Journal of Drug Delivery Science and Technology 30 (2015) 408–416

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

Local delivery of resveratrol using polycaprolactone nanofibers for treatment of periodontal disease

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ARTICLE INFO

Article history:
Received 27 April 2015
Received in revised form
14 July 2015
Accepted 14 July 2015
Available online 15 July 2015

Keywords:
Nanofiber
Electrospinning
Polycaprolactone
Drug loading
Controlled release
Periodontal diseases

ABSTRACT

Periodontal disease is a biofilm-associated inflammatory disease of the periodontium. Resveratrol (RSV) is a promising natural substance for treatment due to its anti-inflammatory and anti-oxidative effects. RSV lacks efficacy under *in vivo* conditions due to low solubility and stability. Our special interest was to evaluate the effect of several selected organic solvent mixtures on polycaprolactone (PCL) nanofibers morphology, RSV incorporation and its release using electrospinning method and scanning electron microscopy, Fourier transform infrared spectroscopy, differential scanning calorimetry, and *in vitro* drug release measurements. The results showed that different organic solvent mixtures and electrospinning parameters strongly influence nanofiber morphology because thick, thin, flat, or circular nanofibers can be produced. RSV incorporated into PCL-nanofibers below 5% is mostly in amorphous form, and at higher loading nanocrystals were seen on the surface of thinner nanofibers, whereas thicker nanofibers can also cover the crystals. PCL nanofibers enabled prolonged release compared to the dissolution of pure RSV in sink condition. A bi-phase release kinetic was the consequence of RSV dissolution and cleavage of hydrogen bonding and hydrophobic interactions between PCL and RSV. The RSV-loaded PCL nanofibers will provide RSV for treatment of periodontal disease in the periodontal pocket even longer due to sustain release, and lowgingival fluid flow.

Periodontal disease is a biofilm-associated inflammatory disease of the periodontium [1]. One of key elements for the development of periodontal disease is colonization of teeth and periodontal tissues by pathologic bacteria, which release lipopolysaccharides that activate the immune system. This leads to inflammation that causes damage to the periodontal tissues due to the increased levels of cytokines and matrix metalloproteases [2]. The host immune response is essentially protective, but when a host is susceptible due to various reasons, such as smoking, poorly controlled diabetes, stress, or genetic factors [3], the response is not normal. Hypo- or hyper-responsive inflammatory pathways lead to enhanced tissue destruction and formation of periodontal pockets. The consequence of an inappropriate body response and the presence of chronic inflammation is the development of chronic periodontal disease [4]. Moreover, some recent studies reported new evidence that periodontal disease could be associated with a systemic oxidative stress state, reduced overall antioxidant capacity, and increased biologic markers for alveolar bone degradation in saliva [4–7].

Periodontal disease is very difficult to treat, because it is a complex disease affected by several factors. Taking these into account, there are three different approaches: (i) removal of dental biofilm with brushing and additional antimicrobial therapy; (ii) modulation of host inflammatory response aiming to suppress inflammation and restore homeostasis; and (iii) regenerative periodontal therapy of a destructed periodontum [8]. Mechanical removal of dental plaque as a gold standard of treatment with additional antimicrobial therapy in the case of persistent periodontitis [1] is not always successful, since disease recurrence is quite common [9]. Because inflammatory response plays a major role in the disease progression and tissue destruction, drugs that can modulate the host inflammatory response represent an important therapeutic approach [10]. The purpose of such therapy is to restore the balance between pro-inflammatory mediators and destructive enzymes on the one hand, and anti-inflammatory

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http://dx.doi.org/10.1016/j.jddst.2015.07.009
1773-2247/

mediators and enzyme inhibitors on the other [11].

One of the promising supporting natural substances for the treatment of periodontal disease is resveratrol (RSV). Its anti-inflammatory and anti-oxidative effects in periodontal disease were shown by reduced production of NO [12], inhibition of vascular endothelial growth factor, decreased vessel permeability [13], and decreased production of inflammatory cytokines, chemokines, and factors for leucocyte differentiation [14]. RSV acting as an antagonist of the aryl-hydrocarbon receptor could have a positive impact on periodontal tissue regeneration [15], and it was also shown that RSV significantly reduces loss of bone tissue [16]. However, RSV lacks the efficacy under *in vivo* conditions due to unfavorable biopharmaceutical properties (low solubility, rapid metabolism, and chemical instability). Therefore, the development of a carrier system that would overcome the biopharmaceutical obstacles and fully realize its therapeutic and prophylactic potential is still a technological challenge [17].

Highly efficacious dosage forms for the treatment of periodontal disease would need to specifically fit to the periodontal pockets, stay there for prolonged time periods, be in tight contact with surrounding tissue, and be able to control the release of drug. However, the investigations oriented towards development of such dosage forms are quite rare [8,18,19]. Currently, the most frequently used dosage forms are solutions, gels, films, or chips [18]. Nanofibers present a promising new alternative drug delivery system with the ability to load a drug and tailor drug release profile by a modification of the composition and the morphology of nanofibers [20]. Their large surface-to-volume ratios increase the tendency to adhere to the tissue in the periodontal pocket. In addition, the nanofibers morphology enable diffusion of gingival crevicular fluid through the matrix of the delivery system, which may decrease the tendency of flushing of the delivery system out of the periodontal pockets, as was the case of some of the other low porosity delivery systems, such as films or chips [18,21,22]. The most appropriate materials for the preparation of nanofibers by electrospinning technique and for the delivery of a wide variety of active ingredients are biodegradable and biocompatible polymers, such as chitosan, poly(-ethylene oxide), poly(vinylalcohol), poly(lactic-glycolic acid), poly(ε-caprolactone) (PCL). A different solution, process, and environmental parameters influence nanofiber formation and their morphology. Water is used for electrospinning of hydrophilic polymers [22]. Conversely, hydrophobic polymers require a careful selection of organic solvents, single or their mixtures with special additives, such as salts that increase the dispersion conductivity [23]. One of the attractive materials for the preparation of nanofibers is PCL, a biodegradable and biocompatible polymer, that also demonstrated sustained drug release kinetics in previous studies of nanodelivery systems [20].

Although RSV was shown to exhibit positive effects for treatment of periodontal disease, to the best of our knowledge, an appropriate delivery system with this substance for the treatment of this disease has not been yet described in the scientific literature. Therefore, the aim of this research was to prepare PCL nanofibers loaded with RSV that could be administrated into the periodontal pocket and there enable slow release. Our special interest was to evaluate the effect of several selected organic solvent mixtures on nanofibers morphology, RSV incorporation and its release. The physicochemical properties of empty PCL nanofibers and pure RSV were characterized and compared with prepared RSV-loaded PCL nanofibers using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), and differential scanning calorimetry (DSC). The release profiles of RSV from nanofibers were also examined using a USP XXV type II (paddle) method.

1. Materials and methods

1.1. Materials

Trans-RSV was purchased from LGC Standards GmbH, Germany with a declared purity of 99.6%, according to the manufacturer. Potassium dihydrogen phosphate, formic acid, sodium iodide, sodium hydroxide, chloroform (C), dichloromethane (DCM), and acetone (A) (for analysis) were obtained from Merck KGaA, Germany. PCL (MW 70,000–90,000 g/mol) and N,N-dimethylformamide (DMF) were purchased from Sigma Aldrich, Germany. Tetrahydrofuran (THF) was purchased from Carlo Elba, Italy. Acetonitrile of HPLC grade was purchased from J.T. Baker, Netherlands. Ultrapure water obtained from a Milli-Q® UF-Plus apparatus (Millipore Corp., Burlington, MA, USA) was used for the preparation of mobile phases.

1.2. Methods

1.2.1. Preparation of the polymer solutions

PCL solutions 10% (w/w) were prepared by dissolving PCL and NaI in different mixtures of solvents (Table 1). The dispersions were stirred with a magnetic stirrer overnight at room temperature to dissolve the polymer. Different quantities of RSV (1, 5, 10, and 20% (w/w) according to dry nanofibers) were added to all solutions and mixed by Ultra Turrax T25 (Janke & Kunkel, IKA Labortechnik, Germany) for 5 min at 15,000 rpm to obtain a homogenous solution.

1.2.2. Conductivity of the polymer solutions

The solution conductivity was measured by a MC226 Conductivity Meter and electrode Inlab 741 (Mettler Toledo, Switzerland).

1.2.3. Electrospinning of the polymer solutions

The polymer solution was placed in a 20 ml plastic syringe fitted with a metallic needle (inner diameter of 0.8 mm). A syringe pump (Model R-99E, RazelTM) was used to feed at a constant rate. High voltage at the needle was achieved by connection to a voltage generator (model HVG-P60-R-EU, Linari Engineering s.r.l., Italy). The electrospinning parameters of each solution are described in Table 1.

1.2.4. The diameter and morphology of the electrospun nanofibers

Nanofibers were examined using a 235 Supra 35VP-24-13 high-resolution scanning electron microscope (SEM, Carl Zeiss, Germany) operated at an accelerating voltage of 1 kV with a secondary electron detector; no conductive coating layer was applied before imaging. The obtained images were used to determine the average fiber diameters using ImageJ 1.44p software (NIH, USA) by measuring 50 nanofibers chosen randomly.

1.2.5. FT-IR analysis

FT-IR was used to qualitatively characterize the interactions between RSV and PCL. The FT-IR spectra of RSV, and empty and loaded PCL nanofibers with 1, 5, 10, and 20% RSV produced from CA0.03 solvent were characterized with FT-IR spectrometer with an attenuated total reflectance accessory (Nexus, Thermo Nicolet, Madison, USA). Spectra in the range of 600–3600 cm^{-1} with a resolution of 8 cm^{-1} were measured. Each recorded spectrum was an average of 16 scans.

1.2.6. Thermal analysis of nanofibers with RSV

Thermal analysis was performed using DSC (Mettler Toledo DSC 1, Switzerland) to determine the physical state of pure RSV and when incorporated in nanofibers. An approximately 5 mg sample