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Prevention of lung cancer recurrence using cisplatin-loaded superhydrophobic nanofiber meshes

Jonah A. Kaplan [a, b, c], Rong Liu [d], Jonathan D. Freedman [a, b, c], Robert Padera [e], John Schwartz [f], Yolonda L. Colson [d, **], Mark W. Grinstaff [a, b, c, *]

[a] Department of Biomedical Engineering, Boston University, 590 Commonwealth Ave., Boston, MA 02215, USA
[b] Department of Chemistry, Boston University, 590 Commonwealth Ave., Boston, MA 02215, USA
[c] Department of Medicine, Boston University, 590 Commonwealth Ave., Boston, MA 02215, USA
[d] Department of Surgery, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115, USA
[e] Department of Pathology, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA
[f] AcuityBio Corp., 200 Upland Rd., Newton, MA 02460, USA

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ABSTRACT

For early stage lung cancer patients, local cancer recurrence after surgical resection is a significant concern and stems from microscopic disease left behind after surgery. Here we apply a local drug delivery strategy to combat local lung cancer recurrence after resection using non-woven, biodegradable nanofiber meshes loaded with cisplatin. The meshes are fabricated using a scalable electrospinning process from two biocompatible polymers—polycaprolactone and poly(glycerol monostearate-*co*-caprolactone)—to afford favorable mechanical properties for use in a dynamic tissue such as the lung. Owing to their rough nanostructure and hydrophobic polymer composition, these meshes exhibit superhydrophobicity, and it is this non-wetting nature that sustains the release of cisplatin in a linear fashion over ~90 days, with anti-cancer efficacy demonstrated using an *in vitro* Lewis Lung carcinoma (LLC) cell assay. The *in vivo* evaluation of cisplatin-loaded superhydrophobic meshes in the prevention of local cancer recurrence in a murine model of LLC surgical resection demonstrated a statistically significant increase ($p = 0.0006$) in median recurrence-free survival to >23 days, compared to standard intraperitoneal cisplatin therapy of equivalent dose. These results emphasize the importance of supplementing cytoreductive surgery with local drug delivery strategies to improve prognosis for lung cancer patients undergoing tumor resection.

1. Introduction

Lung cancer is the leading cause of cancer deaths in North America, with over 200,000 new cases diagnosed each year and a dismal 5-year survival rate of ~18% [1,2]. These survival rates improve to 54% if no metastatic disease is evident at the time of surgery, making surgery to remove the primary tumor the treatment of choice in ~50,000 cases/year. Compared to surgical outcomes for early-stage breast and prostate cancer where 5-year survival rates are 90 and 99%, respectively, early-stage lung cancer survival is surprisingly poor [2]. One of the factors contributing to poor survival is the inability of many patients to tolerate a "wide" local excision of their tumor, i.e. lobectomy, since removal of the estimated 25% of total lung function further compromises already limited pulmonary function. Lesser, i.e. wedge, resections save lung parenchyma but are associated with a two-fold increase in local cancer recurrence as a result of the microscopic disease remaining at the surgical resection margin [3,4]. This is a critical decision since current 2-year survival in patients that develop recurrence drops to ~20% [5] as the majority of these patients are not candidates for additional surgery, and radiation and/or chemotherapy are largely palliative [6]. Platinum-based DNA-adducting agents, such as cisplatin, are the current standard-of-care chemotherapy for lung cancer [7,8]. Although these agents have dose-limiting side-effects such as nephrotoxicity [9] and neurotoxicity [10] with systemic

* Corresponding author. Department of Biomedical Engineering, Boston University, 590 Commonwealth Ave., Boston, MA 02215, USA.
** Corresponding author. Department of Surgery, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA.
E-mail addresses: ycolson@partners.org (Y.L. Colson), mgrin@bu.edu (M.W. Grinstaff).

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administration, the use of cisplatin has achieved some improvement in overall survival for lung cancer patients with metastatic disease. Cisplatin has also been used in combination therapy [7,8,11,12] to achieve a wider therapeutic window and hence better clinical outcomes. Given the limitations in cisplatin dosing, several clinical trials have been or are currently being conducted to improve lung cancer outcomes using cisplatin in conjunction with other agents such as 5-fluorouracil [13], gemcitabine [14], and targeted therapies involving small-molecule inhibitors or monoclonal antibodies [15–18].

Other approaches to improve cisplatin efficacy *in vivo* are also being investigated. Nanoparticles and local drug delivery strategies such as chemotherapy-loaded films, foams, and gels are all being developed to improve drug uptake while minimizing systemic side effects [19]. In particular, cisplatin-loaded nanoparticles have been evaluated in several clinical trials with promising results [20,21], and other cisplatin drug delivery materials such as gels [22], films [23], and glues [24] designed for local administration are gaining traction in the fight against lung and related thoracic cancers. However, many local and systemic drug delivery systems possess burst release kinetics, which exposes drugs to tumors for only a short duration and highlights the need for improved designs for sustained-release chemotherapy depots.

We have recently reported the fabrication of 3-dimensional superhydrophobic microfiber meshes that utilize the metastable air barrier within these porous materials to drastically slow wetting and thereby sustain the release of encapsulated 7-ethyl-10-hydroxycamptothecin [25], an experimental lipophilic anticancer agent, for several weeks. Given the central role of cisplatin therapy in the treatment of lung cancer, this report focuses on our efforts using superhydrophobic materials to deliver this hydrophilic drug. Specifically, the current report describes the fabrication of cisplatin-loaded, three dimensional nanofiber meshes; demonstrates the sustained release of cisplatin *in vitro*; and applies the favorable physical and mechanical properties of these biodegradable meshes to an *in vivo* surgical model of aggressive, early-stage lung cancer and local post-surgical cancer recurrence.

2. Materials and methods

2.1. Chemicals & reagents

Polycaprolactone (MW 70–90 kg/mol), cisplatin (≥99.9%), dichloromethane (DCM, reagent grade ACS), anhydrous N,N-dimethylformamide (DMF, 99.8%), diatomaceous earth (Celite® 545), stearic acid (95%), N,N′-dicyclohexylcarbodiimide (DCC, ≥99.9%), toluene (anhydrous, 99.8%), tin(II) ethylhexanoate (~95%), ε-caprolactone (97%), nitric acid (60–70%), and Triton™-X 100 were purchased from Sigma–Aldrich and used as received. Methanol and tetrahydrofuran (THF) were of reagent grade and purchased from Pharmaco-Aaper. Palladium on carbon (Pd/C, 10% on activated wood carbon, unreduced, ~50% water wet paste) was purchased from Strem Chemicals. DMEM and penicillin/streptomycin were purchased from Gibco, and fetal bovine serum was purchased from Atlanta Biologicals.

2.2. Polymer synthesis

Poly(caprolactone-*co*-glycerol-monostearate) (PGC-C_{18}) was synthesized according to literature procedure [26]. Briefly, the monomer *5*-benzyloxy-*1,3*-dioxan-*2*-one and ε-caprolactone were reacted at 140 °C for 20 h in a ring-opening polymerization catalyzed by tin(II) ethylhexanoate (0.005 eq.). The polymer was cooled, dissolved in DCM, and precipitated into cold methanol (90% yield). After filtration, the polymer was dissolved in THF and hydrogenated at 50 psi for 4 h at room temperature using Pd/C. The catalyst was filtered out through a bed of diatomaceous earth and precipitated into cold methanol (93% yield). Next, stearic acid (1.5 eq.) was grafted onto the free secondary hydroxyl group using standard N,N′-dicyclohexylcarbodiimide (DCC) coupling for 18 h. The mixture was filtered and concentrated three times before precipitating twice into cold methanol, and dried under high-vacuum for 24 h (88% yield). Molecular weight was determined using size exclusion chromatography with a Polymer Laboratories PLgel MIXED-E column (3-μm bead size) and a Rainin HPLC solvent delivery system using THF as the eluent (1.0 mL/min, 25 °C). PGC-C_{18} molecular weight (M_n) was calculated at ~40,000 g/mol (M_w/M_n = 1.5) using polystyrene calibration standards (Polysciences, Inc.). ^{1}H NMR of the polymer agreed with previous reports [26,27].

2.3. Biocompatibility studies of PGC-C_{18}

Biocompatibility testing of PGC-C_{18} involved a series of *in vitro* and *in vivo* studies conducted according to ISO-10993 and FDA G95-1 guidelines. These tests were performed under GLP conditions at Toxikon, Inc. with appropriate protocols and assurances in place.

2.4. Electrospinning

Dichloromethane (1.5 mL) was added to a 20-mL glass scintillation vial containing PCL pellets (910 mg) and PGC-C_{18} (390 mg) and allowed to dissolve overnight. DMF (2.5 mL) was then added to this solution and thoroughly vortexed over 12 h. A solution of cisplatin (40 mg in 2.5 mL DMF) was then added to the polymer solution and vigorously mixed. The solution was loaded into a 10-mL glass syringe equipped with an 18 AWG needle. Solutions of PCL only (1.3 g) with 3% (wt/wt) cisplatin, and polymer solutions without drug, were also prepared.

2.5. Mesh characterization

Scanning electron microscopy (Zeiss Supra V55) was performed to assess the morphology of electrospun meshes and determine fiber diameter. Meshes were cut to 0.3 × 0.3 cm^2, mounted on aluminum stubs using conductive copper tape, and imaged at 2 keV. Advancing and receding deionized water contact angle measurements using a goniometer (Kruss DSA100) were performed to characterize the non-wetting nature of meshes (n = 10 per group). Tensile properties of meshes (1.5 cm × 4 cm) were determined using an Instron 5848 tensile testing apparatus at a 1 mm/s elongation rate and a 10N load cell (n = 3 per group). Surgical stapling was performed using an Endo GIA™ Ultra 12-mm single-use short universal stapler (Covidien, Ltd.). A poly(glycolic acid) surgical mesh (NEOVEIL, Gunze Limited) was stapled for comparison.

2.6. Drug release studies

Sink conditions for release experiments consisted of phosphate-buffered saline (PBS) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. A volume of 15 mL was used for meshes (~10 mg), which was replaced weekly, ensuring >10-fold solubility excess for maintaining sink conditions. Meshes and release medium were sealed in 15-mL polypropylene centrifuge tubes and incubated on a shaker in a 37 °C incubator (n = 4 per group). Aliquots (3 × 100 μL) were withdrawn at predetermined time points and frozen at −20 °C until further use. Cisplatin concentration was determined using flameless atomic absorption spectrophotometry (Varian AA240Z) using pyrolytic graphite-coated, partitioned tubes and Zeeman