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Prevention of Lung Cancer Recurrence Using Cisplatin-Loaded Superhydrophobic Nanofiber Meshes

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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

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Author Contributions

J.A.K and M.W.G designed and conceptualized the polymer chemistry, electrospinning, characterization, and *in vitro* experiments. R.L, M.W.G., and Y.L.C. designed the *in vivo* experiments, and R.L performed the surgeries, monitored/collected animal health/data. R.P. performed the histological evaluation of implanted meshes. JAK wrote the original draft of the manuscript, excluding the final analysis and statements on the *in vivo* study, which was written by R.L. All authors provided insight and substantially contributed to successive revisions that improved and finalized the manuscript.

Conflict of Interest

M.W.G declares a conflict of interest through his receipt of consultation fees and equity position in AcuityBio, Inc. J.S owns equity in AcuityBio, Inc as CEO and President.

with local drug delivery strategies to improve prognosis for lung cancer patients undergoing tumor resection.

Keywords

Drug delivery; electrospinning; nanofibers; lung cancer; surgery; superhydrophobic

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 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 Pharmacia-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-C₁₈) 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 hours 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 hours 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 hours. The mixture was filtered and concentrated three times before precipitating twice into cold methanol, and dried under high-vacuum for 24 hours (88% yield). Molecular weight was determined using size exclusion chromatography with a Polymer Laboratories PLgel MIXED-E column (3- μ m bead size) and a Rainin HPLC solvent delivery system using THF as the eluent (1.0 mL/min, 25 °C). PGC-C₁₈ molecular weight (M_n) was calculated at ~40,000 g/mol ($M_w/M_n = 1.5$) using polystyrene calibration standards (Polysciences, Inc.). ¹H NMR of the polymer agreed with previous reports.[26, 27]

2.3 Biocompatibility Studies of PGC-C₁₈

Biocompatibility testing of PGC-C₁₈ 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-C₁₈ (390 mg) and allowed to dissolve overnight. DMF (2.5 mL) was then added to this solution and thoroughly vortexed over 12 hours. 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 $0.3 \times 0.3 \text{ cm}^2$, mounted on aluminum stubs using conductive copper tape, and imaged at 2 keV. Advancing and receding deionized water contact angle measurements using a goniometer (Krüss DSA100) were performed to characterize the non-wetting nature of meshes ($n = 10$ per group). Tensile properties of meshes ($1.5 \text{ cm} \times 4 \text{ cm}$) 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 ($3 \times 100 \text{ µL}$) 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 background subtraction,[28] using a hot pre-injection of Triton™-X 100 (5 µL) at 50 °C.

2.7 Micro-Computed Tomography (µCT) Analysis of Meshes

Microcomputed tomography studies were performed by incubating meshes in PBS supplemented with 10% FBS and 80 mgI/mL iodixanol (GE Healthcare). Meshes were periodically removed from culture and assessed for water infiltration (i.e., contrast agent solution) using a Scanco Medical µCT40 imaging system as previously described,[29] with an isotropic voxel resolution of 36 µm^3 , 70 kVP tube voltage, 114 µA current, and 300 ms

integration time ($n = 3$ per group). Image slices were converted into the standard image format (DICOM) using proprietary software from Scanco Medical. Data were reconstructed and analyzed using a commercial image processing software (Analyze™, Mayo Clinic).

2.8 Cell Culture

The Lewis Lung Carcinoma (LLC) murine cell line was used to demonstrate *in vitro* efficacy of cisplatin-loaded superhydrophobic meshes. Cells were plated in 12-well plates at a seeding density of 3×10^4 cells/well in DMEM supplemented with 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin and grown overnight at 37 °C in a humidified 5% CO₂ incubator. The following day, meshes were added to transwell inserts (200 µm pore size) and rinsed three times with PBS and aspirated to remove the liquid. Cell culture media was then aspirated from the LLC-containing plates, followed by addition of the transwell inserts (Corning). Fresh complete cell culture medium was then added to the bottom of the well (1.0 mL) and on top of the mesh (0.5 mL) to ensure meshes were submerged in media. After 24 hours' co-incubation with meshes, meshes were removed from each well, and cells were incubated an additional 48 hours before aspirating media and assessing for viability using the colorimetric MTS assay ($n = 3$ per group). Meshes were maintained in complete cell culture medium (1.5 mL) when not co-incubated with cells. Fresh LLC cells were plated every 5 or 10 days to assess the sustained release efficacy of cisplatin-loaded meshes. An additional cell assay using AG02262 lung fibroblast cells — performed as similarly described above for LLC cells with 6×10^4 cells/well — was conducted to study the effect of cisplatin-loaded meshes on a healthy lung cell line.

2.9 In Vivo Murine Model of Local Cancer Recurrence

Animal studies were approved by the Institutional Animal Care and Use Committee at Brigham and Women's Hospital. A total of 36 C57BL/6 female mice were injected subcutaneously (s.c) with 7.5×10^5 LLC cells and allowed 2-3 weeks for tumors to reach more than 300 mm³. Tumors were then resected, and mice were subsequently randomized and split into four groups for subsequent treatment: 1) cisplatin-loaded superhydrophobic meshes (1.2×1.2 cm²) at an equivalent dose of 13.2 mg/kg implanted at the time of surgery; 2) two doses of intraperitoneal (i.p) cisplatin in saline (6.2 mg/kg) administered one week apart; 3) unloaded superhydrophobic meshes implanted at the time of surgery, followed by two i.p cisplatin doses (6.2 mg/kg) administered one week apart; 4) unloaded superhydrophobic meshes implanted at the time of surgery, followed by two i.p saline injections administered one week apart ($n = 9$ per group). Mice were monitored for freedom from local recurrence, with local recurrence defined as tumor(s) present anywhere on the back of animals. Data are presented as the mean $\pm$ SEM. A log-rank (Mantel-Cox) test was used to assess efficacy of the various treatment groups for statistical significance in prolonging tumor-free recurrence, and linear regression was used to compare changes of body weights over time between animals in different treatment groups. Prism 5.0 (GraphPad, La Jolla, CA) was used for all statistical analyses. Histological assessment of the tissue response to implanted unloaded and cisplatin-loaded meshes were performed by excision and fixation in 10% formalin, followed by paraffin embedding. Sections (4 µm thick) were stained with hematoxylin and eosin. Images were obtained at 200X magnification using an Olympus BX41 microscope coupled to an Olympus BP70 digital

camera (Olympus America Inc., Center Valley, PA.) Adobe Photoshop software (Adobe system, San Jose, CA) was used to adjust the white balance of the images.

3. Results

3.1 Electrospinning & Mesh Characterization

Nanofiber meshes containing 3% (w/w) cisplatin were successfully prepared from a 20% (w/v) solution blend of 70% polycaprolactone (PCL) and 30% of the low surface energy copolymer poly(glycerol monostearate-*co*-caprolactone) (PGC-C₁₈) (**Figure 1a**) using the electrospinning process. The operational parameters for electrospinning are presented in **Table 1**. Pure PCL meshes were also fabricated under similar parameters. The resulting fiber jet was deposited onto a grounded, rotating (50 rpm) and linear-translating aluminum cylinder, permitting production of meshes up to ~100 cm² (**Figure 1b**). SEM imaging of electrospun meshes depicted their underlying architecture of ~500 nm diameter entangled fibers (**Figure 1c**), which were significantly thinner than those electrospun from a more commonly used solution of 5:1 chloroform:methanol (6 μ m).[30] Contact angle (CA) goniometry studies revealed pure PCL meshes were moderately hydrophobic (CA = 133°), whereas the addition of 30% (w/w) PGC-C₁₈ rendered meshes superhydrophobic (CA >150°). The PGC-C₁₈ copolymer has a lower surface energy than that of PCL, (CA = 120° and 88° on smooth films, respectively, see Figure S1 in the Supplementary Information, SI, available online), and contributed to the superhydrophobic state.

3.2 In Vitro Release Kinetics & Efficacy

The drug release behavior of cisplatin-loaded nanofiber meshes submerged in PBS (supplemented with 10% fetal bovine serum) and mechanically shaken at 37 °C is presented in **Figure 2a**. While pure PCL meshes rapidly released >95% of their cisplatin payload within 24 hours, less than 1% was released from superhydrophobic PCL/PGC-C₁₈ meshes. The release of cisplatin was linear over ~90 days under these conditions, which corresponded to a dose of ~3 μ g cisplatin/day (film mass = 10 mg). Upon wetting the superhydrophobic PCL/PGC-C₁₈ meshes with a 1 second dip in ethanol, the release of cisplatin occurred rapidly, with >90% releasing over 6 hours (see SI, Figure S2). Using a transwell assay,[25] cisplatin-loaded superhydrophobic meshes were efficacious *in vitro* against the Lewis Lung Carcinoma (LLC) cell line for over 70 days, while cisplatin-loaded non-superhydrophobic PCL meshes were cytotoxic for the first day of exposure only (**Figure 2b**). Ethanol-wetted cisplatin-loaded superhydrophobic meshes were only cytotoxic on the first day of exposure and not at later time points (see SI, Figure S2). Cytotoxicity was observed for cisplatin-loaded meshes incubated with AG02262 lung fibroblast cells (85% viability), while drug-free meshes exhibited no signs of cytotoxicity against these cells (see SI, Figure S3).

Bulk wetting of the unloaded meshes was quantified using contrast-enhanced microcomputed tomography (μ CT) and an iodinated contrast agent dissolved in the surrounding medium (**Figure 3**). PCL meshes wetted rapidly in ~1 day (see SI, Figure S3), while superhydrophobic meshes resisted wetting for >60 days. The wetted volume fraction

also increased in an approximately linear fashion ($R^2 = 0.9$) as a function of submersion duration.

3.3 Mechanical Properties & Surgical Stapling Capabilities

The mechanical behavior of meshes was investigated by subjecting specimens to uniaxial tensile testing. Representative stress-strain curves for cisplatin-loaded PCL and PCL/PGC-C₁₈ meshes and their unloaded counterparts are shown in **Figure 4a**. There were no discernable differences in the stress-strain curves among drug-loaded and corresponding unloaded meshes. Compared to PCL meshes, incorporation of 30% PGC-C₁₈ within the PCL meshes afforded a slightly weaker material in terms of ultimate tensile strength (1200 kPa versus 800 kPa, respectively). Meshes were also cut into strips, loaded into a standard surgical stapler (**Figure 4b**), and then stapled (**Figure 4c**). As shown by SEM, the mesh conforms around the staple (**Figure 4d**).

3.4 Biocompatibility Studies

Given that PCL is a biocompatible and widely-used polymer in FDA-approved devices, we performed a series of *in vitro* and *in vivo* safety studies based on ISO-10993 and FDA G95-1 guidelines for PGC-C₁₈. All tests were performed under GLP conditions at Toxikon with appropriate protocols and assurances in place. The results of these tests are as follows:

- Cytotoxicity: Agar Diffusion (ISO 10993-5). No biological reactivity was observed in L929 mammalian cells at 48 hours post-exposure. The polymer is considered non-cytotoxic. **Pass**.
- Genotoxicity: Ames Assay/Salmonella & E coli (ISO 10993-3). No statistical increase in revertant colonies was observed with the PGC-C₁₈ as compared to the negative control. PGC-C₁₈ was not considered mutagenic. **Pass**.
- Intracutaneous Injection (ISO 10993-10). Injection of the NaCl extract of the polymer in rabbits did not show a significantly greater biological reaction (i.e., irritation) than sites injected with the control. **Pass**.
- Hemolysis (ISO 10993-4). The polymer exhibited 0.00% hemolysis above the level of the hemolysis exhibited by the positive control. The polymer is considered non-hemolytic using ASTM method F756-08. **Pass**.
- Kligman Maximization (ISO 10993-10). The polymer extracts did not elicit any significant allergic or sensitizing response in guinea pigs over the 25-day test period. **Pass**.
- Systemic Injection (ISO 10993-11). Injection of the polymer extract did not induce a significantly greater biological reaction in mice than the control extract. **Pass**.
- Abridged 28 Day Sub-Acute Systemic Toxicity by Implant (ISO 10993-11). This test assessed adverse effects after administration of PGC-C₁₈ films for 28 days. This study included 40 rats (20 male, 20 female), subcutaneous implantation of test or control article, daily health observations, weekly body weights and detailed observations, blood collected at termination for hematology and clinical chemistry, gross necropsy with histopathology of implant sites and up to 9 tissues, and body

and organ weights. For all parameters, the implant did not demonstrate any local or systemic signs of toxicity. The bioreactivity rating for the 28 day time period was 0.0, indicating no reaction. *Pass*.

3.5 In Vivo Lung Cancer Recurrence Model

Cisplatin-loaded meshes were evaluated for their efficacy in preventing local cancer recurrence in C57BL/6 mice using a surgical model of cancer that assesses freedom from local recurrence following surgical excision (**Figure 5**).^[31] This model involved establishing LLC tumors ($>300 \text{ mm}^3$) subcutaneously on the backs of mice, followed by surgical resection and treatment. Treatment groups included: 1) a cisplatin-loaded superhydrophobic mesh (13.2 mg/kg equivalent dose) implanted at the resection site; 2) implantation of an unloaded superhydrophobic mesh; 3) implantation of an unloaded superhydrophobic mesh and intraperitoneal (i.p.) cisplatin (2 doses $\times$ 6.6 mg/kg); or 4) only i.p. cisplatin $2 \times$ 6.6 mg/kg. Without chemotherapy, resected control animals developed local recurrences within a median of 6 days (**Figure 6a**). Cisplatin, administered in two 6.6 mg/kg doses, separated by one week due to single dose toxicity limitations,^[32] had a marginal effect in preventing local recurrence (8 versus 6 days for no additional treatment, $p > 0.05$). Likewise, treatment with unloaded meshes and i.p. cisplatin did not significantly increase median freedom from local recurrence. In contrast, the median freedom from local recurrence was significantly increased for mice treated with cisplatin-loaded meshes compared to those treated with i.p. cisplatin (median not reached, $p = 0.0006$). All animals tolerated systemic and drug-loaded mesh treatments, with no statistically significant differences in changes in mean body weight across all groups (linear regression analysis, $p > 0.05$). Histologic evaluation of all mesh specimens revealed a mild chronic inflammatory infiltrate and granulation tissue immediately adjacent to the material. The overlying normal skin and adjacent skeletal muscle showed no evidence of necrosis or other pathologic change as a result of implantation of the mesh. There was no histologic difference in tissue reaction between unloaded and cisplatin-loaded meshes (**Figure 6b** and **Figure 6c**, respectively).

4. Discussion

Given the need to minimize the amount of lung removed and yet achieve histologically negative margins, a local drug delivery approach that supplements cytoreductive surgery with localized, sustained-release chemotherapy may hold promise by eliminating residual, microscopic tumor cells—particularly in patients unable to tolerate more aggressive resection. Such a local drug-delivery approach is currently clinically implemented in the treatment of only one cancer: high-grade malignant glioma.^[33, 34] After cytoreductive surgery, rigid, brittle carmustine (BCNU)-loaded polyanhydride^[35] wafers are placed in the resected tumor beds, with BCNU release occurring over 2-3 weeks.^[36] For patients with newly diagnosed malignant glioma, this treatment increased their median survival to 64.1 weeks, compared to 49.4 weeks with placebo.^[36, 37] Further success has been limited by the short duration of drug release in this delivery system. Unlike static tissues, the functional difference of reversible tissue extension and contraction present on the lung surface necessitates the use of compliant materials for drug delivery. Therefore, the design

characteristics for drug delivery to the lung required a compliant, flexible material with a prolonged release profile.

Accordingly, we previously developed paclitaxel-loaded PGC-C₁₈ films for preventing local cancer recurrence in a similar LLC tumor resection model.[31] These films relied on their hydrophobicity to provide sustained drug release over ~90 days for lipophilic drugs (such as paclitaxel[31]). However, these films lacked the mechanical integrity to serve as buttressing materials themselves and were therefore cast atop secondary scaffolds composed of decellularized bovine pericardium strips. Ideally, this system should be a single component with dual-functionality—providing both buttressing and sustained drug release without reliance on animal-derived components—so as to facilitate and simplify manufacturing, quality control, and regulatory matters.

In order to fabricate an all-in-one drug-device system that could also be stapled into the lung tissue using standard surgical staplers, we selected the technique of electrospinning. Electrospinning is a polymer processing technique[38-40] that generates non-woven fiber meshes and involves the application of high voltage to a polymer solution at the tip of a syringe pump and needle assembly. The balancing of electrostatic repulsion and droplet surface tension results in a jet emanating from the end of the droplet, with subsequent solvent evaporation as this jet whips through the air toward a grounded collector. The common chloroform/methanol solvent system for electrospinning PCL-based meshes was avoided because cisplatin is insoluble in such nonpolar solvents, and solvent-drug compatibility has been shown to affect drug release rates and/or result in poor encapsulation.[41] Therefore, a solvent mixture of dichloromethane/dimethylformamide was used to ensure solubility of both polymers and cisplatin. Cisplatin-loaded meshes were 600 μm thick and composed of fibers approximately 500 nm in diameter. The electrospinning collector apparatus yielded large meshes (100 cm^2) that could subsequently be cut to specific shapes and sizes to facilitate coverage of the entire tumor bed and to allow surgical buttressing and stapling along the tumor margins. Fabricating meshes by electrospinning improves the mechanical flexibility of the PCL and PGC-C₁₈ polymer blend resulting in strong, yet compliant meshes. Meshes can be rolled or twisted without tearing, and their ultimate tensile strengths are greater than those reported for lung tissue[42, 43] and similar to buttressing materials currently used in the operating room to ensure an air-tight seal after stapling to the resected tissue.[44]

Sustained-release of cisplatin is particularly challenging due to its relatively high solubility in aqueous solution (1 mg/mL, $\log P = -2.35$:[45] compared to < 0.01 mg/mL, $\log P = 3.96$ for paclitaxel,[46] for example). An additional challenge is due to the poor solubility of cisplatin in nonpolar solvents, resulting in burst release kinetics and limiting the methods available to fabricate such drug delivery devices. Previously, the longest reported duration of release for cisplatin-eluting electrospun meshes was ~35 days:[47] and even then release behavior was still largely dominated by burst kinetics. In contrast, our approach relies on slowing the wetting rate of the meshes, and thus the rate of drug release, through the use of a metastable superhydrophobic state present within the meshes. Preventing burst release of water-soluble agents using superhydrophobic materials are being actively explored by other

researchers, where these agents have been incorporated into superhydrophobic polymer electrolyte multilayered films.[48]

In the current formulation, cisplatin release is devoid of burst release, and the profile is gradual and linear for >70 days with 3 μg cisplatin released per day from the superhydrophobic PCL/PGC- C_{18} meshes. In contrast, the hydrophobic meshes composed of PCL release their entire payload within the first several days. Likewise, artificially wetting the superhydrophobic meshes with a 70% ethanol/water mixture causes the rapid release of cisplatin, and the kinetics mirror those of the PCL meshes. Importantly, cisplatin-loaded superhydrophobic meshes exhibit cytotoxicity against LLC cells for a similar duration as the release profile, whereas cisplatin-loaded PCL meshes are not cytotoxic by day 5. Moreover, drug release (and associated *in vitro* cytotoxicity) from the superhydrophobic PCL/PGC- C_{18} meshes mirrors the rate and linearity of their wetting, as measured by contrast-enhanced μCT , and indicates drug release is due to mesh hydration. These results suggest the potential to apply this general strategy to several other potent water-soluble drugs used in the treatment of lung and other cancers, such as doxorubicin hydrochloride, gemcitabine, and pemetrexed, and will be the subject of future studies.

The promising anti-cancer efficacy of these meshes against LLC cells *in vitro* prompted evaluation of their efficacy in preventing local lung cancer recurrence in an *in vivo* tumor resection model. The LLC cell line is murine-derived, aggressively tumorigenic, and responds poorly to chemotherapy. This local cancer recurrence surgical model involves inoculating LLC tumors subcutaneously in the backs of wild-type mice, and then performing tumor resection followed by one of several subsequent treatments (cisplatin-loaded mesh, unloaded mesh and i.p. saline, i.p. cisplatin, and unloaded mesh with i.p. cisplatin). Cisplatin-loaded superhydrophobic meshes prevented local tumor recurrence in 5/9 mice (56%), proving superior to all other groups ($p < 0.001$).

Histological evaluation of excised meshes showed a mild chronic inflammatory response, which is typical and acceptable for implanted biocompatible materials. These results are consistent with the findings from the ISO 10993 biocompatibility study performed on the PGC- C_{18} copolymer, and with the known biocompatibility of PCL and PCL electrospun meshes.[49, 50] Cisplatin-loaded superhydrophobic meshes exhibit some cytotoxic to healthy lung fibroblast cells *in vitro* (AG02262), and this result is expected given the release of cisplatin. Importantly, however, gross observation of animals and histology obtained from implanted cisplatin-loaded mesh explants revealed no major adverse events such as wound healing impairment, acute inflammation, or irritation.

The investigation of the degradation and metabolic fate of PCL and PGC- C_{18} polymers in the implanted mesh is important, however, it is beyond the scope of this work as it requires radio-labeled polymers. This will be the focus of a future study. Degradation studies for PCL have been reported by others[51,52] and shown to proceed by hydrolysis. Degradation of PGC- C_{18} is expected to follow a similar mechanism as other aliphatic polycarbonates[53] and poly(ester carbonate) copolymers such as poly(caprolactone-*cotrimethylene carbonate*), whose metabolites consist of carbon dioxide, 1,3-propanediol, and 6-hydroxyhexanoic acid.

Extended to PGC-C₁₈, the expected metabolites would consist of glycerol, stearic acid, carbon dioxide, and 6-hydroxyhexanoic acid.

Taken together, the current study builds upon our prior work investigating local drug delivery devices for the prevention of recurrence following resection of localized lung cancer. The advances identified in the current manuscript are: 1) an improved form factor to a single system that provides tissue buttressing and drug delivery; 2) the use of electrospinning as a scalable manufacturing method; 3) fabrication of a compliant mesh capable of surgical stapling; 4) the successful completion of FDA-required biocompatibility testing on the PGC-C₁₈ copolymer component of these superhydrophobic meshes; 5) identification of a superhydrophobic mesh composition for controlled and sustained delivery of a hydrophilic drug (i.e., cisplatin) over several months; and 6) superior outcomes in an *in vivo* murine tumor resection model using cisplatin-loaded superhydrophobic meshes compared to equivalent systemic dosing. Lastly, GMP production of 1 kg of PGC-C₁₈ was completed at a contract research organization, and this material will enable us to perform the next crucial pharmacokinetic studies to determine the organ distribution of cisplatin after drug-loaded meshes are applied to resected lung tissue in an ovine model, as well as provide key data on any toxicity arising from stapling of the drug-loaded meshes to healthy lung tissue.

5. Conclusions

The increased risk of recurrence following wedge resection for lung cancer is due to the presence of occult microscopic disease remaining after surgery. Thus, to avoid recurrent disease and yet spare as much “normal” lung as possible, new local treatments at the site of tumor resection are being sought. We addressed this challenge by designing a local drug delivery buttressing device for the sustained release of cisplatin. To overcome the burst release kinetics commonly found with cisplatin delivery systems, we fabricated a biodegradable, biocompatible electrospun nanofiber mesh that exhibits bulk metastable superhydrophobicity, and thus is water repellent and porous. The progressive wetting of these meshes over time acts as a gradual, sustained release mechanism for cisplatin. The macroscopic mesh structure provides mechanical stability and flexibility while being amenable to stapling with a standard surgical stapler used in lung resection procedures. Lastly, the positive results of our *in vivo* murine model of lung cancer recurrence reinforce the concept of using principles of drug delivery, polymer chemistry, and biomaterials science to drive the innovation of multifunctional materials for cancer treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Fabrication and characterization of electrospun meshes composed of the biodegradable polyester polycaprolactone (PCL) and the poly(ester carbonate) poly(caprolactone-*co*-glycerol monostearate) (PGCC₁₈). (a) Chemical structure of PCL and PGC-C₁₈ polymers; (b) photograph of a cisplatin-loaded superhydrophobic electrospun mesh; (c) SEM of mesh showing ~500 nm diameter fibers (inset: water droplet on superhydrophobic mesh exhibiting a contact angle of 151°).

Figure 2.

In vitro studies on hydrophobic PCL and superhydrophobic PCL/PGC-C₁₈ meshes; **(a)** cumulative release of cisplatin from PCL/PGC₁₈ meshes in 10% serum; dashed line shows linear best-fit (0.8%/day, or 3 μg/day); inset shows release from PCL meshes in 10% serum (top curve) or PBS (bottom curve) over 4 days; **(b)** *in vitro* efficacy of meshes against Lewis Lung Carcinoma (LLC) cells in complete cell culture medium using a transwell assay (PCL/PGC-C₁₈ meshes, black; PCL meshes, grey).

Figure 3. Contrast-enhanced microcomputed tomography (μ CT) analysis of mesh wetting. (a) Representative μ CT image cross-sections of superhydrophobic meshes at day 7, 56, and 98 (left to right, respectively). (b) Quantification of volume fraction wetted as a function of submersion duration for superhydrophobic PCL/PGC₁₈ meshes ($n = 3$).

Figure 4. Mechanical (tensile) testing and surgical stapling abilities of superhydrophobic nanofiber meshes. **(a)** Stress-strain curves for electrospun meshes consisting of PCL (black) and PCL/PGC-C₁₈ (grey); solid lines = drug-loaded, dashed lines = unloaded. **(b)** Approximate 1-cm wide strips cut from a larger mesh being loaded into a surgical stapler. **(c)** A stapled superhydrophobic mesh (top) and poly(glycolic acid) (clinical analogue) surgical mesh (bottom). **(d)** SEM of stapled mesh (scale bar = 200 μm).

Figure 5.

Treatment groups for *in vivo* tumor-free recurrence study. Female C57BL/6 mice were injected with LLC cells, and tumors were allowed to reach $> 300 \text{ mm}^3$ before performing resection. Animals were randomly assigned to one of four treatment groups and blinded after treatment. Body weight and days free from local recurrence were measured during the course of this study.

Figure 6.

In vivo assessment of freedom from local tumor recurrence (a) Kaplan-Meier curve showing freedom from local recurrence in mice after surgical resection (* $p = 0.0006$, determined using log-rank test); (b, c) Photomicrographs of excised tissue from implant site for an unloaded PCL/PGC₁₈ mesh and a cisplatin-loaded PCL/PGC₁₈ mesh, respectively, showing surrounding mild chronic inflammation and granulation tissue. Tumor is present deep to the unloaded mesh. Arrowheads represent the top and bottom boundaries of the meshes; yellow arrow denotes tumor tissue. Both images are 200X magnification, H&E stained sections.

Table 1

Electrospinning parameters.

Mesh Composition	Voltage (kV)	Flow Rate (mL/h)	Distance (cm)
PCL + cisplatin	16	3	15
PCL/PGC-C ₁₈ + cisplatin	19	3	13
PCL (unloaded)	15	2.5	13
PCL/PGC-C ₁₈ (unloaded)	15	2.5	13