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Rapidly Biodegrading PLGA-Polyurethane Fibers for Sustained Release of Physicochemically Diverse Drugs

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Abstract

Sustained release of physicochemically diverse drugs from electrospun fibers remains a challenge and precludes the use of fibers in many medical applications. Here, we synthesize a new class of polyurethanes with poly(lactic-co-glycolic acid) (PLGA) moieties that degrade faster than polyurethanes based on polycaprolactone. The new polymers, with varying hard to soft segment ratios and fluorobenzene pendant group content, were electrospun into nanofibers and loaded with four physicochemically diverse small molecule drugs. Polymers were characterized using GPC, XPS, and ¹⁹F NMR. The size and morphology of electrospun fibers were visualized using SEM, and drug/polymer compatibility and drug crystallinity were evaluated using DSC. We measured *in vitro* drug release, polymer degradation and cell-culture cytotoxicity of biodegradation products. We show that these newly synthesized PLGA-based polyurethanes degrade up to 65–80% within 4 weeks and are cytocompatible *in vitro*. The drug-loaded electrospun fibers were amorphous solid dispersions. We found that increasing the hard to soft segment ratio of the polymer enhances the sustained release of positively charged drugs, whereas increasing the fluorobenzene pendant content caused more rapid release of some drugs. In summary, increasing the hard segment or fluorobenzene pendant content of segmented polyurethanes containing PLGA moieties allows for modulation of physicochemically diverse drug release from electrospun fibers while maintaining a biologically relevant biodegradation rate.

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ASSOCIATED CONTENT

Supporting Information

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Supporting Table 1; physicochemically diverse drugs used in release experiments; ¹⁹F NMR spectra of PLGA-PU polymers (PDF)

Author Contributions

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Graphical abstract

Keywords

polyurethanes; PLGA; electrospun fibers; drug delivery; biodegradation

1. INTRODUCTION

Electrospun fibers have been widely explored as drug delivery systems (DDS).¹ Sustained release remains a formidable challenge from these materials, which have large surface areas conducive to rapid diffusive transport. Hydrophobic polymers can sustain drug release from fibers but are typically slow or nonbiodegradable.² The ability to tune the degradation while maintaining sustained release of small molecule drugs will be important for many medical applications. A number of factors are important to consider when designing a delivery formulation and include drug crystallinity,³ polymer degradation rate,⁴ polymer swelling,⁵ and polymer-drug interactions.⁶ Understanding the degradation and mechanisms of release are most important for formulation design,⁷ and in the context of electrospun fibers, the release mechanism may be directly impacted by the electrospinning solution characteristics, polymer chemistry, and polymer-drug compatibility.

Segmented, linear polyurethanes (PUs) allow for design of nontoxic and biocompatible polymers with tunable degradation rate and mechanical properties.^{8,9} Commercially available PUs are synthesized using oligoethers or oligoesters, which are typically based on aromatic diisocyanates that are slow degrading and generate toxic degradation products.¹⁰ A subclass of segmented, linear, biodegradable PU elastomers incorporate hard and soft segments in which backbone chain bonds can be hydrolyzed to biocompatible degradation products.¹¹ Various strategies have been employed to alter the degradation profiles and mechanical properties of biodegradable polyurethanes, including varying the soft^{12–15} or hard¹⁶ segment composition, incorporation of acid-cleavable hydrazone^{17,18} or collagenase-labile¹⁹ linkers, and varying the hard to soft segment ratio.^{20–23} Synthesis of segmented biodegradable PUs allows for engineering the polymer structure, such as the hard to soft segment ratio, or incorporation of pendant groups in order to exert control of the release mechanisms of physicochemically diverse drugs. Topical drug delivery applications, such as antibiotic-loaded wound dressings²⁴ or vaginal delivery of contraceptive or antiretroviral drugs for prevention of unintended pregnancy or HIV,²⁵ require dosage forms with both mechanical integrity during application and tunable drug release in which biodegradable PUs are particularly well suited.

Biodegradable PUs have been previously synthesized using polycaprolactone (PCL)-based soft segments and electrospun for applications such as soft^{26–28} or hard¹⁵ tissue engineering

scaffolds, drug-eluting stents,²⁹ vascular grafts,³⁰ and antibiotic wound dressings.²⁹ The chemical tunability that has been employed for rational design of tissue engineering scaffolds can also be used to modulate the release of physicochemically diverse drugs from electrospun fibers. For example, Hong et al. observed that grafting aminated phosphorylcholine onto the PLC-based poly(ester urethane) urea backbone slightly enhanced the release of paclitaxel from electrospun fibers.²⁹ Changing polymer chemistry may also increase the overall hydrophobicity of the bulk polymer and swelling ratio of the polymer matrix, with generally predictable effects on how the polymer interacts with hydrophilic or hydrophobic drugs encapsulated within the fibers. Similarly, Galperin et al. observed more prolonged release of norfloxacin (NFL) from porous polyHEMA hydrogels with increasing fluorostyrene content in the bulk hydrogel.³¹ In contrast to the hydrogels explored by Galperin et al., it is unclear as to whether minor alterations in polymer chemistry may likewise affect the release of physicochemically diverse drugs from electrospun fibers.

We investigated the release of physicochemically diverse drugs from fibers electrospun with a new class of biodegradable PLGA-based polyurethanes (PLGA-PU) that incorporate PLGA-*b*-tetraethylene glycol (TEG)-*b*-PLGA copolymers. We hypothesized that a polyurethane urea based on PLGA as opposed to PCL would degrade more quickly given the relative degradation rates of these two polymers.³² We also opted to use a PLGA-TEG-PLGA copolymer as the soft segment for increased hydrophilicity. Four small molecule drugs with varying aqueous solubilities and ionization states, but similar molecular weights, were chosen to probe drug-polymer interactions. Levonorgestrel (LNG) is a hydrophobic hormonal contraceptive approved for use in vaginal, mucosal contraception. Maraviroc (MVC) is a hydrophilic antiretroviral drug currently being tested in an intravaginal ring as part of a combination microbicide dosage form.³³ Norfloxacin (NFL) is a slightly hydrophobic antibiotic used in ophthalmic antibacterial eye drops. Tenofovir (TFV) is a hydrophilic antiretroviral drug being investigated for use as a topical microbicide gel for prevention of HIV.³⁴ We synthesized 16 PLGA-based PUs and selected four that showed relatively higher molecular weight and fiber stability at room temperature for further investigation: two with varying hard to soft segment ratios and two with varying molecular content of a fluorobenzene pendant group. The four polymers were characterized for their composition and thermal properties, independently electrospun into fiber mats loaded with each of the four drugs, and then utilized for *in vitro* release and cytotoxicity experiments. Here, we report on the effect of PLGA-PU composition and drug-polymer interactions on the release of physicochemically diverse drugs from PLGA-PU fibers.

2. MATERIALS AND METHODS

2.1. PLGA-based Poly(urethane urea) (PUUr) Synthesis

PLGA-*b*-TEG-*b*-PLGA diol ($M_w = 1260$, LA/GA = 50:50), PLGA-*b*-PEG-*b*-PLGA diol ($M_w = 4706$, LA/GA = 50:50), and PLGA-BDPLGDA diol ($M_w = 1633$, LA/GA = 50:50) were obtained from PolySciTech (West Lafayette, Indiana). Lysine diisocyanate (LDI) was purchased from Kyowa Hakko USA (New York, NY). N,N-dimethylacetamide (DMAc), tin(II) 2-ethylhexanoate (SnOc), 1,4-butanediamine (BDA), 1,3-diamino-2-hydroxypropane

(DAHP), and 4-fluorophenylisocyanate (FPI) were obtained from Sigma-Aldrich (St. Louis, MO). DMAc was dried using 10 wt % of 4 Å molecular sieves. One mole of PLGA-TEG-PLGA diol was dried at 105 °C and ~30 in Hg for 3 h, decompressed with nitrogen, and then placed in a 50 mL round-bottom flask and purged with N₂. A total of 8.77 mL of DMAc was added, and the macrodiol was completely dissolved before adding 2.08 mol of LDI. The mixture was stirred for 3.5 h at 80 °C. The resulting prepolymer solution was cooled to room temperature while stirring. For PU polymers without fluorobenzene pendant groups, 1.08 mol of BDA was added to a 100 mL three-neck round-bottom flask with 16.27 mL of DMAc, which was preliminarily purged with nitrogen. The prepolymer solution was then added to the flask, dropwise, over 10 min, while stirring at room temperature. For the PLGA-PU containing the fluorobenzene pendant groups, 0.96 mol of BDA and 0.12 mol of DAHP were added to a 100 mL three-neck round-bottom flask with 16.27 mL of DMAc at room temperature, until completely dissolved. The prepolymer solution was then added dropwise over 10 min, while stirring at room temperature. Next, 0.1 wt % of SnOc was added to the PUUr solution and then heated to 80 °C and stirred for ~5 min. Then, 0.12 mol of FPI was added dropwise to the OH-modified PUUr solution while stirring and was mixed for 30 min at 80 °C. The final polymers were poured into Teflon trays and dried at 80 °C at atmospheric pressure for 4 h, and then at 80 °C under 30 in. of Hg for 2 h.

2.2. Bulk Polymer Characterization

The values of average molecular weights (*M_w*, *M_n*) and the polydispersity (PDI) of the polymers were determined by gel permeation chromatography (GPC) (SEC Tosoh TSK-GEL R-3000 and R-4000 columns, Tosoh Bioscience, Montgomeryville, PA) connected in series to a Viscotek GPMmax VE2001 and refractometer VE3580 (Viscotek, Houston, TX). Samples were run at a concentration of 5 mg mL⁻¹ in N–N-dimethylformamide (DMF). The mobile phase consisted of DMF containing 0.1 wt % LiBr at a flow rate of 1 mL min⁻¹.

The fluorine content of each polymer was evaluated by quantitative ¹⁹F NMR spectroscopy.^{35,36} Polymers were dissolved in DMSO-*d*₆ containing 2.0 mM fluorobenzene to a final polymer concentration of 10.0 mg/mL. NMR spectra were acquired at 25 °C using a Bruker Avance III spectrometer operating at 376.46 MHz for ¹⁹F with broadband ¹H decoupling. Spectral parameters were as follows: 4096 scans; acquisition time 0.73 s; spectral width 89.286 kHz; spectral size 131 072 points. Spectra were phased and baseline corrected from –110 to –125 ppm using a Whittaker smoothing routine and then referenced to fluorobenzene as an internal standard (–113.15 ppm, normalized area = 1.0). All ¹⁹F resonances arising from polymer incorporated fluorobenzene pendant groups were observed at frequencies spanning –121 ppm to –123 ppm. The combined integrated area for all ¹⁹F resonances arising from polymer incorporated fluorobenzene pendant groups was referenced against the internal standard to quantitate fluorine content in each polymer.

X-ray photoelectron spectroscopy (XPS) was performed using a Surface Science Instruments S-Probe (Al K_{α1,2} X-ray source) at the University of Washington NESAC/BIO surface analysis center. Survey scans and high-resolution scans of carbon, nitrogen, and fluorine were taken for all polymer samples. Peak assignment and integration were

performed using XPS analysis software (Casa XPS). Theoretical atomic percentages for C, N, O, and F were calculated assuming a uniform elemental distribution across materials.

Polymers were dissolved in either dichloromethane (DCM) (PLGA-PU, PLGA-PU-FB, PLGA-PU-2FB) or 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) (PLGA-2PU) at 50% w/v, and 1 mL aliquots of the resulting polymer solutions were cast into thin films on glass slides by drying overnight at 40 °C and atmospheric pressure. Thin films were analyzed using a contact angle goniometer (Ramè-Hart, Model 100, Mountain Lakes, NJ) with 10 µL of deionized water, with $n = 3$ for each sample. Thin film polymer degradation in phosphate buffered saline (PBS) at a concentration of 1 mg mL⁻¹ was monitored over 4 weeks at 37 °C on a rotational shaker. Polymers were cast into thin films, and initial masses were recorded. At each time point (every 7 days), the remaining solid polymer was removed, lyophilized overnight, weighed, and then added to fresh PBS at a concentration of 1 mg mL⁻¹. Spent PBS was stored at -20 °C and utilized for the cytotoxicity assay. Thin films were analyzed for water uptake and were equilibrated in DI H₂O for 72 h. They were then removed from the liquid and lightly blotted to remove extraneous water, and then the swollen film was weighed. Water uptake was calculated using the following equation:

$$\% \text{water weight increase} = 100 \times \frac{(\text{wt. film}_{\text{wet}} - \text{wt. film}_{\text{dry}})}{\text{wt. film}_{\text{dry}}}$$

2.3. Electrospinning and Fabric Characterization

Synthesized polymers were dissolved in DCM (PLGA-PU, PLGA-PU-FB, PLGA-PU-2FB) or HFIP (PLGA-2PU) at 50% (w/v) and allowed to mix for 1 h prior to electrospinning. LNG, MVC, NFL, and TFV (Supporting Information Figure 1) were micronized and added at 15% (wt. drug/ wt. polymer). Polymer solutions were then electrospun at 18 kV over an 8 cm gap, using a flow rate of 20–40 µL min⁻¹. A total volume of 1 mL was dispensed from a glass syringe with a blunt 18-gauge stainless steel needle. Fibers were collected onto a flat, grounded aluminum surface covered by a single sheet of wax paper. After electrospinning, fiber samples were immediately stored in a lyophilizer until further use.

For scanning electron microscopy (SEM), fiber samples were sputter-coated with a mixture of Au and Pd for 90 s and then imaged using a Sirion SEM (NanoTech User Facility, University of Washington). A voltage of 5 kV, spot size of 3, and working distance of 5 mm were used to obtain images at a magnification of 500× and 5000×. Fiber sizes were measured using NIH ImageJ software for $n = 30$ per sample. Fiber degradation was observed by submerging fiber samples still attached to wax paper in PBS and incubating at 37 °C for 4 weeks. At each time point, samples were removed from solution, dried at room temperature for 2 h, and sputtercoated and imaged using SEM as described above.

Fiber samples of ~5 mg were sealed in T_{zero} aluminum pans and analyzed for drug crystallinity, polymer melting temperature, and glass transition temperature using an Auto Q20 differential scanning calorimeter (TA Instruments, UW Materials Science Engineering User Facility). Samples were heated at a constant ramp from 30 to 300 °C with a sampling

interval of 1 s point⁻¹, and heat flow was normalized to sample weight. Data collection and analysis was performed using TA Universal Analysis 2000 software.

2.4. *In Vitro* Release

Temporal release of drugs from electrospun fibers (~5 mg, $n = 3$) was carried out in 2% Kolliphor HS 15 (a nonionic solubilizer and emulsifying agent, Sigma) in deionized water. Prior to release experiments, drug saturation concentrations were calculated by adding excess drug to release media, equilibrating over 24 h, and then analyzing using HPLC with quantification of each drug using a standard curve prepared in DMSO (in which all of the drugs are soluble). Release samples were kept at sink conditions at 37 °C on a rotational shaker, and at each time point, a 100 μ L aliquot was removed for HPLC analysis and replenished with fresh release media. HPLC analysis was carried out on a Shimadzu Prominence LC20AD UH-HPLC system equipped with a Phenomenex Luna C18 column (250 $\times$ 4.6 mm, 5 μ m) using previously described HPLC methods.^{37–40}

2.5. Cytotoxicity of Biodegradation Products and Cytocompatibility of Electrospun Fibers

To evaluate the cytotoxicity of biodegradation products of the synthesized polyurethane polymers, TZM-bl cells, an adherent HeLa cell line, were seeded on a 96-well tissue culture polystyrene plate at a concentration of 15 000 cells well⁻¹. Cells were allowed to culture for 24 h, and then the medium was aspirated and replaced with 100 μ L of cDMEM and 100 μ L of either PBS (negative control) or spent PBS from a 4 week biodegradation study. Cells were then allowed to culture for an additional 72 h and then analyzed for viability using a CellTiter-Blue assay (Promega). Briefly, 20 μ L of CellTiter-Blue Reagent was added to each well and allowed to incubate for 3 h. The plate was then analyzed for fluorescence using a Tecan plate reader at an excitation wavelength of 560 nm and an emission of 590 nm. Percent viability was calculated by normalizing the sample wells to the fluorescence values of the negative control wells.

In order to assess the ability of electrospun PLGA-PU fibers ($n = 3$) to support cellular adhesion, fiber samples were cut and placed at the bottom of a clear-bottomed black 96-well plate. TZM-bl cells were seeded at a concentration of 30 000 cells well⁻¹, allowed to adhere for 24 h, and then analyzed for cellular adhesion relative to a tissue-culture polystyrene (TCPS) control using the same CellTiter-Blue protocol as stated above. Cellular adhesion was expressed as a percentage of TCPS control. PLGA-PU fibers ($n = 3$) were similarly assessed for cell growth: cells were seeded on PLGA-PU fibers at the same concentration and then analyzed after 2, 3, 4, and 5 days of culture using the CellTiter-Blue protocol. The fluorescence signal from each well was normalized to a standard curve of fluorescence signal for a known number of cells. Images of cells were obtained by staining TZM-bl cells with CellTracker Red CMPTX dye (Molecular Probes by Life Technologies, Waltham, ME, USA) according to the manufacturer's protocol. Briefly, cells were incubated in a solution of 20 μ m CMPTX dye in serum-free DMEM for 30 min, centrifuged, and washed with PBS. Cells were then plated at a concentration of 30 000 cells well⁻¹ in cDMEM on PLGA-PU fibers in a black 96-well plate with a clear bottom and incubated for 48 or 96 h. At each time point, the medium was removed from the well. Cells were washed two times with PBS; imaged on a Nikon ECLIPSE Ti microscope at 10 $\times$ magnification in the brightfield, FITC,

and TRITC channels; and merged using Nikon Elements AR software (version 3.0). PLGA-PU fibers exhibited autofluorescence in both the fluorescein isothiocyanate (FITC) channel (519 nm) and tetramethylrhodamine (TRITC) channel (532 nm). Thus, when imaging cells on PLGA-PU fibers, fibers were imaged in the FITC channel while cells were imaged in the TRITC channel, and then the two images were merged.

3. RESULTS

3.1. Characteristics of Synthesized PLGA-PUs

PLGA-based poly(urethane ureas) were synthesized as illustrated in Scheme 1. We chose PLGA-*b*-TEG-*b*-PLGA ($M_w = 1190$ Da) for our polyurethane polymers based on the hypothesis that the bulk polymer would be more hydrophilic than PLGA alone by incorporating TEG into the middle of the two PLGA blocks. The increased hydrophilicity is desirable to promote more rapid hydration of the polymer, which we predicted could result in faster hydrolysis of ester bonds and polymer degradation.⁴¹ Furthermore, incorporating a fluorobenzene pendant has previously been shown to sustain drug release in hydrogels, and we hypothesized that this phenomenon could be translated to electrospun fibers.³¹ Finally, we investigated if increasing the relative amount of hard segment to that of the soft segment would both limit the equilibrium water content of the polymer and sustain drug release.⁴² Polymers were synthesized using standard polyurethane synthesis procedures⁴³ with a yield of >95%.

Sixteen total polymers were synthesized initially using PLGA-PEG-PLGA (1000:1000:1000) and PLGA-BD-PLGA (500:58:500) (Supporting Information Table 1). However, these initial polymers were relatively low in molecular weight and did not produce fibers that retained structural integrity at room temperature. Polymers with similar compositions but based on PLGA-TEG-PLGA (500:190:500) were higher in molecular weight, maintained fiber morphology, and were used for further experimentation. The final polymers include a linear PLGA-based polyurethane urea (PLGA-PU), a linear PLGA-based polyurethane with twice the original hard to soft segment ratio (PLGA-2PU), a PLGA-based polyurethane with an added fluorobenzene pendant group (PLGA-PU-FB), and a PLGA-based polyurethane with twice the original fluorobenzene pendant group content (PLGA-PU-2FB). The molecular weight and PDI for each polymer are reported in Table 1. Prior to electrospinning, all polymers appeared as slightly yellow, semielastic solids at room temperature. All polymers were analyzed by ¹⁹F NMR in order to measure the fluorine content of the bulk polymer (Supporting Information Figure 2), and by XPS to measure the fluorine content at the surface of the polymer cast into thin films. As expected, fluorine was not detected in the PLGA-PU and PLGA-2PU by either technique. Quantitative ¹⁹F NMR analysis of PLGA-PU-FB and PLGA-PU-2FB indicated that PLGA-PU-2FB has ~2.4 times the molar fluorobenzene content of PLGA-PU-FB (Table 2), consistent with complete incorporation of the added pendant group at the target molar ratio. Surprisingly, XPS analysis of PLGA-PU-FB or the PLGA-PU-2FB thin films did not detect any fluorine at the surface (<10 nm sampling depth; Table 3).

XPS was also used to confirm the composition of polymers synthesized with different hard (urethane urea) to soft (PLGA) segment ratios. We observed that polymers with an

equivalent hard to soft segment ratio (PLGA-PU, PLGA-PU-FB, PLGA-PU-2FB) had a carbon content of 74–81% (atomic %), oxygen content of 16–22%, and nitrogen content of 2.4–3.5% (Table 3). The carbon content for these polymers is higher than expected, whereas the oxygen content is lower than expected. This result may be due to more efficient incorporation of BDA into the polymer chain, or molecular positioning of the hard segment toward the surface of the polymer. Doubling the hard to soft segment ratio (PLGA-2PU) resulted in a measured elemental composition of 59% carbon, 34% oxygen, and 0.1% nitrogen. These values of carbon and oxygen content are more reflective of the predicted values, but the nitrogen content is much lower, possibly indicating less incorporation of the nitrogen-rich lysine block or positioning of the lysine block away from the surface of the polymer. Polymer compositions as revealed by ^{19}F NMR and XPS were as expected with only minor deviations.

In order to determine how polymer composition may affect their behavior in aqueous release media, we measured polymer wettability using contact angle goniometry and swelling by equilibrium water content. All polymers were found to have intermediate wettability (both hydrophilic and hydrophobic characteristics, $0^\circ < \theta < 90^\circ$) based on the measured contact angles. The contact angle increased by 15° and 28° when either the hard to soft segment ratio or fluorobenzene pendent content was increased, respectively (Table 4). However, addition of the fluorobenzene alone resulted in a reduced contact angle relative to the PLGA-PU polymer. In general, the contact angle measurements were supported by the equilibrium water content data. For example, the larger contact angle measured for PLGA-PU compared to PLGA-2PU and for PLGA-PU-FB compared to PLGA-PU-2FB also resulted in an observed decrease in equilibrium water content of approximately 40% and 10%, respectively. Similarly, a 20° lower contact angle observed for PLGA-PU-FB compared to PLGA-PU showed almost a 60% increase in water content. In summary, although the polymers had varied hard to soft segment ratios and fluorobenzene pendant content, they showed similar wettability and swelling based on the resolution of the analysis methods, which suggest that they would behave similarly in release media.

3.2. Drug and Polymer Compatibility in Electrospun Fibers

Polymers were electrospun from DCM (PLGA-PU, PLGA-PU-FB, PLGA-PU-2FB) or HFIP (PLGA-2PU) and produced meshes of fibers with diameters of $1\ \mu\text{m}$ with a semi-melted fiber morphology as well as the presence of some nonfiber regions (Figure 1). Nonfiber regions were likely due to the relatively low molecular weights and inherently low melting temperature (T_m) of these polymers. As a consequence, fibers were lyophilized immediately after electrospinning in order to remove residual solvent and prevent meshes from coalescing into films.

Drug solubility in a polymer–solvent system may affect electrospinning as well as the resulting drug crystallinity in the finished fibers. Only MVC was soluble in all the polymer solutions, whereas LNG, NFL, and TFV were either partially soluble or insoluble as assessed by qualitative visual inspection. However, analysis of drug-loaded electrospun fibers, which all had $>90\%$ loading efficiency, using differential scanning calorimetry (DSC) showed no crystalline drug present in any of the samples. A representative thermogram of

LNG and PLGA-PU-FB polymer shows a broad polymer melting peak present in samples with and without a drug but a lack of any detectable crystalline drug (Figure 2a). PLGA-PU, PLGA-PU-FB, and PLGA-PU-2FB showed a polymer melting temperature of approximately 260 °C, and increasing the hard to soft segment ratio of the polymer (PLGA-2PU) decreased the T_m by ~20 °C (Figure 2b). Unchanged or increased polymer T_m indicates good drug–polymer compatibility, or physicochemical interactions between components of the formulation that facilitate drug dissolution and dispersion within the solid matrix.⁴⁴ Based on this criterion, PLGA-PU and PLGA-2PU showed good compatibility with all four drugs despite physicochemical differences in the compounds. In contrast, PLGA-PU-FB had poor compatibility with MVC and NFL but was compatible with LNG and TFV. PLGA-PU-2FB had poor compatibility with LNG and MVC but was compatible with NFL and TFV. Glass transition temperature (T_g) is also an indicator of drug–polymer compatibility where a decrease in T_g upon incorporation of a small molecule drug is evidence of more mobile polymer chains due to the plasticizing effect of the drug.⁴⁵ All drugs caused a decrease in T_g for PLGA-2PU but had little effect on the T_g of PLGA-PU, PLGA-PU-FB, and PLGA-PU-2FB. We observed an increase of ~5 °C in the T_g when MVC was incorporated into PLGA-PU and PLGA-PU-FB and when TFV was incorporated into PLGA-PU-2FB (Figure 2c). Overall, PLGA-2PU showed good compatibility with all drugs, while PLGA-PU, PLGA-PU-FB, and PLGA-PU-2FB showed minor deviations in T_m and T_g upon incorporation of LNG/MVC, MVC/NFL, and LNG/MVC, respectively.

3.3. Release of Physicochemically Diverse Drugs from PLGA-PU with Higher Hard to Soft Segment Ratio

We measured the effect of polymer hard to soft segment composition on the release of our four physicochemically diverse drugs. We observed that increasing the hard to soft segment ratio (PLGA-PU and PLGA-2PU) had a significant effect on the *in vitro* release rate of all the drugs except for LNG (Figure 3). We observed that MVC and NFL had a 20–30% reduction in the burst release phase with PLGA-2PU compared to PLGA-PU, but similar pseudolinear release rates resulting in a lower cumulative percent release after 72 h. In contrast, increasing the hard to soft segment ratio had the opposite effect on TFV release where the amount of burst release almost tripled, but the pseudolinear phase was similar or slightly higher. The burst and pseudolinear phases were almost identical between PLGA-PU and PLGA-2PU for release of LNG.

3.4. Release of Physicochemically Diverse Drugs from PLGA-PU with Higher Fluorobenzene Pendant Group Content

We measured the effect of increased fluorobenzene pendant content of the polymer has on drug release. We observed that doubling the fluorobenzene pendant content of the PLGA-PU enhanced sustained *in vitro* release of LNG and NFL, but not MVC or TFV (Figure 4). MVC and TFV showed an initial burst of ~40% followed by similar pseudolinear release rates between PLGA-PU-FB and PLGA-PU-2FB. LNG and NFL showed a similar trend of reduced burst release with PLGA-PU-FB compared to PLGA-PU-2FB but comparable pseudolinear release rates. However, a 20% reduction in burst release was measured for LNG, whereas the reduction in NFL burst release was ~60%. Overall, increasing

fluorobenzene pendant content did not affect MVC or TFV release but resulted in a greater burst release for both LNG and NFL.

3.5. Biodegradation and Cytocompatibility of PLGA-PU Fibers and Degradation Products

Biodegradability and cytocompatibility are essential considerations for polymers intended for use in topical drug delivery. We measured biodegradation in PBS of all polymers cast as thin films over the course of 4 weeks. Thin films were used in place of fibers to ensure that all samples had equivalent thickness and surface area. We observed that thin films of the four polymers showed similar linear degradation profiles (Figure 5a). PLGA-PU and PLGA-2PU degraded at similar rates of $-21.3\% \pm 1.4\%$ and $-19.3\% \pm 2.4\%$ per week, respectively. Incorporation of the fluorobenzene pendant group enhanced degradation by $<1\%$ to 4% and yielded rates of $-22.1\% \pm 3.4\%$ and $-23.5\% \pm 1.8\%$ per week for PLGA-PU-FB and PLGA-PU-2FB, respectively. To complement the PLGA-PU thin film biodegradation studies, we also measured biodegradation of electrospun PLGA-PU fibers in PBS (Figure 5c). Fibers were predicted to show faster degradation rates than the films due to their comparatively higher porosity and surface area, and indeed this was confirmed (see below). After 24 h, all fibers had almost completely lost the fibrous structure and had started to relax into films. The fibers then appeared to be melded into a thin, smooth layer of polymer film, which was present after 1 and 2 weeks. After 4 weeks, only small droplet-like structures of polymer were present on the wax paper.

The cytocompatibility of PLGA-PU was evaluated using viability as an indicator of metabolic activity and cellular adhesion and growth as indicators of cellular function. Polymer degradation products were cytocompatible with measured cellular viability of $>80\%$ for all time points (Figure 5b). PLGA-PU, PLGA-PU-FB, and PLGA-PU-2FB degradation products showed increased cytotoxicity over time. However, PLGA-2PU degradation products were cytocompatible over the entire four-week time course. All PLGA-PU fiber had similar cellular adhesion to TCPS (Figure 6a) and cell growth over 5 days (Figure 6b), indicating that these materials are cytocompatible. In order to visualize the cytocompatibility of PLGA-PU fibers, TZM-bl was stained with CellTrackerRed CMPTX dye and cultured on PLGA-PU fibers for 4 days. CMPTX-stained TZM-bl cells exhibit fluorescence in the TRITC channel (Figure 6c). Cells appeared to be attached to and surround the fibers after both 48 and 96 h, further confirming the cytocompatible nature of PLGA-PU. For imaging purposes, the PLGA-PU fibers used in this experiment were very thin, and as observed in the fiber degradation experiments, the fibers had begun to meld into films after 48 h and appear as polymer droplets.

4. DISCUSSION

PLGA-based polyurethanes exhibit biologically relevant degradation profiles, are noncytotoxic, produce amorphous solid dispersions when electrospun into drug-loaded nanofibers, and can be tuned to sustain release of a variety of physicochemically diverse drugs. While all synthesized polymers had relatively low molecular weight ranging from 7 to 27 kDa, this did not preclude their ability to be electrospun into fibers. Repeated batches of the synthesized PLGA-PU polymers yielded similar molecular weights, suggesting that it

may not be possible to achieve higher molecular weight polymers with these commercially sourced oligo-PLGA molecules (Scheme 2, Supporting Information Table 1). At room temperature, the polymers and electrospun meshes were soft and pliable, likely due to the relatively low molecular weight of the polymers. In order to maintain the structural integrity of the fibers and remove any residual solvent, the fabrics were immediately lyophilized after electrospinning to preserve the fiber morphology. However, the materials were generally easy to handle and could feasibly be used as a topical dosage form for drug delivery.

NMR was used to measure bulk fluorine content, and XPS measured the fluorine content in the outermost <10 nm of surface.⁴⁶ Although ¹⁹F NMR indicated that the PLGA-PU-2FB bulk polymer had approximately 2-fold higher fluorine content compared to PLGA-PU-FB, XPS was unable to detect fluorine at the surface of either of these polymers cast as thin films. XPS is highly sensitive to fluorine, so it can be concluded that the fluorobenzene pendant group is present at <0.1 atomic percent at the surface of the polymer when the polymer is cast as a thin film. The low surface energy of fluorine typically causes it to surface-localize in polymer thin films,^{47–49} and we predicted that XPS would measure it at the stoichiometric atomic ratio or concentrated at the surface. In another study, phase separation forced a lower surface energy soft segment to the surface, which decreased the XPS signal from the fluorine-containing hard segment.⁵⁰ However, our XPS data of carbon enrichment and oxygen depletion suggests surface enrichment of the hard segment in which the fluorobenzene pendant group is attached. This phenomenon could potentially alter the release of certain drugs that partition within the polymer matrix and preferentially localize within the hard segment. XPS analysis also revealed unexpected ratios of carbon and oxygen in the PLGA-PU containing the lower hard to soft segment ratio (PLGA-PU, PLGA-PU-FB, and PLGA-PU-2FB). The carbon to oxygen ratio for these polymers is much closer to that of butane and lysine, components of the hard segment, and further indicates enrichment of the hard segment at the surface of the polymer thin film. Hydrocarbon contamination, indicated by higher than expected carbon content and lower than expected nitrogen content,⁵¹ was not apparent for any samples. On a bulk polymer level, there were no major differences in wettability, and all polymers exhibited a mixed hydrophobic and hydrophilic nature.⁵² This is supported by our XPS and polymer degradation results, as enrichment of fluorine on the film or fiber surface would likely cause an observable increase in hydrophobicity and slower degradation; however, the polymers did not exhibit differences in wettability or degradation. In particular, our PLGA-based biodegradable PU all degraded more quickly than previously reported PCL-based polyurethanes^{26,53,54} and showed <20% mass remaining after 4 weeks. Given the observed mass loss profiles (Figure 5a) and visually observed fiber degradation (Figure 5c), these polymers likely undergo bulk matrix degradation, as opposed to surface erosion, which is expected for PLGA-based polymers.^{55,56}

Despite the physicochemically diverse drugs having variable solubility within the polymer solution prior to electrospinning, all electrospun fibers were found to have undetectable drug crystallinity. This may be due to the electrospinning process where solvent evaporation takes place rapidly and limits the amount of time for the formation of crystalline pockets of drug. Thus, the PLGA-PU drug-loaded electrospun fibers are considered amorphous solid dispersions, which may allow for enhanced control over release properties via polymer

engineering, since drug release from fibers does not depend on solubilizing crystalline drug.⁵⁷ The melt temperature of the polymer, as determined by DSC, can be used as an indicator of drug–polymer compatibility.⁴⁴ Unchanged or increased T_m of the polymer upon incorporation of the drug indicates good compatibility owing to the superior ordering of drug and polymer molecules within the solid matrix. Despite varying solubility, all drugs had good compatibility with PLGA-PU and PLGA-2PU. However, only TFV had good compatibility with PLGA-PU-FB, while both NFL and TFV had good compatibility with PLGA-PU-2FB. This difference may be due to the higher number of hydrogen bonding groups on TFV and NFL compared to LNG and MVC, which may facilitate interaction with the added fluorobenzene pendant group.

The differences in bulk polymer properties and drug–polymer compatibility manifest themselves in varying levels of sustained release of the physicochemically diverse drugs into aqueous media. Increasing the hard to soft segment ratio had no effect on the release of LNG, which has no ionizable groups, suggesting the release is not controlled by charge-based interaction with the polymer. The release of both MVC and NFL was prolonged by increasing the hard to soft segment ratio, while the opposite is true for TFV. Increasing the hard to soft segment ratio caused TFV to essentially burst release over 48 h. The enhancement of sustained release of MVC and NFL upon the augmentation of the hard segment may be due to the positive ionization state of MVC and NFL interacting with the more electronegative hard segment, whereas TFV has a negative charge when ionized, which facilitates more rapid release.⁵⁸ Increasing the fluorobenzene pendant group content had a minimal effect on the sustained release of MVC and TFV, while it caused more rapid release of LNG and NFL. This could be because LNG and NFL have incompatible interactions with the pendant group, and when it is increased the drugs may be forced to the surface since our XPS data indicates the pendant is internalized within the bulk polymer matrix. The greater presence of drug at the surface may allow for more rapid diffusion and dissolution from the fibers. These release profiles may also be observed for similar small molecule drugs, depending on hydrogen bonding and the degree of ionization. Our data demonstrates the wide overall applicability of this promising new class of polymers, which degrade on a biologically relevant time scale and can sustain release of a variety of small molecule drugs relevant to topical drug delivery applications.

5. CONCLUSIONS

A novel class of faster biodegrading polyurethanes with PLGA-moieties was synthesized with varying hard to soft segment ratios and fluorobenzene pendant group content. Though increasing the fluorobenzene pendant group content increased the total fluorine content of the bulk polymer, the fluorine was not present at the surface. Resulting polymers had both hydrophilic and hydrophobic surface properties, were noncytotoxic and biodegrade *in vitro* within one month. PLGA-PUs were successfully electrospun and loaded with LNG, MVC, NFL, and TFV to produce amorphous solid dispersions. Increasing the hard to soft segment ratio augmented the sustained release of positively charged drugs (NFL, MVC), while increasing the fluorobenzene pendant group content caused the drugs repelled by the fluorobenzene pendant (LNG, NFL) to be released more quickly. Increasing the hard to soft segment ratio or fluorobenzene content of PLGA-PUs allows for modulation of drug release

of both hydrophobic and hydrophilic drugs from electrospun fibers while maintaining a biologically relevant biodegradation rate.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

DSC	differential scanning calorimetry
GPC	gel permeation chromatography
LNG	levonorgestrel
MVC	maraviroc
NFL	norfloxacin
NMR	nuclear magnetic resonance
PCL	polycaprolactone
PLGA	poly(lactic- <i>co</i> -glycolic acid)
PU	polyurethane
SEM	scanning electron microscopy
TEG	tetraethylene glycol
TFV	tenofovir
XPS	X-ray photoelectron spectroscopy

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Figure 1. Scanning electron micrographs of electrospun polyurethane polymers: (a) PLGA-PU, (b) PLGA-2PU, (c) PLGA-PU-FB, and (d) PLGA-PU-2FB. Magnification = 5000 $\times$ and scale bar = 5 μ m.

Figure 2.

Thermal properties of polymers in blank and drug-loaded electrospun fibers as determined by differential scanning calorimetry. (a) Representative differential scanning calorimetry thermograms of crystalline LNG, electrospun PLGA-PU-FB polymer, and electrospun PLGA-PU-FB polymer loaded with LNG; (b) melting temperatures; and (c) glass transition temperatures of PLGA-PUs.

Figure 3. Release of LNG (a), MVC (b), NFL (c), and TFV (d) from electrospun fibers from polyurethanes PLGA-PU and PLGA-2PU (both sans fluorobenzene pendant) into 2% solutol in deionized water at sink conditions over 72 h. Each point represents the mean $\pm$ standard deviation for $n = 3$.

Figure 4. Release of LNG (a), MVC (b), NFL (c), and TFV (d) from electrospun fibers from polyurethanes PLGA-PU-FB and PLGA-PU-2FB (2 and 4 mol % fluorobenzene pendant, respectively) into 2% solutol in deionized water at sink conditions over 72 h. Each point represents the mean $\pm$ standard deviation for $n = 3$.

Figure 5. Degradation characteristics of PLGA-PU fibers over 4 weeks: (a) mass loss degradation profile, (b) the cytotoxicity of corresponding biodegradation products in TZM-bl cells, and (c) scanning electron micrographs of degrading electrospun PLGA-PU fibers in PBS at 37 °C. Scale bar = 200 μ m. Inset shows a higher-resolution view of the formation of polymer droplets.

Figure 6.

TBM-bl cell cytocompatibility with electrospun PLGA-PU fibers. (a) Cellular adhesion after 24 h, normalized to tissue culture polystyrene (TCPS), (b) cell growth on TCPS and electrospun PLGA-PU fibers over the course of 2–5 days, fluorescence normalized to a standard curve of known number of cells, and (c) CellTracker Red CMPTX stained TBM-bl cells on PLGA-PU fibers after 2 and 4 days of culture. TCPS = merge of FITC, TRITC, and brightfield channels of cells on TCPS only, Brightfield = brightfield channel of cells of PLGA-PU fibers, FITC = FITC channel of cells on PLGA-PU fibers, and TRITC = TRITC channel of cells on PLGA-PU fibers. Day 2 and 4 images are a merge of the FITC and TRITC channels. PLGA-PU fibers exhibit autofluorescence in both the FITC and TRITC

channels, while cells stained with CMPTX exhibit fluorescence in the TRITC channel only.
Scale bar = 100 μm .

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Scheme 1.
PLGA-based Polyurethane Urea Synthesis

Scheme 2.
Evolution of PLGA-PU Compositions

Table 1
Polymer Molecular Weights and Polydispersity Index (PDI) As Determined by Gel Permeation Chromatography^a

polymer ID	composition						M_n (g/mol)	M_w (g/mol)	PDI (M_w/M_n)	hard segment	soft segment
	PLGA-TEG-PLGA	LDI	BDA	DAHP	FPI						
PLGA-PU	1.00	2.00	1.00	0	0		20 960	27 140	1.295	22%	78%
PLGA-2PU	1.00	3.00	2.00	0	0		3226	7805	2.419	31%	69%
PLGA-PU-FB	1.00	2.00	0.92	0.08	0.08		5099	8189	1.606	22%	78%
PLGA-PU-2FB	1.00	2.00	0.84	0.16	0.16		6325	10 790	1.706	23%	77%

^a A value of 0.07 was used for the dn/dc of all tested polymers.

Table 2

Fluorine Content of PLGA-PU Polymers Containing a Fluorobenzene Pendant Group

polymer ID	polymer ^a (moles)	¹⁹ F content ^b (moles)	¹⁹ F mole ratio (moles ¹⁹ F/ mol polymer)
PLGA-PU-FB	6.39×10^{-7}	7.60×10^{-7}	1.19
PLGA-PU-2FB	4.63×10^{-7}	1.31×10^{-6}	2.83

^aMole quantity calculated based on the average M_w of PLGA-PU-FB of 8189 g/mol and PLGA-PU-2FB of 10 790 g/mol.^bMeasured based on quantitative ¹⁹F NMR.

Table 3
Composition of Polymers As Determined by X-Ray Photoelectron Spectroscopy

polymer ID	theoretical atomic %				measured atomic % average $\pm$ standard deviation			
	C	O	N	F	C 1s	O 1s	N 1s	F 1s
PLGA-PU	58.4	38.7	2.9	0	75.7 $\pm$ 0.006	20.8 $\pm$ 0.005	3.5 $\pm$ 0.002	n.d.
PLGA-2PU	59.6	36.5	3.8	0	58.5 $\pm$ 0.009	34.3 $\pm$ 0.01	0.07 $\pm$ 0.003	n.d.
PLGA-PU-FB	58.3	38.5	3.1	0.0006	73.9 $\pm$ 0.06	22.7 $\pm$ 0.06	3.3 $\pm$ 0.0009	n.d.
PLGA-PU-2FB	58.3	38.4	3.2	0.001	81.2 $\pm$ 0.03	16.3 $\pm$ 0.02	2.4 $\pm$ 0.006	n.d.

Table 4

Wettability and Equilibrium Water Content of Polymers

polymer ID	contact angle	water mass uptake (72 h)
PLGA-PU	$67^{\circ} \pm 2^{\circ}$	$34\% \pm 6\%$
PLGA-2PU	$82^{\circ} \pm 3^{\circ}$	$19\% \pm 1\%$
PLGA-PU-FB	$47^{\circ} \pm 1^{\circ}$	$54\% \pm 8\%$
PLGA-PU-2FB	$75^{\circ} \pm 3^{\circ}$	$49\% \pm 2\%$