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Development of a single-jet electrospray method for producing quercetin-loaded poly (lactic-co-glycolic acid) microspheres with prolonged-release patterns

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ABSTRACT

Quercetin has been reported to possess unique biological effects. Here, we describe an electrospraying method for the preparation of poly (lactic-co-glycolic acid) (PLGA) microspheres, which serve as a platform for the long-term release of quercetin. Optimal microspheres had a narrow size distribution (1–4 μm), as determined by scanning electron microscopy. Quercetin was successfully incorporated into the PLGA matrix in the amorphous or molecularly dispersed state, as evidenced by XRD and DSC spectra. The FTIR spectra revealed no chemical interaction between quercetin and PLGA. The drug encapsulation efficiency and loading capacity of microspheres were 81.84 ± 1.60% and 7.77 ± 0.15%, respectively. Importantly, quercetin exhibited a prolonged-release pattern for 30 days *in vitro* with little evidence of burst release. Finally, cytotoxicity study revealed that the PLGA microspheres exhibited no toxic effects when treated to INS-1 cells. The study indicates that the developed electrospray method may be suitable for the preparation of quercetin-loaded PLGA microspheres.

1. Introduction

Quercetin is found in various plants and possesses many interesting biological effects [1], such as anticarcinogenic, anti-inflammatory, antiviral, and antioxidative effects [2–4]. Recently, researchers reported quercetin promotes osteogenic differentiation of stem cells for bone formation, and inhibits cellular senescence, especially in a background of diabetes [5–13].

However, quercetin is sparingly soluble in water and lipid-soluble, and it has been suggested that its oral bioavailability is poor and highly variable (~2%) in human. In addition, quercetin is rapidly cleared from the body due to its metabolism in the liver. In fact, its half-life is 1–2 h after intravenous administration, which adds to the variability of its effects *in vivo* [4,14]. Many research groups have developed means of preparing quercetin-based particles that enhance the stability, absorption, and bioavailability of quercetin *via* oral, topical and parental routes [15–30].

However, when systemically administered, it is difficult to achieve targeted quercetin concentrations in tissues or in environments of interest. Thus, a long-term release system is required to enable the efficient use of quercetin. This formulation can be applied by a local injection into intended tissues such as subcutaneous space or intramuscular space where inflammation occurs or can be co-delivered with therapeutic cells. This strategy is attractive because it would require single administration for the treatment and reduce patient inconvenience [31–33]. A literature search revealed one report on the preparation of polycaprolactone biodegradable polymer-based quercetin-loaded microspheres for the local treatment of rheumatoid arthritis. In this study, the authors prepared microspheres using an emulsion-evaporation technique, which produced large microspheres (> 60 μm) with relatively low quercetin loading capacities (2–4%) [34].

Electrospraying provides an attractive means of preparing micro- and nanoparticles for drug delivery. This technique does not require surfactants, such as PVA, to stabilize produced particles because their relative hardening before collection in the plate makes them not fuse to each other. Furthermore, electrospraying enables straightforward collection, and purification and is known for its excellent batch-to-batch reproducibility, which facilitates scale-up to industrial levels [35–39]. Poly (lactic-co-glycolic acid) (PLGA), which is approved by the FDA, is the most common polymer used in electrospraying processes because of its biocompatibility, biodegradability and its potential for enabling the prolonged release of active agents.

The aim of present study was to optimize electrospraying parameters to produce prolonged-release quercetin-loaded PLGA microspheres suitable for the local delivery of quercetin that could maximize

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Table 1
Properties of solvents used in electrospraying process.

Solvent	Boiling point (oC)	Dielectric constant	Surface tension (at 20 °C, dyn/cm)	Viscosity (at 20 °C, cP)	Density (at 20 °C, g/ml)	Solubility of quercetin (mg/ml)
ACE	56	20.7	23.3	0.36	0.788	24
DMF	153	36.7	36.8	0.92	0.949	30
Water	100	80.1	72.8	1.0	0.998	0.06

the effect of quercetin *in vivo*.

2. Materials and methods

2.1. Materials

Poly (lactic-co-glycolic acid) (PLGA; MW: 38–54 kDa, 50:50 LA: GA) was from Evonik Industries AG (Darmstadt, Germany). Quercetin (Q4951-10G), tween 20, acridine orange, and propidium iodide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Reagent-grade acetone (ACE), and dimethylformamide (DMF) were from Junsei Chemicals (Tokyo, Japan) (see Table 1). 1,4-Dithiothreitol (DTT) was from Promega (Madison, WI, USA).

2.2. Microsphere fabrication by electrospraying

Microspheres were fabricated using an electrospray system equipped with a high voltage generator (NanoNC, Seoul, Republic of Korea). Briefly, PLGA and quercetin were dissolved by vortexing in ACE or an ACE/DMF mixture (Table 2). Solutions were filled into a 10-mL plastic syringe fitted with a stainless steel nozzle G25 (inner diameter: 0.26 mm), which was then placed in a syringe pump system (KD Scientific Inc., Holliston, MA, USA). Microspheres were collected into an aluminum foil-covered tray (15 cm × 15 cm) using distilled water (80 mL) as the collection medium(see Fig. 1. Subsequently, microspheres were separated from the water by centrifugation (2700 rpm, 5 min) and lyophilized overnight. The experiments were carried out at room temperature and electrospray parameters, including flow rate, applied voltage, and tip-to-collector distance, were evaluated.

2.3. Scanning electron microscopy

A field emission scanning electron microscope (S-4100, Hitachi, Japan) was used to examine the surface topography of the PLGA microspheres. Briefly, lyophilized microspheres were placed on a stainless-steel plate using adhesive tape and then coated with a thin layer of platinum. At least 200 particles were randomly observed to figure out size distribution of microspheres using a Quartz PCI Lite software (version 5.10 Asia).

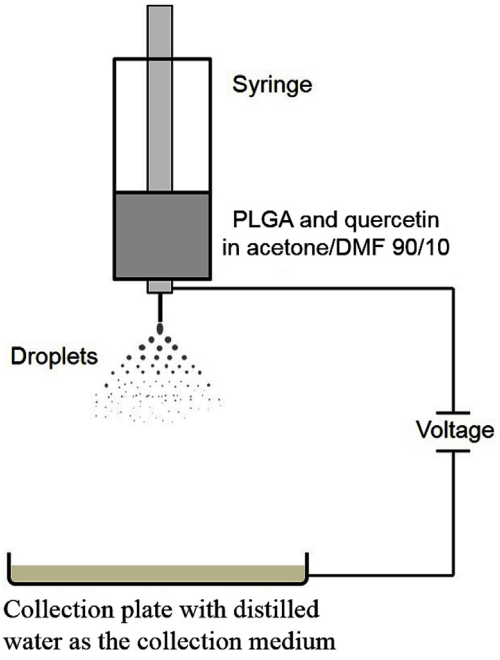

Fig. 1. Schematic of the electrospray process used to prepare microspheres.

2.4. Fourier transform infrared spectroscopy

To confirm chemical composition of microspheres before and after quercetin loading, Fourier transform infrared (FTIR) spectroscopy operated in a Nicolet Nexus 670 FTIR Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) was performed. Data were recorded at wavenumbers from 4500 to 800 cm^{-1} at room temperature.

2.5. X-ray diffraction

To explore the phase of quercetin in PLGA microspheres, X-ray powder diffraction (XRD) spectroscopy was conducted in an X-ray Diffractometer (X'Pert PRO MPD, PANalytical, Almelo, the Netherlands) using a copper anode Cu Kα, (λ = 1.5406 A^o, Kα2/Kα1 = 0.5). The samples put on a flat glass sample holders were scanned at 2θ values from 10° to 60° with a scan step size of 0.026, a generator voltage of 40 kV, and a tube current of 30 mA.

2.6. Differential scanning calorimetry

A differential scanning calorimeter (DSC-Q200, TA Instruments,

Table 2
Composition of prepared drug-polymeric solutions and electrospray parameters.

Formulation	Total solutes concentration (w/v, %)	Ratio of drug and polymer (w/w)	Ratio of ACE and DMF (v/v, %)	Flow rate (ml/hour)	Voltage (kV)	Collection distance (cm)
F1	10	0:100	100:0	1.2	12	15
F2	10	0:100	90:10	1.2	12	15
F3	10	0:100	90:10	1.2	12	12
F4	10	0:100	90:10	1.2	15	15
F5	8	0:100	90:10	1.2	15	15
F6	8	0:100	90:10	1.5	15	15
F7	10	10:90	90:10	1.2	12	15
F8	10	10:90	90:10	1.2	15	15
F9	8	10:90	90:10	1.2	12	15
F10	8	10:90	90:10	1.5	12	15
F11	8	10:90	90:10	1.5	15	15
F12	8	15:85	90:10	1.5	15	15