Journal of Controlled Release 214 (2015) 23–29

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Layered superhydrophobic meshes for controlled drug release

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ARTICLE INFO

Article history:
Received 16 April 2015
Accepted 30 June 2015
Available online 6 July 2015

Keywords:
Drug delivery
Superhydrophobic
Electrospinning
Lung cancer
Electrospun mesh

ABSTRACT

Layered superhydrophobic electrospun meshes composed of poly(ε-caprolactone) (PCL) and poly(glycerol monostearate-co-ε-caprolactone) (PGC-C18) are described as a local source of chemotherapeutic delivery. Specifically, the chemotherapeutic agent SN-38 is incorporated into a central 'core' layer, between two 'shield' layers of mesh without drug. This mesh is resistant to wetting of the surface and throughout the bulk due to the pronounced hydrophobicity imparted by the high roughness of a hydrophobic polymer, PGC-C18. In serum solution, these meshes exhibit slow initial drug release over 10 days corresponding to media infiltrating the shield layer, followed by steady release over >30 days, as the drug-loaded core layer is wetted. This sequence of events is supported by X-ray computed tomography imaging of a contrast agent solution infiltrating the mesh. In vitro cytotoxicity data collected with Lewis Lung Carcinoma (LLC) cells are consistent with this release profile, remaining cytotoxic for over 20 days, longer than the unlayered version. Finally, after subcutaneous implantation in rats, histology of meshes with and without drug demonstrated good integration and lack of adverse reaction over 28 days. The drug release rates, robust superhydrophobicity, in vitro cytotoxicity of SN-38 loaded meshes, and compatibility provide key design parameters for the development of an implantable chemotherapeutic-loaded device for the prevention of local lung cancer recurrence following surgical resection.

1. Introduction

Lung cancer is the most commonly diagnosed cancer and the most common cause of cancer deaths, with more than 1.61 million new diagnoses and 1.38 million deaths worldwide [1]. For the roughly 80,000 patients diagnosed with early-stage (stages I and II) disease each year in the U.S. [2], surgery is the most effective treatment, whereas chemotherapy is the primary treatment for later stage patients [3,4]. Surgeons must remove the cancer while preserving as much lung tissue as possible, particularly in cases where lung function is limited. In these cases, a wedge of tissue is removed including a small rim of lung tissue around the cancer. This results in a "limited margin" between the tumor and the resection line, with a subcentimeter margin correlating with an increased risk of local cancer recurrence [5]. The prognosis of recurrent NSCLC is extremely poor, and surgery to remove recurrent disease is rarely performed due to clinical and technical limitations [4]. Overall, patients with early stage I or II, non-small-cell lung cancer (NSCLC) have a five-year local recurrence rate of 23% [6]. Accordingly, a strategy to prevent cancer recurrence at the surgical margin would benefit thousands of patients annually, as shown by the improved outcomes following the application of brachytherapy seeds along the surgical margin at the time of limited surgical resection. In stage IA NSCLC patients with larger (2–3 cm) tumors, this treatment significantly increased survival following surgery (from 44.7 to 70 months) [7]. However, clinical acceptance of brachytherapy has been limited by the concerns of radiation exposure to health care professionals and the technical and regulatory challenges of this approach [8]. Building off this success, our goal is therefore to design an easy to use, regulatory-friendly, chemotherapeutic delivery system to prevent local recurrence.

We envision the use of a drug-loaded buttressing device that is stapled into the resection margin as the wedge resection is performed using a standard surgical stapler. In order to achieve this goal, the following design criteria are required for the drug-loaded buttressing material: a) elute minimal drug during the first 10 days of wound healing; b) subsequently elute drug over several weeks in order to expose any remaining tumor cells to the drug over several cell cycles; c) be readily processed into a polymeric structure that can be stapled into tissue by the surgeon; and d) not elicit an adverse reaction after implant. We are exploring two potential form-factor solutions for this unmet clinical need — films [9,10] and meshes [11,12]. Both form-factors are

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http://dx.doi.org/10.1016/j.jconrel.2015.06.042
0168-3659/

composed of hydrophobic, biocompatible, and biodegradable polymers poly(glycerol monostearate-co-ε-caprolactone) or PGC-C18, and poly(ε-caprolactone) or PCL, to prolong the delivery of anticancer agents. The former represents a cast film of a drug loaded polymer solution on a collagen buttressing material used to prevent air-leaks. In contrast, and as a means to further control drug delivery, meshes are electrospun into a fibrous morphology where the porosity and inherent hydrophobicity create a superhydrophobic material that slows wetting and subsequent drug release. Thus, these meshes offer the potential benefit of being both a lung buttressing material as well as a delivery device to tailored drug release, which can be fabricated in a single processing step.

Superhydrophobicity is a property of several natural materials such as lotus leaves, water strider legs, and gecko feet [13,14]. These surfaces possess micrometer and/or nanometer features, resulting in roughness that magnifies their hydrophobic character by creating an energetically unfavorable increase in liquid-air surface area before wetting. If the apparent contact angle (θ^*) exceeds 150°, the materials are commonly called superhydrophobic [13]. When roughness is increased sufficiently, the air–liquid interface is stabilized, which inhibits or stops wetting, and the material enters what is known as the Cassie–Baxter state. Superhydrophobic materials are useful in applications such as nonfouling coatings, low-drag surfaces, and microfluidics [15–25]. In our case, we are interested in 3D bulk structures (i.e., meshes) composed of layers of electrospun fibers that are superhydrophobic throughout. In contrast to most other approaches [26] to superhydrophobic materials, our design does not depend on indefinite maintenance of the Cassie–Baxter state; instead it is designed to be metastable with a controlled wetting rate where fibers in the core release drug once wetted.

Control of drug release from a porous superhydrophobic material has been demonstrated in earlier papers [11,12] from our group using SN-38 and CPT-11, and by the Lynn group using hydrophilic small molecules TMR and 2-ABI [27]. Layered electrospun meshes have been designed for sequential and delayed drug release, but without intent to use superhydrophobicity as a means of control [28,29]. Building off of these results we are evaluating layered superhydrophobic material for controlled drug release. As drug release will be dependent on wetting, we hypothesized that by controlling the superhydrophobic metastable state the rate of wetting will be controlled (and thus drug release). In this study we report the design, fabrication, and evaluation of layered electrospun polymeric meshes containing a chemotherapeutic agent within the fibers of a central core layer surrounded by layers of unloaded fibers. This design was intended to slow initial drug release, while later providing robust delivery of local chemotherapy. In this study we employ a chemotherapeutic agent that has proved difficult to deliver in traditional formulations due to low aqueous solubility: 7-ethyl-10-hydroxycampothecin (SN-38). SN-38 is the active metabolite of irinotecan, which is used clinically in the treatment of colon, rectal, and lung cancer but is 1000 fold less active than SN-38 [30–32]. Specifically, we describe the fabrication of layered meshes, the resistance to mesh wetting as measured by X-ray CT imaging, the elution of a sustained and controlled amount of SN-38 in saline and serum solutions under both static and agitated conditions, the cytotoxic activity against lung cancer cells in vitro, and the results from a 28 day subcutaneous implant biocompatibility study. Finally, we discuss these results in relation to our drug-device design requirements, the potential limitations of this system, and propose solutions for further testing.

2. Materials and methods

Please see the Supplementary Information section for complete details on materials, methods, mesh fabrication, and characterization. For each mesh layer, thickness vs. time was calibrated immediately beforehand by electrospinning a mesh for a known time and measuring thickness. Fiber diameters ranged from 1.4 to 5.3 μm, as detailed in Table S1. The meshes were named as shown in Table 1.

Table 1
Names, compositions, and thicknesses of selected meshes are presented. The subscripts refer to core layer thicknesses in micrometers, and number, for example 30, refers to a polymer blend of 30% PGC-C18 with 70% PCL by mass. The complete listing with contact angles and fiber diameters is shown in Table S1.

Mesh name	Core	Shield
PCL_{90} core	90 μm PCL	None
PCL–PCL_{90}–PCL	90 μm PCL	150 μm PCL
10–PCL_{90}–10 (75 μm)	90 μm PCL	75 μm 10% PGC-C18
10–PCL_{90}–10	90 μm PCL	150 μm 10% PGC-C18
10–PCL_{90}–10 (300 μm)	90 μm PCL	300 μm 10% PGC-C18
30–PCL_{90}–30	90 μm PCL	150 μm 30% PGC-C18
30_{300} core	300 μm 30% PGC-C18	None
30–30_{300}–30	300 μm 30% PGC-C18	150 μm 30% PGC-C18
30–PCL_{300}–30	300 μm PCL	150 μm 30% PGC-C18

3. Results and discussion

As discussed below, our approach to locally tuned chemotherapeutic delivery, with the ultimate goal of preventing local tumor recurrence, entails using a triple-layered electrospun mesh containing an inner drug core with two outer non-drug layers. The resultant control of the wetting rate of the mesh leads to a marked delay in drug release with prolonged kinetics. Given that the average doubling times of rapidly growing NSCLC tumors are reported to be between 46 and 181 days [33–36], it is important that cell-cycle specific drugs such as SN-38 are present for many weeks in order to prevent local growth of any occult tumor cells. Further, low local drug concentrations resulting from systemic chemotherapy are correlated with higher cancer recurrence [37], highlighting the need to increase drug concentration locally via the use of an implantable drug loaded device at the resection margin. Therefore, as a proof of concept for our studies utilizing these superhydrophobic meshes, we have chosen to target 60 days of chemotherapeutic delivery using SN-38 as a model drug. We begin with a discussion of mesh fabrication, characterization, and wettability, followed by drug release from layered and non-layered meshes under static and dynamic conditions in the presence of saline or saline with serum, the cytotoxicity of these meshes in vitro, and, finally, the in vivo response of these meshes after implantation.

3.1. Mesh fabrication and characterization

The meshes were prepared by electrospinning chloroform:methanol solutions of PCL and various mixtures (0, 10 or 30 wt.%) of PGC-C18 (Fig. 1A). For drug-loaded layers, SN-38 was dissolved in the polymer solution at 1 wt.% to polymer and the three layers of mesh (two empty shield layers and one drug load core layer) were fabricated in a continuous manner. The structure of a representative layered mesh is shown in Fig. 1B. Scanning electron microscopy shows the drug-loaded PCL layer (slightly thicker fibers) between two shield layers of 30% PGC-C18 on the left and right. The two compositions (unloaded and drug loaded) are co-electrospun for 15 s between layers to minimize delamination. The apparent contact angle of the different mesh formulations as measured with deionized water is shown in Fig. 2, where the upper points (solid symbols) indicate advancing contact angles which increase as a function of more PGC-C18 content and smaller fiber diameter. The lower set of points (open symbols) denotes the receding angles, and a similar trend is observed. The vertical lines represent the difference between the two angles, hysteresis, which decreases with higher PGC-C18 content. Hysteresis is caused by "asymmetry of wetting and dewetting and the irreversibility of the wetting–dewetting cycle" [13], and greater values indicate a less robust Cassie–Baxter state.

3.2. Drug release into saline and serum solutions

The first set of drug release studies tested the effect of shield layer thickness on SN-38 release. A 90-μm thick core of PCL (PCL_{90} core)