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Design and characterization of biofunctional magnetic porous silicon flakes ☆

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ABSTRACT

Magnetic porous silicon flakes (MPSF) were obtained from mesoporous silicon layers formed by multi-step anodization and subsequent composite formation with Fe oxide nanoparticles by thermal annealing. The magnetic nanoparticles adhered to the surface and penetrated inside the pores. Their structure evolved as a result of the annealing treatments derived from X-ray diffraction and X-ray absorption analyses. Moreover, by tailoring the magnetic load, the dynamic and hydrodynamic properties of the particles were controlled, as observed by the pressure displayed against a sensor probe. Preliminary functionality experiments were performed using an eye model, seeking potential use of MPSF as reinforcement for restored detached retina. It was observed that optimal flake immobilization is obtained when the MPSF reach values of magnetic saturation $>10^{-4}$ A m^2 g^{-1}. Furthermore, the MPSF were demonstrated to be preliminarily biocompatible in vitro. Moreover, New Zealand rabbit in vivo models demonstrated their short-term histocompatibility and their magnetic functionality as retina pressure actuators.

1. Introduction

Porous silicon (PS) emerged in recent decades as a promising material for the development of optoelectronic devices [1–3]. Nevertheless, the biomedical potential of this material for the development of highly biocompatible silicon-based materials [4] was only described several years later. The study of the optimal chemical conditions for the preparation of PS with improved viability for determined tissues is actually a topic under intense research [5].

PS possesses many outstanding properties beyond its biocompatibility, such as its high surface area and luminescence in the visible range, both of which can be tuned by varying pore morphology and total porosity. Recently, strategies have been developed to obtain PS particles from formerly prepared PS layers [6]. A huge number of methods have been described to obtain PS particles with very different morphologies and sizes: spheres [6,7] or irregular shapes [8] at very different length scales [9].

In the biological area, PS particles have been obtained to perform dermatological functions in light-absorption-based hyperthermia [10] or in ophthalmological applications as optical probes [11]. Furthermore, some works describe the use of PS particles as a food additive [12] or as a drug carrier [13].

The properties of the particles produced in the current work have been designed to support, by a magnetic sandwich, the fixation of repaired detached retina. In fact, retinal detachment is a pathology that affects more than 10 million people every year with a rate of recidivism of ~40% [14]. Moreover, the post-operative period requires a very specific and uncomfortable posture. This post-operative period usually takes several weeks and causes both social and economic problems.

The current work presents the preparation and properties of magnetic porous silicon flakes (MPSF). These flakes were designed to fit a particular mechanical requirement in the internal eye, supported by an external permanent magnet. The physical, chemical and functional features were studied as well as the cytotoxicity and inflammatory response in both in vitro and in vivo assays.

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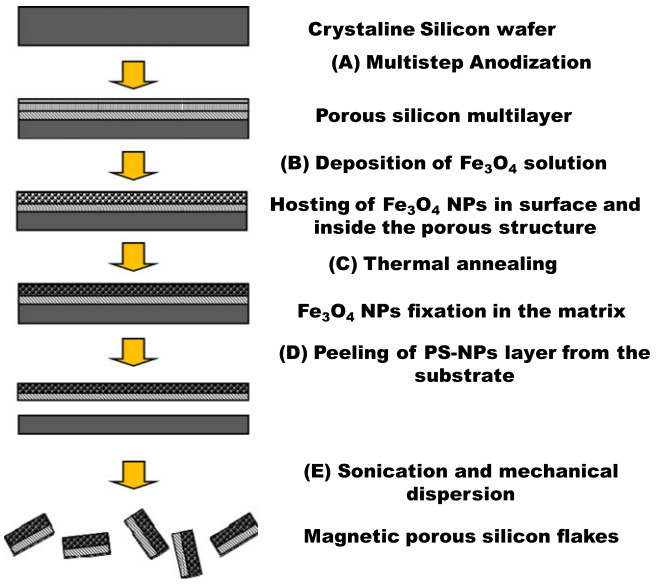

Fig. 1. Scheme of the fabrication process of the MPSF.

2. Materials and methods

2.1. Fabrication of MPSF

MPSF were fabricated by a multistep process, which is summarized in Fig. 1. The first step (A) consists in the preparation of a multilayered PS host matrix. The PS matrix is obtained by the anodization of a crystalline p-type (100) oriented silicon wafer in a HF:ethanol (1:2, from commercial aqueous 48% HF) solution. The anodization is carried out controlling the current density applied between the electrodes in an electrochemical cell with a Pt electrode as cathode. The selection of current density values allows tailoring of the porosity of the PS layer according to studies performed by both optical and Rutherford backscattering measurements [15–18]. A multilayer of different porosities is achieved by creating an anodization program consisting in several steps of different current density (porosity) and time (layer thickness). For the preparation of MPSF, a program consisting of four steps is prepared: 100 mA cm^{-2} for 100 s, 125 mA cm^{-2} for 200 s, 80 mA cm^{-2} for 400 s and 150 mA cm^{-2} for 10 s. The first top layer is designed with a porosity in the 60% range and pore diameter 40 nm (pore size and total porosity optimized according to previous studies [18]) to open the path to magnetic nanoparticles (MNP), which can accumulate preferably in the second layer with an estimated 5–10% higher porosity and an equivalent pore size [17,18]. The third layer presents a lower porosity to act as mechanically stable scaffold for the flake structure. A final layer, obtained just below the electropolishing limit, is a sacrificial layer, allowing the removal of the whole multilayer system from the substrate. The silicon substrate is placed horizontally, and a 40 μl droplet of the Fe_3O_4 nanoparticle solution (10 nm average diameter, oleic acid as surfactant with a relative content <1% and toluene as solvent; Sigma-Aldrich) is spread over the PS surface (B). This droplet is left to dry, giving rise to a stable structure, after which the droplet deposition can be repeated several times to increase the MNP load in the PS layer. After the MNP loading, the system is annealed in an open atmosphere for 2 h at temperatures ranging from 100 to 300 °C (C). This annealing removes the solvent and organic surfactants of the MNP and oxidizes the PS, favoring the fixation of the MNP inside the matrix and enhancing the biocompatibility of PS according to previous reports [1–7]. The last preparation step consists in peeling the system from the silicon wafer (D) by immersion in the solvent (distilled water). The surface tension is high enough to lift the whole layer. The fragmentation of the detached layer in flakes is finally achieved by sonication in water (E). Once this first suspension is obtained, a magnetic separation is carried out with a permanent magnet, to separate the MPSF from non-magnetic flakes. The remaining suspended particles are pipetted away, and the equivalent volume replaced by distilled water. This process is repeated three times to remove non-magnetic flakes. A final separation cycle and subsequent dispersion in physiological saline solution is performed for further bioassays. For all the magnetic characterization, functionality assays and bioassays, the MPSF were prepared by the method described, setting an annealing temperature of 200 °C.

2.2. Characterization

Scanning electron microscopy (SEM) images were acquired in a Hitachi S-3000N scanning electron microscope equipped with a conventional thermionic filament. The operating voltage was set to 20 keV. Qualitative chemical information was retrieved by energy dispersive X-ray analysis (EDX). Field emission scanning electron microscopy (FESEM) images were obtained in a XL 30S-FEG (Philips) instrument. No metallization was required to observe the samples.

X-ray absorption spectroscopy (XAS), X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) spectroscopy measurements at the Fe K-edge energy were performed at room temperature in fluorescence mode at the BM25 Spanish CRG Beamline (SpLine) of the European Synchrotron Radiation Facility. An INCA 13-element X-ray detector was used to measure in fluorescence mode. A metallic Fe foil and FeO, alpha-Fe_2O_3, gamma-Fe_2O_3 and Fe_3O_4 powders were measured as references. Data were normalized, applying the same normalization parameters for all the spectra by means of Athena Software [19].

X-ray diffraction (XRD) measurements were performed in an X'Pert PRO-Panalytical, with a graphite secondary monochromator. Cu K_α radiation was used in $\theta/2\theta$ configuration, obtaining diffractograms in the 40–100° range with a step of 0.02° and 5 s integration time.

Alternating gradient field magnetometry (AGFM) was performed by a Micromag 2900 alternating gradient magnetometer system (Princeton Measurements Corporation). Nine measurements were taken for each sample at room temperature. Measurements were carried out applying a magnetic field from −100 mT to 100 mT, a time pass of 100 ms, and a field pass of 800 μT.

2.3. Evaluation of the magnetic functionality

In order to evaluate the magnetic response of MPSF, commercial permanent magnets of NdFeB (Magnet Experts Ltd., model F3600, 42 MGOe max. energy, 200 ± 10 mT performance, Ni–Cu–Ni coating and 80 °C max. temperature performance) as well as two different types of particles were used: commercial (Chemicell, 2 μm diameter, maghemite core and spherical non-porous silica shell (MCSP)) and MPSF.

For testing the biomechanical behavior of the MPSF, some experiments were performed to estimate how much pressure they could exert over the retina. This characterization was performed using a previously described pressure sensor [20]. To perform the tests, 20 μl of MPSF and MCSP, both in a concentration of 25 mg ml^{-1}, were applied with a micropipette over the sensor. In order to emulate the interaction between particles and the permanent magnets in the rabbit's eye, the magnet was placed at different distances from the bottom side of the pressure sensor (distances below, similar and above the separation existing between the sclera and the retina, i.e.,<1, ~2.5 and >7 mm).