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Development and *in vitro* evaluation of acyclovir delivery system using nanostructured porous silicon carriers

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ABSTRACT

The present work describes the preparation, surface modification, and characterization of three different porous silicon (PSi) carriers for the acyclovir delivery. Acyclovir was loaded into these carriers and *in vitro* release behaviour has been studied. Such carriers were characterized by a combination of scanning electron microscopy, attenuated total reflectance Fourier transform infrared spectroscopy, and X-ray diffraction. *In vitro* drug release studies showed slower release (up to 8 h), and immediate release (up to 3 h) from native and thermally oxidized PSi, respectively. Drug release kinetics studies of thermally oxidized PSi suggested diffusion controlled drug release whereas native PSi indicated a combination of both diffusion of acyclovir and erosion of the silicon scaffold as drug release mechanisms.

1. Introduction

Herpes simplex virus (HSV) type 1 and type 2 are widespread human infectious agents that affect approximately 60–95% of adult population worldwide (Chayavichitsilp et al., 2009). HSV type 1 (HSV-1) is typically transmitted nonsexually during childhood causes oropharyngeal mucosal lesions; whereas HSV type 2 (HSV-2) is almost always transmitted sexually and typically causes genital lesions. The infection may cause blindness, encephalitis, aseptic meningitis and life threatening neonatal herpes (Bradley et al., 2014). In addition, a close relationship has been observed between HSV-2 and HIV acquisition. An epidemiological study reveals that prevalent HSV-2 infection is associated with 2- to 3-fold increased risk of acquiring HIV infection (Freeman et al., 2006). The drugs that are commonly used for the treatment of HSV are acyclovir (ACV), valacyclovir, and famciclovir.

ACV is a synthetic nucleoside analogue of guanosine and one of the most widely used drugs for the treatment of HSV infections. The mode of action of the activated form of ACV is *via* inhibition of viral DNA polymerase. ACV is activated by viral thymidine kinase to ACV monophosphate which is then converted to the diphosphate and finally to the triphosphate by cellular kinase of host cells and inhibits DNA synthesis by acting as a chain terminator (Elion, 1982). In addition to that, anticancer and antihepatitis B activity of ACV has been reported in recent studies (Yao et al., 2013; Huang et al., 2011). ACV is currently available as capsules, tablets, suspension and topical ointment for the treatment of epidermal ocular or systemic herpetic infections. However, the conventional therapy either the parenteral or the oral administration has been associated with number of drawbacks. The absorption of ACV in gastrointestinal tract is slow, variable, and incomplete and its average oral bioavailability is very low (10–20%). Approximately, 80% of the administered dose is not absorbed and therefore require high doses which results in systemic toxicity and other adverse reactions (O'Brien and Campoli-Richards, 1989). The low oral bioavailability of ACV

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is due to the low diffusivity and short residence time of dosage forms at the absorption site. Furthermore, because of plasma elimination half-life of the ACV is 2.5 h, and frequent administrations are required to maintain therapeutic drug concentration (Wagstaff et al., 1994). This has emphasized the urgent need to develop more patient compliant and efficient drug delivery system.

Over the years, different technological approaches have been proposed to improve the efficacy of ACV treatment and reduce its adverse side effects. However, nanoparticulate based drug delivery system of ACV has attracted much research attention in recent years. For example, the use of modified amphiphilic β-cyclodextrin nanoparticles in combination with ACV and liposome based sustained delivery system for vaginal administration of ACV are reported (Cavalli et al., 2009; Pavelic et al., 2005). Niosomal formulation containing ACV has also been prepared with the aim of improving the drug's poor and variable oral bioavailability (Mukherjee et al., 2007). Furthermore, Labrafac-based microemulsion formulations have been developed for improvement of oral bioavailability of ACV (Ghosh et al., 2006).

To the best of our knowledge, nanostructured porous silicon (PSi) has not been exploited yet as a possible ACV delivery carrier and only feeble information is available in a patent (Ashton et al., 2010). PSi is prepared by electrochemical etching of single-crystal silicon (Si) wafers in ethanolic hydrofluoric acid solution (Uhlir, 1956). The etching parameters such as current density and etching time can be varied to produce porous layer with desired porosity, pore size, and thickness (Maniya et al., 2013). PSi offers several unique physical and optical properties, advantageous as drug delivery carriers including simple top-down production method, controllable pore size, high surface area to volume ratio (800 $m^2 g^{-1}$), and easy surface modification for stabilization (Zhang, 2004; Buriak, 2002; Maniya et al., 2014). In addition, PSi is biocompatible and degrades completely to orthosilicic acid in physiological fluids (Canham, 1995). Optical properties of PSi also offer additional benefit of drug release monitoring in real time (Wu et al., 2011). Poorly water-soluble drugs can be loaded into the porous matrix of PSi by simple adsorsption to improve the bioavailability. PSi has already been investigated for loading and release of anticancer agents (Wu et al., 2011; Li et al., 2000; Chhablani et al., 2013), poorly water-soluble drugs (Wang et al., 2010; Salonen et al., 2005), peptides, proteins (Kilpelainen et al., 2009; Jarvis et al., 2010), and small interfering RNA (Tanaka et al., 2010).

In the present work, nanostructured PSi prepared by electrochemical anodization of Si wafer was used for the development of ACV delivery system. The study was focused on porous films still attached to the Si wafer in order to have more accurate quantification of drug loading and release behaviour. Native PSi surface was stabilized by partial and complete thermal oxidation. ACV was then loaded into the three types of PSi films by immersing in drug loading solution. The effect of surface chemistry on ACV loading efficiency and its release behaviour were studied.

2. Materials and methods

2.1. Materials

Si wafers were purchased from Siltronix Corp., France. ACV was kindly provided by Arochem Industries (Thane, India). Phosphate buffered saline was purchased from Sigma-Aldrich. Sulphuric acid, hydrogen peroxide, and hydrofluoric acid were purchased from Finar Chemicals, Ahmedabad, India. Ethanol was purchased from Changshu Yangyuan, China.

2.2. Preparation of PSi

Immediately before etching, the wafers were cleaned using piranha solution (3:1 (v/v) ratio of sulphuric acid (99%) and hydrogen peroxide (30%)) to remove the organic residues and other impurities present on the Si surface. PSi films were prepared by electrochemical etching of highly boron-doped p^+ type (100) Si wafers (resistivity of 0.01–0.02 Ω cm) in a 1:2 (v/v) hydrofluoric acid (40%) and ethanol (99.9%) solution using a two electrode configuration. Etching was performed in Teflon etch cell (electrode area of 2 cm^2) by applying constant current density of 50 mA cm^{-2} for 3 min. After etching, PSi films were thoroughly rinsed using ethanol and dried under a stream of nitrogen.

2.3. Thermal oxidation of PSi

Surface passivation of the PSi was carried out by thermal oxidation. The native PSi films were partially and completely thermally oxidized by heating at 300 °C for 2 h and 800 °C for 1 h, respectively. After oxidation, PSi films were allowed to cool to room temperature (37 °C).

2.4. PSi characterization

Scanning electron microscopy (SEM) analysis of PSi films were carried out by using a field emission gun scanning electron microscope (JEOL, JSM-7600F, Japan) with an accelerating voltage of 10 kV. PSi samples were sputter coated with thin layer of gold and palladium prior to SEM analysis. ImageJ software was used to quantify average pore diameter of porous layer from the SEM image (ImageJ software, 2014). Surface modification and drug loading of PSi carriers were characterized by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) using Hyperion 3000 microscope with micro ATR device coupled with Vertex 80 FTIR system (Bruker, Germany). PSi films attached with the substrate were directly placed on the Hyperion 3000 microscope and spectra were recorded. The X-ray diffractograms of ACV, PSi and ACV loaded PSi were obtained using a PANalytical model PW3050/60 goniometer and XPERT-PRO diffractometer system (X'Pert Pro PANalytical, The Netherlands) with a copper electrode. The samples were scanned continuously from 5.02° to 99.98° (2θ) at 40 kV and 30 mA.

2.5. Drug loading methodology

PSi was loaded using the simple immersion method. Drug loading solution of 4 mL of ACV dissolved in de-ionised water (solution concentration 1 mg mL^{-1}) was added to the PSi. The films were kept for 3 h in loading solution (37 °C) and then rinsed with de-ionised water to remove excess ACV from the surface of the PSi films and dried at room temperature (37 °C). ACV loading within PSi films was determined by completely dissolving the porous layer in an aqueous sodium hydroxide solution (1 M) and absorbance was measured using a UV–VIS spectrophotometer (HACH, DR6000, USA). The absorbance maximum at 255 nm (Jain et al., 2011) was used to determine