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Prolonged controlled delivery of nerve growth factor using porous silicon nanostructures

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ABSTRACT

Although nerve growth factor (NGF) is beneficial for the treatment of numerous neurological and non-neurological diseases, its therapeutic administration represents a significant challenge, due to the difficulty to locally deliver relevant doses in a safe and non-invasive manner. In this work, we employ degradable nanostructured porous silicon (PSi) films as carriers for NGF, allowing its continuous and prolonged release, while retaining its bioactivity. The PSi carriers exhibit high loading efficacy (up to 90%) of NGF and a continuous release, with no burst, over a period of > 26 days. The released NGF bioactivity is compared to that of free NGF in both PC12 cells and dissociated dorsal root ganglion (DRG) neurons. We show that the NGF has retained its bioactivity and induces neurite outgrowth and profound differentiation (of >50% for PC12 cells) throughout the period of release within a single administration. Thus, this proof-of-concept study demonstrates the immense therapeutic potential of these tunable carriers as long-term implants of NGF reservoirs and paves the way for new localized treatment strategies of neurodegenerative diseases.

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1. Introduction

Nerve growth factor (NGF) is essential for neuronal differentiation [1] and is critical for the development and maintenance of neurons in both the peripheral and central nervous systems [2]. NGF deficiency leads to brain pathologies and therefore NGF delivery presents high pharmacological potential for treating central neurodegenerative diseases, including Alzheimer's and Parkinson's [3–6]. Moreover, NGF exhibits protective properties for damaged neurons, stimulating axonal regeneration, and has been demonstrated as effective therapy for peripheral and spinal cord injuries [7,8]. However, NGF effectiveness in therapeutics is limited by its short biological half-life; in vivo pharmacokinetics studies show its rapid enzymatic degradation [9]. Current therapeutic approaches to administer NGF include intracerebroventricular infusion of NGF, gene therapy and implants of NGF-producing cells, with several ongoing clinical trials [10–14]. Some of these methods are highly invasive and may cause serious adverse effects [15]. For example, intracerebroventricular infusion, requires a continuous catheter maintenance in the brain [10].

Thus, there is an immense need for designing delivery systems for sustained and prolonged release of NGF in a controlled manner. Such systems should increase the protein's stability and availability over time and consequently minimize the toxicity risks and adverse side effects, associated with NGF administration for treating neurodegenerative diseases [10,16]. Various NGF delivery systems based on synthetic and natural polymer conjugates have been developed [17–22]. These polymers, including different polyphosphoesters (e.g., P(BHET-EOP/TC) and P(DAPG-EOP)) as well as poly(lactide/glycolic acid) (PLGA), have demonstrated effective sustained release profiles for several days [17–22]. Yet, in some of these polymeric systems (e.g., PLGA), NGF bioactivity was lost during the encapsulation process, requiring the use of different stabilizing agents [23,24] and sophisticated fabrication techniques [21].

Porous Si (PSi) has emerged over the past several years as a promising nanomaterial for biomedical applications in general [25–28] and drug delivery in particular [29–31]. Its tunable nanostructure, ease of fabrication, biocompatibility and degradability in physiological environment allow to design versatile drug delivery systems [32–38]. Importantly, following its synthesis, PSi can be rendered into thin films, microparticles, or nanoparticles to meet specific application needs [37,39,40]. We have recently demonstrated the ability to deliver loaded

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PSi particles into both cells and tissues using biolistics [41], opening new possibilities for non-invasive therapeutic routes [42]. The simple fabrication process of PSi enables tailoring the material structural properties such as porosity and pore size for controlling drug loading and release kinetics [32,36]. In particular, PSi ability to carry and release various payloads of small molecules, oligonucleotides and proteins for therapeutics has been demonstrated [39,43–48]. Recent work by several groups has shown that oxidized PSi ($PSiO_2$) films have a high loading capacity for different antibodies (e.g., bevacizumab (Avastin) [43] and Infliximab [47]) and extended antibody release in vitro. Notably, tuning the pores dimensions of the PSi scaffold and modifying its surface chemistry, according to the properties of the payload (e.g., molecule size, structure and charge), allow optimizing drug loading and release from these nanostructures [31,32,49,50]. This is also the case when designing a PSi-based delivery system for proteins [43,44,48,51] and various studies highlighted that PSi surface chemistry plays a vital role in minimizing their possible denaturation [47,48].

The aim of this work is to develop $PSiO_2$ carriers for NGF payloads, allowing its continuous release and prolonged delivery, while retaining its bioactivity. The porous carriers, prepared by electrochemical etching and subsequent thermal oxidization, demonstrated high loading efficacy (of up to 90%) of NGF. The carriers show a continuous NGF release over a period of 26 days while their degradation is monitored by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The released NGF functionality is compared to that of free NGF in both PC12 cells and dissociated dorsal root ganglion (DRG) neurons. We show that the NGF has retained its bioactivity and induces neurite outgrowth and differentiation within a single administration. Its bioactivity is determined by measuring morphometric parameters and by molecular analysis of known neuronal specific markers (GAP43 and β3-tubulin). This proof-of-concept study demonstrates the immense therapeutic potential of these tunable carriers as long-term implants of NGF reservoirs and thus paves the way for new treatment strategies of neurodegenerative diseases.

2. Results and discussion

2.1. Fabrication and characterization of $PSiO_2$ carriers

Single-crystalline Si wafers were electrochemically etched at a constant current density of 250 mA/cm^2, followed by thermal oxidation, as illustrated in Fig. S1 (see Supporting information). The current density was adjusted [52] to yield cylindrical pores of approximately 40 nm in diameter (Fig. 1a, b) that can easily accommodate the NGF payload (molecular weight of 26.5 kDa [53] and a diameter of ~4 nm [54]) within the nanostructure. Anodization time was varied between 20 and 67 s to produce PSi films with different thicknesses. HR-SEM of the cross-sectioned films (Fig. 1b) revealed that their thickness is 2.9 or 10 μm, referred to as thin or thick $PSiO_2$ carriers, respectively. Following anodization, the films were thermally oxidized to render the PSi into porous SiO_2 layers ($PSiO_2$). The porosity of the films was determined by gravimetric studies [52,55], confirming their high porosity of ~77%. A detailed physical characterization of the $PSiO_2$ films is summarized in Table S1 (Supporting information).

2.2. NGF loading and release from $PSiO_2$ carriers

The $PSiO_2$ carriers were loaded with a NGF solution (50 μg/mL in PBS) by physical adsorption mechanism, in which the positively charged protein infiltrates into the porous nanostructure by electrostatic attraction to the negatively charged surface. NGF loading was quantified using NGF ELISA kit, with an average loading of 2.8 ± 0.2 μg or 2.0 ± 0.3 μg for the thin or thick $PSiO_2$ carriers, respectively (corresponding to a loading efficacy of 90% and 65% (w/w), respectively), as

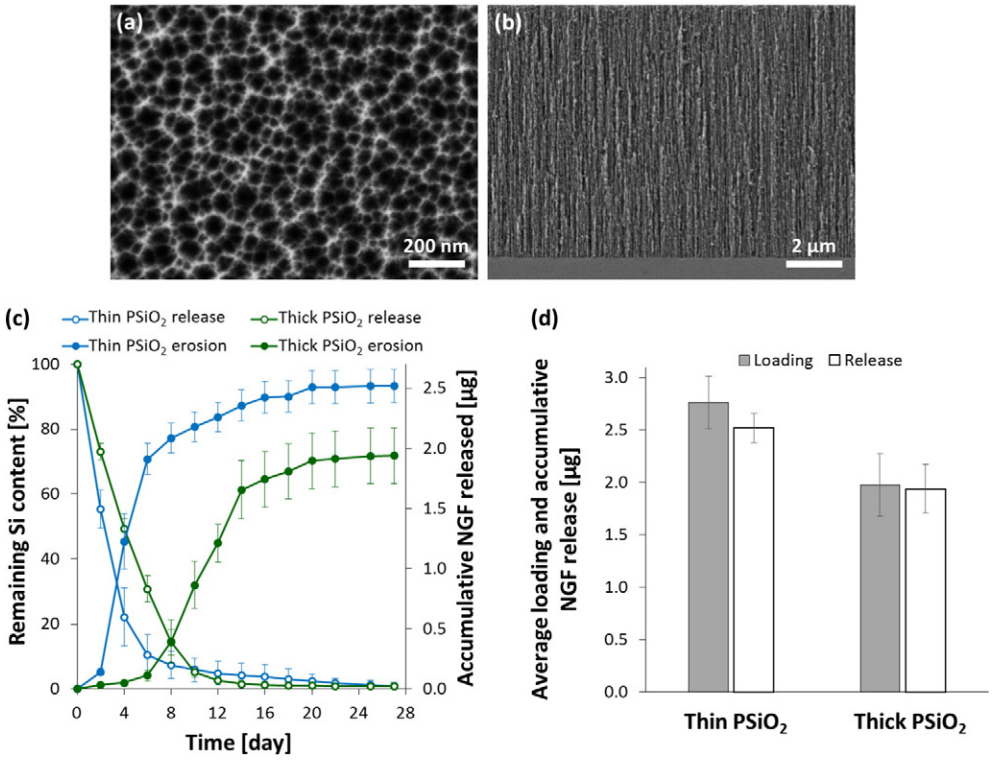

Fig. 1. Characterization of $PSiO_2$ films and their loading and release behavior. (a–b) Top-view and cross-section HRSEM images of a typical $PSiO_2$ film etched at a current density of 250 mA/cm^2 for 67 s (referred as thick film), respectively. (c) NGF release and Si degradation profiles of thin and thick $PSiO_2$ carriers. The Si erosion kinetic profile is expressed as a percentage of the total Si contents released. (d) Average values of NGF loading (gray) for the different $PSiO_2$ carriers and the corresponding accumulative values of NGF release (white). Data represent mean ± SD, n = 3.

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