Journal of Colloid and Interface Science 508 (2017) 112–120

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

Regular Article

Phloretin-loaded fast dissolving nanofibers for the locoregional therapy of oral squamous cell carcinoma

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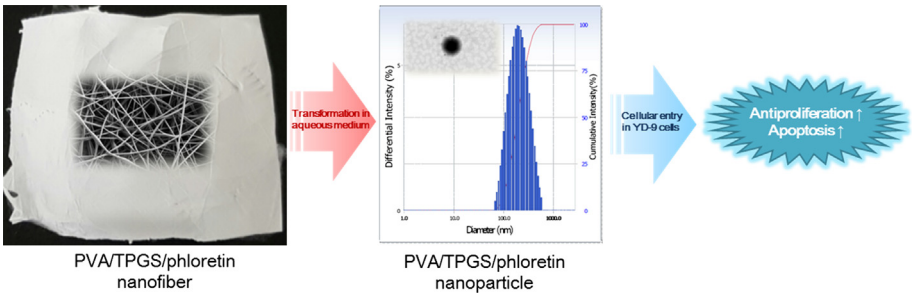
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 2 July 2017
Revised 8 August 2017
Accepted 9 August 2017
Available online 12 August 2017

Keywords:
Antiproliferation
Apoptosis
Fast dissolving nanofiber
Oral cancer
Phloretin

ABSTRACT

Fast dissolving nanofiber (NF) composed of poly(vinyl alcohol) (PVA) and D-α-tocopheryl polyethylene glycol succinate (TPGS) was developed for locoregional delivery of phloretin to oral cancers. PVA/TPGS/phloretin NF with 321 nm mean diameter and >90% drug entrapment efficiency was fabricated by an electrospinning method. Transformation of drug from crystalline to amorphous state was identified by solid-state studies. NF structure was changed to nanoparticles after its dispersing in the aqueous medium. PVA/TPGS/phloretin NF exhibited fast wetting property and smaller hydrodynamic size of dispersion, compared with PVA/phloretin NF. The amphiphilic property of TPGS also contributed to the improved drug release from PVA/TPGS/phloretin NF. The anticancer activities of phloretin, *via* the inhibition of glucose uptake into the cancer cells, in NFs were assessed in YD-9 cells (oral squamous cell carcinoma from buccal cheek). The antiproliferation efficacy of PVA/TPGS/phloretin NF was significantly higher than that of phloretin solution and PVA/phloretin NF ($p < 0.05$). Higher apoptotic events were also observed in PVA/TPGS/phloretin NF group rather than phloretin solution and PVA/phloretin NF groups ($p < 0.05$). All these results support that PVA/TPGS/phloretin NF can be a promising fast dissolving formulation for the treatment of oral cancers.

1. Introduction

Oral cancer is ranked at top 10 in the incidence of cancers and there have been considerable progresses in the development of therapeutic modalities [1]. However, the survival rate was not significantly improved yet [1]. Oral cancers generally occur in the region of lips, cheeks, tongue, floor of the mouth, palate, sinuses, and pharynx. Common symptoms of oral cancers are as follows; swellings, thickenings, lumps, crusts, white and red patches, unexplained bleeding, persistent sores, and difficulty in chewing, swallowing, and speaking. Smoking tobacco and drinking alcohol are considered as co-morbidity factors in almost cases [2]. There are several histologic types of oral cancers, such as adenocarcinoma,

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http://dx.doi.org/10.1016/j.jcis.2017.08.030
0021-9797/

lymphoma, melanoma, and teratoma, and approximately 90% of oral cancers was classified as squamous cell carcinomas [3]. Although chemotherapy, radiotherapy, surgery, and targeted-therapy have been adopted for the treatment of oral cancers, the prognosis is not good due to metastasis and recurrence [4,5].

Besides synthetic small chemicals, various phytochemicals and herbal extracts have shown chemotherapeutic and chemopreventive activities against oral cancers [3,6–11]. In our previous study [3], the antiproliferation effect of ethanol extract of *Angelica gigas* Nakai in oral squamous cell carcinoma was demonstrated. Phloretin was selected as an anticancer agent in this study for the treatment of oral cancers. It has an inhibitory effect of glucose transport and has been used as one of anticancer agents for several types of cancers [12–16]. By inhibiting the intracellular uptake of glucose *via* glucose transporters (GLUTs), phloretin can induce cell cycle arrest and apoptosis in malignant tumor cells and they seemed to be related to anticancer activities [17–19]. With chemoprevention effects of phloretin, it is expected that it can exert chemotherapeutic efficacies against oral squamous cell carcinoma.

Several pharmaceutical formulations have been developed for the delivery of drug cargos to the oral cavity [20–22]. Conventional dosage forms, such as semisolid dosage forms (*e.g.*, gels, creams, and pastes), liquid dosage forms (*e.g.*, solutions and suspensions), chewing gum, patches, films, and strips, have been used and some of them were commercially available in the market. Film formulations were also popular due to easy storage and conventional administration [3]. It was reported that a couple of processing methods (*i.e.,* hot-melt extrusion, casting, and compression) can be applied to prepare film formulations [23–25]. As one of film formulations, nanofiber (NF) mat was developed for its application to the oral cavity [3,26–30]. Thin fibril structure and high surface area can provide quick wetting and immediate drug release from formulations. In this study, NFs composed of poly(vinyl alcohol) (PVA) and D-α-tocopheryl polyethylene glycol succinate (TPGS) were fabricated by an electrospinning method for the delivery of phloretin. The poor aqueous solubility of phloretin can be overcome by its incorporation into the NF structure. Of note, PVA has been widely used as one of hydrophilic polymers to fabricate fast dissolving NF films [3,31,32]. The influences of the incorporation of various materials (*i.e.,* alginate, non-ionic detergents, organosilanes, and polyethylene glycol or its derivative) on the physicochemical properties of PVA NFs have been studied [33–35]. In this study, the effects of TPGS on PVA NFs were investigated. TPGS, which has an amphiphilic property, can enhance the aqueous solubility of drug *via* forming a micellar structure [36,37]. The combinatorial use of PVA and TPGS may produce nano-sized particles from NFs in the aqueous environment by reducing the surface tension. It is anticipated that increase of drug solubility, fast dissolution, immediate drug release, and efficient cellular entry can be accomplished with an electrospun PVA/TPGS NF structure. Herein, phloretin-incorporated PVA/TPGS NF was prepared by an electrospinning method and its anticancer effects were demonstrated. Remarkably, the relationship between the addition of TPGS to PVA NF and the physicochemical properties and anticancer activities of NF was systemically investigated.

2. Materials and methods

2.1. Materials

Phloretin and Tween 80 were acquired from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). PVA (molecular weight: 30–70 kDa) was purchased from Sigma–Aldrich (Saint Louis, MO, USA). TPGS was kindly provided by BASF SE (Ludwigshafen, Germany). RPMI 1640 (developed by Roswell Park Memorial Institute), penicillin, streptomycin, and heat-inactivated fetal bovine serum (FBS) were purchased from Gibco Life Technologies, Inc. (Grand Island, NY, USA). All other chemicals were of analytical grade and used without further purification.

2.2. Preparation and characterizations of NFs

NFs were prepared by an electrospinning method as reported [3]. PVA (30%, w/v) solution was prepared by dissolving PVA in distilled water (DW) with heating. For preparation of PVA NF, 30% PVA solution (0.8 mL) was mixed with ethanol (EtOH, 1.2 mL). To fabricate PVA/TPGS NF, 30% PVA solution (0.72 mL) and DW (0.08 mL) were mixed with TPGS (24 mg) in EtOH (1.2 mL). For fabricating PVA/phloretin NF, 30% PVA solution (0.76 mL) and DW (0.04 mL) were blended with phloretin (12 mg) in EtOH (1.2 mL). In case of PVA/TPGS/phloretin NF, 30% PVA solution (0.68 mL) and DW (0.12 mL) were mixed with TPGS (24 mg) and phloretin (12 mg) in EtOH (1.2 mL).

Each working solution was filled to the syringe (10 mL volume) linked to a stainless steel needle (25G) for an electrospinning process. The distance from the bottom to the apex of syringe was set as 15 cm. The solution was sprayed, at 1 mL/h flow rate, to the stainless steel sheet by a syringe pump at 25 kV. NFs were detached from the stainless steel sheet and stored at −20 °C for further uses.

The morphology of NFs was observed by a variable pressure-field emission-scanning electron microscope (VP-FE-SEM; SUPRA 55VP, Carl Zeiss, Oberkochen, Germany). The diameters of NFs were directly read from SEM images. NFs were mounted on stubs and coated with Au under vacuum prior to observation by SEM.

Entrapment efficiency of phloretin in NFs was determined by high-performance liquid chromatography (HPLC) analysis according to the reported method [13]. Each NF was dispersed in DW and diluted with the mobile phase of HPLC assay for the measurement of phloretin content. It was quantitatively analyzed using an HPLC system equipped with a pump (PU-2089 Plus; Jasco, Tokyo, Japan), an automatic injector (AS-2050 Plus), and an UV/Vis detector (UV-1575). A reverse phase C18 column (Gemini, 250 mm × 4.6 mm, 5 μm; Phenomenex, Torrance, CA, USA) was used for the analysis of phloretin and the injection volume was 20 μL. The mobile phase was comprised of acetonitrile, water, and phosphoric acid (50:50:0.08, v/v/v) and the flow rate was 1 mL/min. The absorbance of each sample was detected at 288 nm. The quantitative HPLC analysis method was validated.

2.3. Solid state studies

2.3.1. X-ray powder diffractometer (XRD) analysis

XRD patterns of phloretin, PVA/phloretin NF, and PVA/TPGS/phloretin NF were obtained by using D8 ADVANCE with DAVINCI (Bruker AXS GmbH, Karlsruhe, Germany) equipped with LYNXEYE detector. Operating conditions were as follows: 1.5418 Å wavelength of CuKα-radiation, 5–50° of 2θ range, 40 mA and 40 kV (generator conditions), 0.02° of step size, and 0.5 s/step of scan speed.

2.3.2. Differential scanning calorimeter (DSC) analysis

DSC curves of phloretin, PVA/phloretin NF, and PVA/TPGS/phloretin NF were measured by using DSC-Q100 model (TA Instrument, New Castle, DE, USA). Each material was analyzed using aluminum sealed pans and the heat flow was scanned from 25 to 295 °C at 10 °C/min temperature increasing speed under the supply of nitrogen gas (50 mL/min).