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A novel pressed porous silicon-polycaprolactone composite as a dual-purpose implant for the delivery of cells and drugs to the eye

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ABSTRACT

Dysfunction of corneal epithelial stem cells can result in painful and blinding disease of the ocular surface. In such cases, treatment may involve transfer of growth factor and normal adult stem cells to the ocular surface. Our purpose was to develop an implantable scaffold for the delivery of drugs and cells to the ocular surface. We examined the potential of novel composite biomaterials fabricated from electrospun polycaprolactone (PCL) fibres into which nanostructured porous silicon (pSi) microparticles of varying sizes (150–250 μm or <40 μm) had been pressed. The PCL fabric provided a flexible support for mammalian cells, whereas the embedded pSi provided a substantial surface area for efficient delivery of adsorbed drugs and growth factors. Measurements of tensile strength of these composites revealed that the pSi did not strongly influence the mechanical properties of the polymer microfiber component for the Si loadings evaluated. Human lens epithelial cells (SRA01/04) attached to the composite materials, and exhibited enhanced attachment and growth when the materials were coated with foetal bovine serum. To examine the ability of the materials to deliver a small-drug payload, pSi microparticles were loaded with fluorescein diacetate prior to cell attachment. After 6 hours (h), cells exhibited intracellular fluorescence, indicative of transfer of the fluorescein diacetate into viable cells and its subsequent enzymatic conversion to fluorescein. To investigate loading of large-molecule biologics, murine BALB/c 3T3 cells, responsive to epidermal growth factor, insulin and transferrin, were seeded on composite materials. The cells showed significantly more proliferation at 48 h when seeded on composites loaded with these biologics, than on unloaded composites. No cell proliferation was observed on PCL alone, indicating the biologics had loaded into the pSi microparticles. Drug release, measured by ELISA for insulin, indicated a burst followed by a slower, continuous release over six days. When implanted under the rat conjunctiva, the most promising composite material did not cause significant neovascularization but did elicit a macrophage and mild foreign body response. These novel pressed pSi–PCL materials have potential for delivery of both small and large drugs that can be released in active form, and can support the growth of mammalian cells.

1. Introduction

Porous silicon (pSi) shows potential as a biocompatible scaffold for applications in orthopaedics (Li et al., 1998; Coffer et al., 2005; Whitehead et al., 2008; Anderson and Olsen, 2010), oncology (Zhang et al., 2005; Mann et al., 2011; Park et al., 2011) and ophthalmology (Low et al., 2006, 2009; Cheng et al., 2008; Kashanian et al., 2010). For use in ophthalmic implants designed to deliver drugs or cells to the eye, the advantages of pSi include its large surface area for drug-loading and cell attachment, and good

Abbreviations: BSS, ophthalmic balanced salt solution; DMEM, Dulbecco's Modified Eagle's Medium; EGF, epidermal growth factor; FBS, foetal bovine serum; FDA, fluorescein diacetate; PBS, phosphate buffered saline; PCL, polycaprolactone; pSi, nanostructured porous silicon; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

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ocular biocompatibility (Low et al., 2009; Kashanian et al., 2010). However, pSi is somewhat inflexible, is opaque, and shards of the membrane form of the material exhibit sharp edges. We have previously demonstrated that a composite material comprising a soft polymer such as polycaprolactone (PCL) encasing pSi particles mitigated some of these issues (Kashanian et al., 2010). Nevertheless, challenges with drug loading and release remained. Here, we describe a novel dual-function composite of pSi and PCL, pressed pSi–PCL. The composite consists of an electrospun (non-woven) PCL fabric with nanostructured pSi microparticles pressed into the outside of the fibres. An advantage of the newer composites is that drugs, including biologics such as proteins and peptides, can be loaded into the pSi particles (McInnes et al., 2015) *after* fabrication of the PCL material, thereby avoiding exposure to heat and solvents.

Our focus is on disorders that result in loss or dysfunction of the adult corneal epithelial stem cells normally residing at the limbus, at least in humans (Notara et al., 2010; Ordonez and Di Girolamo, 2012; Joe and Yeung, 2014). A number of biomaterials have been assessed as scaffolds (Feng et al., 2014) for transfer of corneal epithelial stem cells and transient-amplifying cells to the ocular surface (Joe and Yeung, 2014), and silk fibroin shows promise for corneo-limbal reconstruction (Harkin et al., 2011; Bray et al., 2012). We aimed to engineer a biomaterial suitable for ocular surface repair. Herein, we assessed the loading and release of both small molecule and macromolecular drugs into pressed pSi–PCL composite materials, and their ability to support mammalian cell attachment and growth. Implantation of the material beneath the conjunctiva of rats was performed to assess biocompatibility.

2. Materials and methods

2.1. Reagents

Ophthalmic balanced salt solution (BSS) was obtained from Alcon Laboratories Inc (Fort Worth, TX, USA). Dulbecco's Modified Eagle's Medium (DMEM) was sourced from JRH Bioscience (Lanexa, KA, USA). L-glutamine, penicillin, streptomycin sulphate, insulin, transferrin and sodium selenite were all obtained from Invitrogen (Mount Waverley, VIC, Australia). Epidermal growth factor (EGF) was obtained from ProSpec-Tany TechnoGene Ltd. (Ness-Ziona, Israel). Chloroform, fluorescein diacetate (FDA) and ethanol were obtained from Sigma Chemical Co. (St Louis, MO, USA). Foetal bovine serum (FBS) was obtained from Bovogen Biologicals (Essendon, VIC, Australia). Hoechst 33342 dye was purchased from Molecular Probes (Eugene, OR, USA).

2.2. Fabrication of pressed pSi–PCL composites

PCL was obtained from Aldrich (Milwaukee, WI, USA). Nonwoven fabrics of PCL were prepared as described previously (Kashanian et al., 2010; Fan et al., 2011). Briefly, a 25% by weight solution of PCL (MW 65 kDa) in chloroform was placed in a 5 ml glass syringe fitted with a 21 gauge needle, with an applied potential of 20 kV and tip to grounded Al collector distance of approximately 20 cm. Samples of pSi were generously provided by Professor L Canham and Dr A Loni, pSiMedica Ltd. Films of pSi were first produced by electrochemical anodization of p-type (0.01–0.02 Ohm cm) single-crystal silicon wafers in hydrofluoric acid, followed by separation of a pSi membrane from the bulk Si and subsequent milling of the membrane. The particles were sieved to two size ranges: 150–250 μm, and <40 μm. For fabrication of composite materials, two different methods were evaluated. *Method A*. To embed pSi microparticles in the outer surface of microfibres of PCL, the particles were warmed to a temperature above the polymer melting point, followed by brief exposure to the PCL fabric, forcing a modest blending between the contacting interfaces. Specifically, pSi particles were heated in an oven at 110 °C, then immediately transferred to a glass plate. A 1 × 1 cm^2 piece of PCL fabric was physically pressed on to the hot particles. The hot particles caused localized melting of the PCL fibres and thus resulted in the pSi being partially embedded in the polymer. Loadings of the order of 5–6% pSi by mass were obtained, with retention of PCL fibre morphology. *Method B*. Improved embedding of pSi microparticles in the PCL was achieved by the brief addition of chloroform to pSi microparticles after removal from the oven, resulting in a surface etch/dissolution of the PCL surface and better adhesion of the pSi particles to the PCL matrix. This processing step was followed by an additional electrospinning event, to physically entrap more pSi into the composite. Specifically, pSi particles were heated in an oven at 220 °C for 10 min (min), then transferred to a cold glass plate. One drop of chloroform was added to the pSi and quickly mixed. After ~10 s, a 1 × 1 cm^2 piece of PCL fabric was physically pressed on to the moist pSi particles. An additional thin layer of PCL fibres was then generated on to the pSi–PCL, resulting in the formation of a "net". Loadings of the order of 32–34% pSi by mass were obtained, with retention of PCL fibre morphology.

2.3. Characterization by electron microscopy

Nanoporous features in individual pSi microparticles were imaged by transmission electron microscopy (TEM) using a JEOL JEM 2100 and by scanning electron microscopy (SEM) using a JEOL JSM 6400. Fibre diameter and scaffold morphology in the pSi–PCL composites were also characterized by SEM.

2.4. Characterization of biomaterial tensile strength

Evaluation of tensile strength was carried out on selected pSi–PCL composites (as well as PCL fibre only controls) with an approximate cross-sectional area of 1 × 10^{-6} m^2 using a Hampden H-6310 tension-testing machine (Hampden Engineering Corp., East Longmeadow, MA, USA).

2.5. Small molecule loading into pSi microparticles and quantification of drug release

For loading of FDA, 10 mg of pSi microparticles were immersed in a solution of 250 g/L FDA in chloroform for 1 h at 70 °C. Percent loading of FDA by mass was assessed by thermogravimetric analysis (TGA) using a Seiko Instruments TG/DTA 220 unit. To quantify FDA release (Mogal et al., 2014), FDA-loaded pSi microparticles were immersed in 100 μL of PBS pH 7.4. Samples were assayed in triplicate and stored in an incubator at 37 °C between sampling events. For each sampling event, the microparticles were centrifuged, and the entire supernatant was withdrawn for analysis and replaced with 100 μL of fresh PBS. The sample to be tested was diluted into 900 μL of 0.1 M NaOH and the fluorescein peak at 490 nm was read on a NanoDrop UV–Vis (Thermo Scientific, Wilmington, DE, USA) under ambient conditions. The total FDA concentration was determined by summation of the 490 nm readings at all time-points. The earlier time points were then presented as a percentage of total accumulated release.

2.6. Cell attachment and culture on pSi–PCL composite material

Pieces of composite material were sterilised by immersion in 70% ethanol for 5 min, then washed three times with sterile Dulbecco's A phosphate buffered saline (PBS), pH 7.2. The pieces were then transferred into sterile 96-well plates (Becton Dickinson Labware, Franklin Lakes, NJ, USA) and held in place by