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Submicron polycaprolactone particles as a carrier for imaging contrast agent for in vitro applications

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ABSTRACT

Fluorescent materials have recently attracted considerable attention due to their unique properties and high performance as imaging agent in biomedical fields. Different imaging agents have been encapsulated in order to restrict its delivery to a specific area. In this study, a fluorescent contrast agent was encapsulated for in vitro application by polycaprolactone (PCL) polymer. The encapsulation was performed using modified double emulsion solvent evaporation technique with sonication. Fluorescent nanoparticles (20 nm) were incorporated in the inner aqueous phase of double emulsion. A number of samples were fabricated using different concentrations of fluorescent contrast agent. The contrast agent-containing submicron particle was characterized by a zetasizer for average particle size, SEM and TEM for morphology observations and fluorescence spectrophotometer for encapsulation efficiency. Moreover, contrast agent distribution in the PCL matrix was determined by confocal microscopy. The incorporation of contrast agent in different concentrations did not affect the physicochemical properties of PCL particles and the average size of encapsulated particles was found to be in the submicron range.

1. Introduction

Recently, biomedical imaging has received immense attention due to its extensive applications in diagnosis of disease at an early stage [1,2], tracking of therapeutic carrier, monitoring disease changes and determining a proper end state to therapy [3]. In many cases, imaging is performed for diagnosis of a disease state prior to initiation of therapy. Several imaging techniques such as, computed X-ray tomography (CT), optical imaging, magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon-emission computed tomography (SPECT), and ultrasound are being used for diagnosis of disease including cancer and neurodegenerative diseases. These are noninvasive techniques and allow the visualization of target tissues [4,5]. Various imaging technologies (Magnetic resonance, optical etc) depend on contrast agent to visualize the organ of interest [6]. Contrast agents could augment the efficiency of imaging techniques by highlighting the differences between tissues [3], without contrast agent such information-rich images would be unobtainable. The contrast agents currently used for diagnosis faces problems of poor target specificity, instability and low concentration at target site, which consequently affect image quality. Thus, it is essential to deliver high payload of contrast agent specifically to an organ of interest in order to obtain beneficial images. Due to their specific size and shape, submicron particles offer multifunctional capability. Polymeric particles, incorporated with contrast agents (polymeric encapsulation of contrast agent) are emerging as a new class of imaging agent for detecting human diseases [7]. These particles have shown many potential benefits, such as, (i) they restrict the delivery of imaging agent to a small area thus reducing the systemic side effects (ii) they can deliver high payload of imaging agent at target site selectively (iii) they can travel through blood vessels and protect the encapsulated agent until delivery (iv) polymeric particles provide high surface area that allows the attachment of appropriate targeting agent and enhance the release properties (v) they can modify the biodistribution of active agent in controlled manner. Moreover, polymeric materials have the ability to encapsulate different contrast agents and active molecules in a single particle enabling multifunctional particles possibilities [6–9], with a capacity for targeted site imaging and delivery of therapeutic agents [8]. Standard process allow

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for the encapsulation of lipophilic molecules into a multitude of particulate materials, however their application to hydrophilic compounds encapsulation is limited due to uncontrolled leakage of entrapped compounds during the preparation process [10–12]. However, double emulsion technique is an appropriate method for the encapsulation of hydrophilic molecules as well as hydrophobic molecules, additionally, it allows flexibility in particle size by adjusting process parameters, the process is independent of special laboratory equipment, the operation costs are low and preferable for low scale production [13,14]. Several polymeric materials such as polystyrene, dextran, chitosan poly (lactic acid) and poly (lactic-co-glycolic acid) has been used to develop multi-target and multifunctional particle loaded with fluorescent agent for optical imaging [15]. The characteristics (size, surface charges, and structures) of these particles can be controlled by polymeric backbone and process parameter during preparation, in order to improve the fluorescent agent entrapment, blood circulation time, target site accumulation and target specificity of imaging particles probe [16,17]. Polycaprolatone is a biodegradable polymer with low glass transition temperature and melting point and the polymer metabolites are eliminated from the body by innate metabolic process [18]. Due to biodegradable, biocompatible and non-toxic nature of PCL, it is extensively studied for control drug delivery system in several formulations including nanoparticles, implants, nano-fibers, microspheres etc. Its compatibility with wide range of drug and its slow degradation to release drug for extended period of time (months–years) makes it an appropriate candidate for controlled drug delivery systems. Moreover, PCL versatility is due to the fact that, it allows the modification of its physicochemical and mechanical properties by copolymerization, which intern affect all other properties of PCL such as solubility, ionic property and degradation pattern [19–21]. Though, PCL has been extensive investigate in drug delivery system [22–24], but its applications in imaging technologies are studied too little, especially for encapsulation of contrast agent in optical imaging techniques.

Confocal laser scanning microscopy (CLSM) can be used as potential tool for characterization of polymeric particles. It allows visualization of structures not only on surface, but also inside the particles without prior sample destruction and can be used to visualize the encapsulated compounds. CLSM has ability to acquire in-focus images from selected depths, allowing three-dimensional reconstructions of topologically complex objects, by assembling several coplanar cross sections and already has been used for evaluation of different formulations [25,26]. Conversely, SEM does not allow the visualization of internal structures (encapsulated phase) of intact particle, and requires mechanical section of particle to observe the internal structures, which may result in loss of encapsulated phase. Moreover, CLSM enable us to evaluate the encapsulation of fluorescent contrast agent into submicron particles as well as its distribution in biological samples. dual fluorescence technique enable us to record images at two individual wavelength couplets (excitation/emission), subsequently the confocal images of fluorescent particles (visualized under laser scanning) can be superimposed on images of submicron carrier particles (under normal observation) using the same sample plane [27]. Hence, the main goal of this work was to develop a polymeric submicron carrier for fluorescent contrast agent that might enhance stability, augment the imaging efficiency, and restrict contrast agent accumulation to specific area and could be used for delivery of imaging agent and therapeutic agent simultaneously. We studied the encapsulation of contrast agents in/on to PCL particles using double emulsion evaporation technique. Several formulations with different concentrations of contrast agent were characterized regarding particles size, morphology, zeta potential, encapsulation efficiency etc. Average size of particles was found to be in submicron range with smooth surfaces, spherical shapes and high encapsulation efficiency. We also evaluated the penetration of the particles into excised human skin.

2. Materials and methods

2.1. Materials

Polycaprolactone (PCL) (Mw = 14000 g/mol), polyvinyl alcohol (PVA) (Mowiol® 4–88, Mw = 31000 g/mol), and dichloromethane (DCM) were obtained from Sigma–Aldrich, Germany and used as such. Water was deionized using (Aquadem® from Veolia Water, France). Ultrasonic homogenizer system "CY-500" ivymen® (500W, 20 kHz) from SELECTA GROUP, Switzerland. Analytical balance (Acculab ALC-110.4) was supplied by Sartorius group, Germany. Hitachi S-800 FEG Scanning Electron Microscope from Hitachi Japan, Zetasizer Nano-ZS (Malvern, UK), red fluorescent (580/605) labeled carboxyl-functionalized polystyrene particles (FluoSpheres®) was purchased from Molecular Probes® F-8786 (Oregon, USA). CM 120 Transmission electron microscope was obtained from Philips, Netherlands. Eppendorf 5415C Centrifuge, was obtained from Eppendorf, Germany, and Rotary Evaporator (1500 W) was supplied by Nahita. Cary Eclipse Fluorescence Spectrophotometer (Fluorometer) was obtained from Agilent Technologies (Malaysia).

2.2. Methods

2.2.1. Preparation of submicron particles incorporated with contrast agent

The fluorescent contrast agent was encapsulated by the modified double emulsion ($W_1/O/W_2$) solvent evaporation process, via two-step emulsification technique using power ultrasound as described by Iqbal et al. [28]. Briefly, before preparing the first emulsion, the inner aqueous phase (W_1), was prepared by incorporating different concentrations of fluorescent contrast agent (FluoSpheres®) in deionized water and the volume was made up to 1.5 ml. Similarly, oil phase was prepared by dissolving 3 g of polycaprolactone (PCL) in 12 ml of dichloromethane properly to form a clear solution. And PVA solution (0.5%) was prepared to be used as outer aqueous phase (W_2), by taking 5 g of PVA in 1000 ml flask and sufficient amount of deionized water was added to make up the volume. PVA was dissolved under magnetic stirring at 60 °C for 40 min, which resulted in a clear PVA solution.

Then, in the first step of emulsification, the inner aqueous phase (W_1) was added to PCL solution and this mixture was homogenized properly to form a primary emulsion (W_1/O) using ultrasonic homogenizer "CY-500" ivymen® at a 70% amplitude for 5 min. In the second step, the primary emulsion (W_1/O) was dispersed in 150 ml of the outer aqueous phase (W_2) containing 0.5% PVA as stabilizer in a 250 ml glass beaker. This mixture was homogenized by an ultrasonic probe at 70% amplitude for 8 min, to produce a double emulsion ($W_1/O/W_2$). The ultrasonic horn was positioned 2 mm above the oil-water interface in the system. This position was kept constant for all the experiments. Afterward, the organic solvent evaporation from the dispersion with the help of rotary evaporator has led to the formation of solidified PCL particles. These dispersed particles were then recovered by centrifugation at 10000 rpm for 10 min and washed three times with deionized water properly. The ultrasonic transducer (homogenizer) consisting of titanium alloy probe (5.6 mm diameter and 60 mm height) used has power of 500W and frequency of 20 kHz. The above mentioned conditions were same for all the experiments, only the concentration of fluorescent agent was changed in each formulation.