be optimal. The process was further modified by manipulating the collection environment temperature (~25 to 50 °C). Based on this, NaAlg/GLP micro-particles were engineered with variable surface morphologies (porous and crinkled), without effecting the chemical composition of either material (GLP and NaAlg). *In-vitro* release studies demonstrated pH responsive release rates. Modest release of GLP from micro-particles in simulated gastric fluid (pH ~1.7) was observed, while rapid release was exhibited under simulated intestinal conditions (pH ~7.4). Release of GLP from NaAlg beads was the greatest from samples prepared at elevated environmental temperatures. These findings demonstrate a facile route to fabricate GLP-NaAlg loaded micro-particles with various shapes, surface topographies and release characteristics *via* a one-step ES process.

1. Introduction

Ganoderma lucidum (GL) is a basidiomycete white rot fungus belonging to the *Aphyllophorals* family. It has been deployed in preventative medicine for more than 2000 years (Ma et al., 2015). The benefits of GL have been documented in several studies; as a potential therapy for hepatitis, chronic bronchitis, gastritis, various tumors and immunological disorders. The fungus also displays anti-neoplastic, anti-inflammatory, antioxidant and immune-regulation properties (Ma et al., 2013; Zhang et al., 2016). The main bioactive substances associated with GL are polysaccharide and triterpenes; which are easily isolated from fruit bodies, mycelia and spores (Pan et al., 2013). Several reports indicate *Ganoderma lucidum* polysaccharide (GLP) to exhibit the aforementioned properties (Shi et al., 2013a,b; Wang et al., 2009), while several other studies show GLP's potential in other global healthcare challenges (Jie et al., 2009; Shi et al., 2013a,b; Teng et al., 2012). *In-vivo* experiments utilizing GLP exhibit little to no toxicity (Zhang et al., 2016), implying their relatively high biocompatibility. Despite these benefits, β-D-glucan (a form of polysaccharide isolated from GL) is extremely sensitive to oxidative degradation (including the reactions of active oxygen substances e.g. hydroxyl radicals). This environmental damage is most likely to occur during the storage process and subsequently

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limits GLP efficacy (Kivelä et al., 2012). Hence, it is essential to protect GLP after its extraction from GL and post formulation in order to preserve function. This limited longevity has impeded its potential use in the pharmaceutical arena.

Sodium alginate (NaAlg) is a natural polysaccharide comprising D-mannuronic and L-gulunronic acids, and is commonly extracted from marine brown algae (Hamid et al., 2008). NaAlg has been widely used as an encapsulating matrix material because of cost effectiveness, process ability and biocompatibility. Ionic cross-linking is used to yield three dimensional structures which reduces undesirable interactions between encapsulated moieties and external environmental factors (e.g. oxidizing agents and UV light) (Iliescu et al., 2014). Moreover, because of NaAlg's ionic nature, the release of encapsulated materials can be controlled based on external pH environments. In acidic conditions (pH ~2) release is retarded, whereas enhanced release is observed at more neutral pH values (~6 to 8) (Zhao et al., 2016). For these reasons, several studies utilizing NaAlg have focused on protective and controlled release functions using encapsulated oils (e.g. peppermint (Koo et al., 2014)) and viruses (e.g. adenovirus) *via* cross-linked beads (Park et al., 2012).

The electrospray (ES) technique is a one-step preparation method which has been widely used to engineer particles ranging from tens of nanometers to hundreds of micrometers (Gao et al., 2016). The diameter and surface morphology of generated particles is regulated by process parameter manipulation (e.g. flow rate, applied voltage and collector distance) (Yuan et al., 2016). In addition, the process has been used to fabricate particles with varied surface topographies through vapor-induced phase separation (Meng et al., 2009), non-solvent collecting mediums (Gao et al., 2014) selective coating layers (Yao et al., 2016b) and by altering the environmental humidity and polymer molecular weight (Casper et al., 2003; Wu and Clark, 2007). Interestingly, topographical and surface area-to-volume ratios (arising from particle shape) have been shown to impact active release from an embedded matrix (Gao et al., 2015). This provides a shape driven drug delivery approach, although the effect of environmental process temperature at the site of ES deposition on particle shape and surface topography is limited. This is crucial since the ES process is an atomization method and therefore solvent vaporization effects need to be explored further.

In this study, we demonstrate two aspects relating to GLP encapsulation which have potential to enable its pharmaceutical function and suitability. Firstly, a new combination of process (electrospray) and materials (GLP and NaAlg) is shown incorporating optimization and the demonstration of a crucial process factor (i.e. the environmental process temperature). This is an important find for electrohydrodynamic processing using this material, but also very relevant to other pharmaceutical technologies which deploy elevated or variable temperatures for particle engineering. Secondly, directly linked to this process parameter, particle morphology and release dynamics of GLP from micro-capsules are varied; providing a route to modulate such properties during an unexplored engineering step. Since the particle size is coarse and comparable in dimension to systems deployed for oral delivery, GLP release behavior is shown over a pH range; typical of what is expected at selected points in the GI tract once administered orally.

2. Materials and methods

2.1. Materials

Sodium alginate (NaAlg, A1112) and calcium chloride dehydrate ($CaCl_2$, C4901) were purchased from Sigma Aldrich (St. Louis, Mo., USA). Phosphate buffer solution (PBS) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Deionized water was produced using a Millipore Milli-Q Reference ultrapure water purifier (USA). High purity-grade wood-log cultivated GLS and commercial broken spore products were obtained from TianHe Agricultural Group (Zhe Jiang Long Quan, China). All chemicals used were analytical grade without additional purification.

2.2. Extraction of GLP *from* Ganoderma Lucidum *spores*

Ganoderma Lucidum spore powder was added to DI water to prepare a 5 w/v% mixture. The mixture was subjected to ultrasound irradiation (υ = 40 kHz) by using an ultrasonic device (KS-300 EI, Kesheng Co., Ltd, Zhejiang, China) for 50 min at 70 °C, followed by centrifugation for 15 min at 12000 rpm. The supernatant was then removed and stored. The process was repeated three times by further additions of DI water (to make up to the primary volume) followed by ultrasound and centrifugation. All (stored) supernatant was placed into a flask and then rotary distillated (50 °C). The resulting sample was freeze dried which yielded solid polysaccharide.

2.3. Preparation of electrospraying solutions

Pure NaAlg solution (2 w/v%) was prepared by dissolving NaAlg powder into DI water. GLP was then added into the 2 w/v% NaAlg solution at a ratio of 2:1 (weight of NaAlg solute: weight of GLP) to achieve a homogeneous mixture. Known quantities of $CaCl_2$ were dissolved into DI water to prepare several $CaCl_2$ solutions (1, 2, 5, 10, and 20 w/v%), which were subsequently used as collection mediums. A magnetic stirrer (VELP ARE heating magnetic stirrer, Italy) was used to achieve complete dissolution of the powder. The solutions were individually mechanically stirred at ~300 rpm at ambient temperature (25 °C) for 1 h.

2.4. Fabrication of electrosprayed micro-particles

Micro-particles were fabricated using the ES technique as illustrated in Fig. 1a. The set-up includes a high power voltage supply, a high-precision syringe pump, a stainless steel needle and a ring-shaped ground electrode. The formulated liquid was propelled by a syringe pump (KD Scientific KDS100, USA) into the metallic needle (the inner and outer diameters were 0.8 mm and 1 mm, respectively) using a feeding rate range between 6.0 to 10.0 mL/h. An electric field was applied (Glassman high voltage Inc. series FC, USA), and the voltage ranged from 14.0 to 16.0 kV. Selected $CaCl_2$ solution (collector medium) was placed directly below the metallic needle at a distance of 500 mm, while the ground electrode was set at 150 mm below the needle orifice. For each condition, a collection time of 2 h was deployed which enabled sufficient particle collection for further analysis. During the ES process, the environment temperature was controlled using a heating apparatus (FH-06A, Liqi electric appliance Co., Ltd., Zhejiang, China) and temperature readings were observed around the collection area to ensure uniformity. A high-speed camera (Baummer TXG02C, Germany) was used to observe ES jetting modes. Collected micro-particles were dried prior to further analysis using an electric vacuum drying oven (D2F-6020AF, Gongxing Laboratory Instrument Co., Ltd, Tianjin, China) at a pressure of 0.1 MPa (25 °C) for 24 h.

2.5. Particle morphology assessment

Optical (OM, Pheonix BMC503-ICCF, China) and field emission scanning electron microscopy (SEM, SU 8000 SEM, Hitachi, Japan) were used to study the size distribution and surface morphology of