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Surface modification of poly(lactide-co-glycolide) nanoparticles by D-α-tocopheryl polyethylene glycol 1000 succinate as potential carrier for the delivery of drugs to the brain

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ABSTRACT

The potential health benefits of vitamin E (D-α-tocopheryl polyethylene glycol 1000 succinate, TPGS), particularly, in curing of the neurological symptoms associated with vitamin E deficiency have been reported. Hence, vitamin E containing carriers for delivery of drugs to the brain might be useful from different points of view. Herein, in order to obtain desired surface morphology and particle size of poly(lactide-co-glycolide) (PLGA) nanoparticles (NPs) and high emulsifying effects, TPGS-modified PLGA NPs were optimized as a potential carrier for the delivery of drugs to the brain. The particle sizes, surface morphology, phase composition correlated with different emulsifiers and different stirring times were characterized. Also, the *in vitro* cytotoxicity of the samples using PC12 cell line was investigated. According to the obtained results, by increasing the percentages of TPGS, the average particle size decreased and the distribution of particle diameters came closer by further addition, and the larger particles did not create. In addition, no obvious cytotoxicity was observed at various TPGS amounts, and the modified PLGA NPs were considered biocompatible since they show little decrease in cellular viability. With the increase of TPGS ratio, more effective *in vitro* therapeutic effects could be observed, which achieved the highest cell viability, because the degradation of NPs may release the most amounts of TPGS components that have synergistic activity. Furthermore, it was found that TPGS as a water-soluble derivative of natural source of vitamin E could be a perfect emulsifier for making PLGA NPs as potential carrier for delivery of drugs to the brain.

1. Introduction

For many years, the delivery of drugs to the brain has been a challenging subject for many researchers, because this tissue benefits from a very efficient protective barrier. The same mechanisms that protect the brain from foreign substances also restrict the entry of many potentially therapeutic agents [1]. Actually, the blood–brain barrier separates the brain parenchyma from the circulating blood and maintains the homeostasis of the brain by protecting it from harmful blood-borne substances and microorganisms [2]. Nevertheless, this self-protecting mechanism also poses an insurmountable obstacle when attempting to deliver drugs directly to the brain. It has been also shown that all of the high molecular weight drugs and nearly all of the low molecular weight drugs cannot cross the blood–brain barrier [3]. Therefore, many neurological disorders remain under treated. In this field of research, as the years have passed, many brain-targeted delivery strategies have been developed to overcome the blood–brain barrier.

As the drug vector, a significant amount of the work exploring nanotechnology for crossing the blood–brain barrier has focused on nanocarriers. Among these nano carriers, polymeric nanoparticles (NPs) are promising candidates because they are capable of opening the tight junctions of the blood–brain barrier, effectively disguising the membrane barrier limiting characterizations of the drug molecule, prolonging drug release and protecting against enzymatic degradation [4]. In fact, the brain distribution of many inherently impermeable drugs, have been improved after being incorporated into NPs [5,6].

Various drug delivery systems such as liposomes, emulsions, micelle, and polymeric micro/nano-particles have shown many advantages in controlled and targeted drug delivery. These

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techniques are capable of controlling the rate and duration of drug delivery and/or targeting the drug to the cell or tissue. Among them, nanospheres of biocompatible and biodegradable polymers have shown remarkable progress [7,8].

Polymeric NPs, in which an active agent can be dissolved, entrapped, encapsulated, adsorbed, attached or chemically coupled, are an exciting new area of research which can provide sustained, controlled and targeted drug delivery. Among them, poly(lactide-co-glycolide) (PLGA) is a FDA-approved biodegradable polymer, which is used most often in the recent drug delivery researches. PLGA nanospheres are usually produced by the solvent evaporation/extraction technique. In such a process, a number of fabrication parameters can affect the nature of the nanospheres obtained. One of the most important parameters is emulsifier, which is necessary as surfactant stabiliser in the process to form nanospheres. Emulsifier plays a key role in separation of the two (oil/water) phases to form the emulsion or particles [8,10]. The emulsifier stabilises the dispersed-phase droplets formed during emulsification, inhibits coalescence of droplets and determines the particle size and distribution, the morphological properties and thus the release characteristics of the nanospheres [7]. The emulsifier should be a kind of amphiphilic compound possessing two distinct groups, i.e., hydrophobic and hydrophilic groups in the same molecule. The traditional and most popular emulsifiers are chemical macromolecules such as poly(vinyl alcohol) (PVA), which their usage have some disadvantages. The chemical emulsifiers may have side effects for health care products. In fabrication process, they are difficult to be completely removed from the nanospheres. This often causes the difficulty for purification of the product [11,12]. Hence, finding new emulsifiers that could be also helpful for delivery of drugs to the brain would be interesting.

Vitamin E (D-α-tocopheryl polyethylene glycol 1000 succinate, TPGS) has been utilized for numerous applications in pharmaceutical dosage forms. Its chemical structure contains both the lipophilic and hydrophilic moieties, making it similar to a conventional surface-active agent. The chemical properties of this distinctive compound have suggested its use as an emulsifier [13]. Several studies have also demonstrated the effects of TPGS as an absorption enhancer [14–19] conducted a multi-center trial of TPGS for treatment of vitamin E deficiency in children with chronic cholestasis. These researchers also reported that TPGS appeared to be a safe and effective form of vitamin E for reversing or preventing vitamin E deficiency during chronic childhood cholestasis. Since TPGS can form micelles, it can cross from the intestinal lumen into the intestinal cells. This mechanism has suggested the use of TPGS as a controlled drug delivery carrier. Ismailos et al. [20] concluded that TPGS increased the solubility of cyclosporine, resulting in an increased bioavailability and thus an increased absorption.

Potential health benefits of vitamin E supplementation that are in addition to the essential effects on reproduction were suggested in the 1950s. In particular, the neurological symptoms associated with vitamin E deficiency were reported to improve with vitamin E supplementation [21]. The central nervous system (CNS) shows an exceptionally high degree of vulnerability to reactive oxygen species. Considerable evidence suggests that free radical formation and oxidative stress might play an important role in the pathogenesis of Parkinson's disease (PD) [22–26].

Once investigators realized that oxidative stress and lipid peroxidation play an important role in the etiology of Parkinson's disease (PD), vitamin E was investigated as a potential treatment for PD, both clinical and experimental models [27]. It is also worth to note that vitamin E can trap free radicals and interrupt the chain reactions that damage the cells [28,29]. Moreover, it has been reported that the levels of glutathione and vitamin E increase in the brain of patients with PD as a compensatory mechanism to deal with oxidative stress [22,30,26,31]. Since vitamin E is an effective free radical scavenger in the brain [32], its neuroprotective function is the issue of new therapeutic approaches in neurodegenerative diseases. In clinical trials, the vitamin E therapy might have retarded the progression of degenerative process in patients with PD [33].

In the recent researches, PLGA NPs were usually prepared by using chemical emulsifiers such as PVA, which has been found of disadvantages including low emulsification efficiency, side effects and difficulties to wash away in the formulation process. Instead, TPGS has high emulsification efficiency (67 times higher than PVA). It can also greatly improve the drug encapsulation efficiency (up to 100% EE achieved) and enhance cellular uptake of NPs and thus increase the cancer cell mortality [34–38]. Besides, its bulky structure and large surface area characteristics makes it as an excellent emulsifier.

2. Materials and methods

2.1. Preparation of PLGA NPs

PLGA (50:50, Resomer-RG 503H; Boehringer Ingelheim) was dissolved in 1 mL of dichloromethane. The PLGA NPs were prepared by a one-step oil-in-water emulsion, solvent evaporation method. In brief, 50 mg of PLGA in dichloromethane was added in to the dispersing phase (0.02%, 0.06%, 0.12% and 0.18% TPGS aqueous solution, 50 mL) under moderate magnetic stirring and NPs were formed immediately upon mixing. The formed emulsion was then stirred with 1250 rpm on a magnetic stirrer plate at room temperature for 2 h to evaporate dichloromethane (DCM). In this new method, the particles were frizzed for one day. The final product was obtained after freeze-drying for 24 h.

2.2. Characterization

2.2.1. Particle size and morphology

In the pharmaceutical industry, scanning electron microscope (SEM) may be used as a qualitative tool for the analysis of drug substance and drug product in order to obtain information on the shape and surface structure of the material. More than just qualitative analysis, SEM plays an important role in the quantitative analysis for characterization of size, shape and distribution of nanoscale and sub-micron particulate systems. Herein, the particle size, morphology and structure of the synthesized samples were evaluated using SEM. The samples were coated with a thin layer of gold (Au) by sputtering (EMITECH K450X, England) and then the morphology of them were observed by a scanning electron microscope (SEM-Philips XL30) that operated at the acceleration voltage of 15 kV.

2.2.2. Chemical bonding

The IR spectra of the samples were obtained using the reflection absorption spectroscopy technique involving the use of an ARO (all reflective objectives) lens while simultaneously viewing under a 10× eyepiece. Briefly, the samples for FTIR analysis were prepared by grinding 90% KBr (infrared grade) with 10% polymer and then pressing the mixture under 4000 psi pressure to form into a transparent tablet. A total of 20 scans were recorded for each sample at a resolution of 4 cm^{-1} and the spectra recorded from 500 to 4000 cm^{-1}. The spectral data was collected using the Bomem Software and the numerical values transferred to Microsoft Excel® Software for graphical representation.

2.2.3. In vitro studies in PC12 nerve cell line

The *in vitro* cytotoxicity of the TPGS-modified PLGA NPs was tested using PC12 cell line. The line was kept in continuous culture in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and streptomycin/penicillin 100 U/mL (1%). The cells were detached with trypsin/EDTA before seeding on