Journal of Colloid and Interface Science 409 (2013) 245–254

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Preparation of multicompartment sub-micron particles using a triple-needle electrohydrodynamic device

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ARTICLE INFO

Article history:
Received 27 June 2013
Accepted 12 July 2013
Available online 30 July 2013

Keywords:
Electrohydrodynamic
Multilayered structures
Polymer
Drug delivery
Sub-micron particles
Human osteoblast cells

ABSTRACT

Control over the size and morphology of polymeric carriers for drug delivery systems is essential to optimize their functionality. In the current study, we demonstrate the feasibility of using an electrohydrodynamic process with a triple-needle device to prepare nearly mono-dispersed, spherical, tri-layered sub-micron particles. Three biocompatible polymer solutions of poly (lactic-*co*-glycolic acid) (PLGA), polycaprolactone (PCL) and polymethylsilsesquioxane (PMSQ) were used to prepare particles with three distinct layers. Optimized particles were shown to be spherical with an average size ranging from 320 nm (±80 nm) to 220 (±8 nm), which varied with a change in the working distance in the electrohydrodynamic processing. The surface and internal structure and morphology were studied using confocal, transmission and scanning electron microscopy combined with focused ion beam sectioning. Cytotoxicity was shown to be negligible in an *in vitro* assay. The ability to fabricate such multilayered particles in a single step, under ambient conditions has considerable potential for a range of applications in particular controlled release drug delivery system.

1. Introduction

Despite global interest, the development of single step processes for the preparation of effective drug delivery systems still faces numerous challenges. There is great demand for processing methods that are efficient, flexible, scalable and economical for the generation of a wide range of encapsulated nanostructures. A number of processes that have been established for the generation of multicompartment particles for medical applications include self-assembly [1], emulsion polymerization [2], layer-by-layer adsorption onto solid core particles [3], templated polymerization [4,5] and electrohydrodynamic atomization [6]. All these methods have their strengths and weaknesses, and technologies with the potential to create custom-tailored multicompartment particles are on the horizon. Nevertheless, over the past few decades, electrohydrodynamic (EHD) processing has received significant attention as a new method for the preparation of complex structures with attractive features for therapeutic applications. This technique avoids many of the processing problems that may interfere with drug encapsulation encountered with other techniques such as high temperature, agglomeration, protein denaturation and also the use of surfactant and/or organic solvents that may remain in the structures following processing leading to undesirable reactions with cells [7,8].

EHD processing enables the production of nanoscale particles with a controlled size distribution. Encapsulation studies using coaxial EHD processing, whereby two or more concentric liquid jets are formed simultaneously, present great potential for delivery systems i.e. carrier vehicles and multilayered capsules [9]. The ultimate aim of a drug delivery system is to have a regulated release profile from the desired drug carriers. Hence, preparation of multicompartment structures will enable more controlled release of drug over longer period of time. For this purpose, often biodegradable and biocompatible polymers are used in delivery vehicles to encapsulate or entrap therapeutic agents. Such materials are widely used as they allow sustained and controlled release of the encapsulated drug through diffusion and degradation in vivo [10,11]. Delivery vehicles loaded with an entrapped therapeutic agent can transport the substance through blood vessels to release their payload at a target site [12]. The co-delivery of synergistic drug combinations, with spatial and temporal control is important in targeted drug delivery. The current study focuses on the use of three different biocompatible polymers; poly (lactic-*co*-glycolic acid) (PLGA), polycaprolactone (PCL) and polymethylsilsesquioxane (PMSQ) to prepare tri-layered sub-micron particles. Recently,

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http://dx.doi.org/10.1016/j.jcis.2013.07.033

PLGA and PCL have both been shown to be attractive candidates for biomedical applications due to their ability to degrade into natural metabolites and their non-cytotoxic nature [13]. PMSQ has also received considerable attention for the preparation of drug carriers as it is also non-toxic and has superior chemical and physical stability during drug release [14,15].

During EHD processing, the simultaneous flow of liquids in co-axially arranged needles under electrical potential differences is used to generate stable structures with a controlled size distribution [16]. By incorporation of an extra co-axial needle it is possible in principle to produce further layers. Although EHD processing is considered relatively easy to perform, attaining a specific size, size distribution, monodispersity and morphology of a desired type of particle is still challenging. Various parameters such as the magnitude of the electrical potential difference, flow rate, the collection plane and the physical properties of processed solution play an important role in creating an optimized structure [8,17,18]. The current study utilizes the EHD method combined with a triple-needle device for the preparation of multilayered structures using different polymeric materials. The study describes the use of the device to prepare unique structures, through a single step process, that holds great potential for the development of future drug delivery systems.

The co-axial method enables direct encapsulation of different drugs inside polymeric carriers, with desired size, in a single step. To the best of our knowledge, to date, only Ahmad et al. [18] have applied the EHD method combined with a coaxial triple-needle device to prepare a variety of multilayered structures using materials such as olive oil and glycerol. However, based on the data presented, the fabricated particles were agglomerated [18]. This led to difficulties in distinguishing individual layers. Therefore, the main aim of the current study was to investigate the feasibility of using triple-needle device, in combination with changes in the working distance, to design and produce an optimized electrospraying process that would allow the production of discrete, spherical, tri-layer sub-micron particles with a narrow size distribution via a single stepprocess using biomaterials. Importantly, the cytotoxicity of tri-layered sub-micron particles was assessed in order to confirm the absence of any toxic residue that may have remained following electrospraying. These are crucial aspects of the technology that need to be studied and understood prior to progressing to drug loading.

2. Experimental section

2.1. Materials

Three different polymers were used in this study; polymethylsilsesquioxane (PMSQ) powder provided by Wacker Chemie AG, GmbH, Burghausen, Germany. Poly (lactic-*co*-glycolic acid) (PLGA-co-polymer 50:50 Resomer RG503H, molecular weight 33,000 g/mol) was purchased from Boehringer Ingelheim, Germany. Calcein blue, Methyl green, Evans blue, polycaprolactone (PCL) (Mn 45,000) and solvents including ethanol (EtOH), dimethyl carbonate (DMC), dichloromethane (DCM) were all purchased from Sigma–Aldrich Poole, UK.

2.2. Solution preparation

PLGA, PCL and PMSQ solutions were prepared by mixing appropriate amounts of polymer with the relevant solvents (Table 1) and stirring for 900 s at the ambient temperature (21 °C), pressure and humidity.

Table 1
Physical properties of the solutions and solvents used in the study.

Sample	Viscocity (mPa s)	Surface tension ($mN\ m^{-1}$)	Electrical conductivity $\times 10^{-4}$ ($S\ m^{-1}$)
DMC:PLGA 95:5	3.1	27.7	0.2
DCM:PCL 94:6	6.3	24.7	0.1
EtOH:PMSQ 88:12	1.6	22.5	9

2.3. Characterization of polymer solutions

For the proposed study, viscosity, surface tension and electrical conductivity were measured as follows: Viscosity was measured using a VISCOEASY rotational viscometer (Brookfield Rheometer). Surface tension was measured using a Kruss Tensiometer (Standard Wilhelmy's plate method). Electrical conductivity was estimated using a HI-8733 (Hanna Instrument, USA) conductivity probe. The polymer solution properties are listed in Table 1. In all cases the mean value of five consecutive readings was taken.

2.4. Electrohydrodynamic (EHD) processing

In EHD processing an electric field is applied to a liquid droplet and the body of the liquid becomes charged; electrostatic repulsion offsets the surface tension and a droplet forms at the end of the needle (Fig. 1a). At a specific applied voltage (generated in this study by a high voltage power supply, Glassman Europe Ltd., Tadley, UK) and flow rate (controlled using high precision syringe pumps, Harvard Apparatus, Edenbridge, UK), the droplet adopts a conical shape and a fine jet emerges from its apex. The jet subsequently breaks up to form droplets. This is known as the stable 'cone-jet' mode [19] and is typically required in order to achieve fine particle production [20]. In this study, three coaxial liquid streams were produced simultaneously to form multilayereddroplets. It is believed that minimum droplet size can be achieved by increasing the applied voltage to the highest possible value and reducing flow rate to the lowest possible value whilst obtaining a stable cone-jet [21]. Moreover, according to Hartman et al [22], the shape of the cone-jet is as a result of an equilibrium between several forces of liquid pressure, liquid surface tension and the electrical stresses in the liquid surface. To form a stable 'cone-jet' the surface tension has to be overcome by the electric stress.

The dimensions of the needles used are shown in Fig. 1b. PLGA solution was introduced through the outer needle whilst the central and inner needles were supplied with PCL and PMSQ solutions, respectively. 10 ml volume capacity syringes were connected to each needle accordingly and were set at a specific flow rates (Table 2). Details of the applied voltage and the distance from the exit of the outer needle to the collector (known as working distance) for the current study are also shown in Table 2. All samples were collected in a petri dish filled with ethanol. Following droplet formation, the solvent evaporates leading to particle formation in the collecting media. All studies were carried out at the ambient temperature (21 °C), pressure and humidity.

2.5. Characterization techniques

Dynamic light scattering (DLS, Malvern instrument 2000) was applied to investigate the size distribution of multilayered nanospheres in 100% EtOH.

In order to confirm the presence of three different polymers in the produced particles, Fourier Transform Infrared (FTIR) spectra and Nuclear Magnetic Resonance (NMR) were both applied. FTIR