Trimethoprim-Loaded Microspheres Prepared from Low-Molecular-Weight PLGA as a Potential Drug Delivery System for the Treatment of Urinary Tract Infections

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ABSTRACT: Commonly, therapy of urinary tract infections suffers from increasing resistance to antibiotics and the ability of uropathogenic Escherichia coli (UPEC) to invade bladder cells and cause recurring infections. As an alternative strategy for instillation into the bladder, trimethoprim-loaded microparticles with poly(D,L-lactic-co-glycolic acid) (PLGA) as a matrix were prepared. To reduce particle loss by washout, their surface was grafted with bioadhesive wheat germ agglutinin, providing biomimicry akin to UPEC. Since PLGA 503H has shown a slow drug release profile, the low-molecular-weight PLGA 2300 was studied. Whereas the drug loading of PLGA 503H particles amounted to 2.8%, the drug content of PLGA 2300 particles was twice as high. Although the drug release pattern started with an initial burst of 30% after 24 h for both PLGA types, half of the trimethoprim content was released after 4 days from PLGA 503H microparticles as opposed to 2 days in the case of PLGA 2300. Higher drug loading and accelerated release render PLGA 2300 a viable alternative to PLGA 503H.

1. INTRODUCTION

One of the most common infectious diseases is the urinary tract infection (UTI).1 Classiﬁed into uncomplicated and complicated UTI, it can affect the upper tract, provoking pyelonephritis, and the lower tract, causing cystitis.2−4 Typical symptoms include urination pain, dysuria, pollakiuria, and hematuria; in the case of nephrotic inﬂammation, fever can occur additionally.5,6 Risk factors are female gender, sexual activity, vaginal infections, and chronic diseases such as diabetes.7 Most UTIs are caused by Escherichia coli (uropathogenic E. coli, UPEC); in addition, other Enterobacteriaceae such as Klebsiella pneumoniae or various types of Staphylococcus can elicit UTI as well.3,4,8

The urothelium represents a dense barrier between urine and the rest of the body.9,10 However, bacterial adhesins such as FimH, part of type 1 pili of UPEC, can interact with the urothelial cells, which can lead to bacterial invasion. UPEC then forms bioﬁlm-like intracellular communities, which can cause recurring infections.3,5,11

Standard therapy of UTI is a 1 to 5 day oral antibiotic treatment. In severe cases, antibiotics such as fosfomycin, pivmecillinam, and trimethoprim are administered parenterally.12 Increasing resistance rates require alternative treatment options such as intravesical therapy.13,14 Intravesical instillation is a safe and effective alternative to oral or intravenous application: the drug is administered directly into the bladder cavity via a catheter. In response to a higher local concentration and thus efﬁcacy, a lower dose of antibiotic is necessary and less systemic side effects are provoked.9,14

Major problems associated with intravesical therapy are (i) the dilution and washout process due to constantly secreted urine, (ii) the dense barrier of the urothelium, and (iii) possible premature degradation of the antibiotic.14−16 These limits might be overcome by a biomimetic drug delivery system, such as lectin-modified poly(D,L-lactic-co-glycolic acid) (PLGA) microparticles.

PLGA is a copolymer commonly used as a matrix for particles because of its biocompatibility and biodegradability.17 It is a polyester of lactic acid and glycolic acid and varies in its monomeric ratio. The higher the amount of lactic acid, the slower the degradation process. However, at a 50:50 ratio of both hydroxy acids, as represented by PLGA 503H, degradation is the fastest. Another parameter of PLGA determining the release rate is its molecular weight. Non-speciﬁc hydrolysis of low-molecular-weight PLGA yields faster water-soluble oligomers and, ﬁnally, faster drug release than high-molecular-weight PLGA.18,19 Furthermore, PLGA micro-particles as a drug carrier can provide retardation and protect the active pharmaceutical ingredient against undesired...
An additional advantage of the H-type of PLGA, such as PLGA 503H, is the presence of free carboxyl groups that might be useful as anchors for further surface functionalization. The surface of microparticles can be modified with lectins such as wheat germ agglutinin (WGA) to increase their adhesion rate to bladder cells, exploiting the same binding sites as UPEC. Adhesion of the drug delivery system to the bladder wall is expected to reduce dilution by urine and limit rapid washout of the drug by micturition, hence increasing dwell time in the bladder and shortening the diffusional pathway to the diseased cells, resulting in prolonged exposure and higher drug efficacy. In addition, those factors might reduce the duration and frequency of instillitative interventions as well.

In the present study, microparticles were prepared using two different types of PLGA and loaded with trimethoprim, an antibiotic commonly used for the treatment of UTI. Considering the rather slow degradation and, therefore, limited bioavailability of the incorporated drug upon instillation of commonly used PLGA 503H (MW 24,000–38,000 Da) formulations, the novel, low-molecular-weight PLGA 2300 (MW 2000–2500 Da) was investigated. While low-molecular-weight PLGA has been evaluated, e.g., 4 kDa PLGA for nanoparticles or 8 kDa PLGA for microparticles, PLGA with even lower molecular weight might offer certain advantages such as faster drug release, solubility in different solvents, and higher entrapment of active pharmaceutical ingredients. PLGA 2300 was investigated to evaluate trimethoprim loading and release in comparison to PLGA 503H and its bioadhesive characteristics with and without WGA modification. Thus, the aim of this study was to disclose the advantages of a low-molecular-weight PLGA in comparison to an already established one, potentially providing faster drug release.

2. RESULTS AND DISCUSSION

2.1. Optimization of the Particle Preparation Process.

To prepare microparticles with a drug content as high as possible, various parameters of the preparation process have been modified. To evaluate the influence of the type and concentration of the surfactant, both were varied and the results are presented in Table 1 and scanning electron images in Figure 1.

Regarding the surfactant type, changing the default Pluronic F68 (F68-A) to the higher-molecular-weight F127 type resulted in 25% lower drug loading ($p \leq 0.001$) and negligibly increased particle size ($p \leq 0.001$). The crack in the particle surface shown in Figure 1 is a damaged material caused by exposure to the electron beam. Thus, there is no improvement over the final protocol.

Variations of the surfactant concentrations, however, significantly impacted the size and drug loading of the microparticles. Using 3% in both emulsions (F68-B) yielded the largest and most inhomogeneous particle population with the lowest trimethoprim content. This is reflected in the microscopic image as well (Figure 1). Reducing the concentration to 1% in both emulsions (F68-C) yielded the smallest particles with medium-ranking drug load. Overall, the microparticles where spherical and porous, possibly due to trimethoprim leakage in suspension or due to humidity evaporated by the vacuum/electron beam. Since 1% surfactant in emulsion 1 and 3% in emulsion 2 provoked the highest drug loading and a particle size distribution most appropriate for instillation, we adhered to this protocol.

Considering the drug loading capacity of 2–3%, a suspension containing 70–100 mg particles per liter would be required for instillation to reach the minimal inhibitory concentration of trimethoprim amounting to 0.5–2 mg/L.

To further increase the drug loading, the amount of trimethoprim used in the preparation process was varied, and besides the commonly used PLGA503H, the lower molecular weight-type PLGA 2300 was used. 

Table 1. Type and Concentration of Surfactant Used for Preparation of PLGA 503H Microparticles

| Surfactant type | Emulsion 1 | Emulsion 2 | Size D50 (μm) | Size D90 (μm) | Drug loading capacity % | Span (μm) |
|-----------------|------------|------------|---------------|---------------|-------------------------|-----------|
| F68-A           | 1%         | 3%         | 15.06 ± 0.07  | 44.08 ± 0.95  | 18.70 ± 0.28            | 2.88 ± 0.05 | 2.75%       |
| F68-B           | 3%         | 3%         | 19.55 ± 6.13*** | 92.55 ± 31.63*** | 75.81 ± 16.77***         | 4.74 ± 1.69*** | 1.83%***   |
| F68-C           | 1%         | 1%         | 11.03 ± 0.087 | 27.15 ± 0.26  | 12.66 ± 0.13            | 2.40 ± 0.01  | 2.44%***    |
| F127            | 1%         | 3%         | 16.43 ± 0.31* | 49.85 ± 1.43* | 20.74 ± 0.52*           | 2.99 ± 0.04*  | 2.02%***    |

*Respective size ($n = 10$) and %loading capacity ($n = 3$). Using F68-A as a control, ***$p \leq 0.001$, *$p \leq 0.05$, and no star $p \geq 0.05$. 

Figure 1. Overview and detailed scanning electron microscopic images of microparticles prepared from PLGA 503H with different surfactant concentrations and types (see Table 1).
In the case of PLGA 503H, the particle size decreased with increasing amount of trimethoprim added ($p \leq 0.001$), but the drug loading improved by only 0.05% ($p \leq 0.001$) (Figure 2 and Table 2).

Thus, the smallest particles contained the highest amount of antibiotic. Since the difference in drug loading between the 40 mg batch and the 60 mg batch was miniscule ($p \leq 0.001$), the maximum drug loading seemed to have been achieved using the 40 mg protocol. While the maximum drug loading of PLGA 503H microspheres was about 2.8%, PLGA 2300 generally displayed a higher loading capacity ($p \leq 0.001$). The 6.4% drug loading of the 60 mg batches was about 2-fold higher than that of the PLGA 503H counterpart ($p \leq 0.001$).

As the drug loading concurrently increased with the initial amount of trimethoprim added, an additional batch with 100 mg of trimethoprim was prepared, however, resulting in a quite lower drug loading (4.4%). The higher amount of trimethoprim required more solvent and, therefore, a change in glassware. Apparently, these alterations in solvent volume and interfaces resulted in comparatively lower entrapment rates of trimethoprim.

The micro Particles of the 40 mg and 60 mg batches of PLGA 503H and PLGA 2300 were comparable in size ($p \leq 0.001$). Especially, the 60 mg batches were not only almost similar in size but also allowed encapsulation of the highest amounts (6.36%) of trimethoprim for both polyesters. PLGA 503H particles were spherical with a few pores (Figure 3). The crack visible in the detailed image of PLGA 503H micro-

### Table 2. Effect of Trimethoprim Amount Used in Preparation on Size and Drug Loading of Microparticles Made from PLGA 503H and PLGA 2300

| Trimethoprim | PLGA 503H | PLGA 2300 |
|--------------|-----------|-----------|
|              | $D_{[4,3]}$ ($\mu$m) | %LC | $D_{[4,3]}$ ($\mu$m) | %LC |
| 20 mg        | 20.57 ± 0.65*** | 1.78%*** | 20.57 ± 0.65*** | 1.78%*** |
| 40 mg        | 18.70 ± 0.28*** | 2.75%*** | 18.70 ± 0.28*** | 2.75%*** |
| 60 mg        | 15.87 ± 0.87*** | 2.80%*** | 15.87 ± 0.87*** | 2.80%*** |

$D_{[4,3]}$ ($n = 10$) and %loading capacity ($n = 3$). Statistical comparison was done with 40 mg as a control within a group and between groups with the same amount of trimethoprim. $P$ values in the table for both comparisons, if not noted otherwise. ***, $p \leq 0.001$.

Figure 2. Microparticle sizes ($D_{[4,3]}$; $n = 10$) and trimethoprim content ($n = 3$) when using different amounts of trimethoprim during preparation (20—60 mg).

Figure 3. Overview and detailed scanning electron microscopic images of microparticles prepared from PLGA 503H with different trimethoprim amounts added during preparation (see Table 2).
spheres prepared with 20 mg of trimethoprim occurred during acquiring of the image due to the energy of the electron beam. While PLGA 2300 microparticles were spherical as well, overall higher porosity was visible (Figure 4), which was expected to offer fast degradation and free diffusion pathways for improved drug release.

The release profile of trimethoprim from PLGA 503H and PLGA 2300 microparticles was analyzed in two different media, artificial urine (Figure 5) and HEPES/PLU (Figure 6). The assay was performed for 2 weeks at 4 °C and room temperature to evaluate trimethoprim leakage in possible storage conditions and at 37 °C to mimic the physiological conditions in the bladder.

2.2.1. Release Profile at 4 °C. The maximum release of trimethoprim at 4 °C from both PLGA 503H and PLGA 2300 microparticles was below 20% of the entrapped drug in either media (Figures 5 and 6).

The highest correlation to mathematical models for PLGA 503H in artificial urine was to Weibull (adjusted $R^2 = 0.974$) and Higuchi and Korsmeyer–Peppas (adjusted $R^2 = 0.973$ and 0.967, respectively). In buffer, the adjusted $R^2$ values were 0.984 to both Weibull and Higuchi and 0.976 to Korsmeyer–Peppas. The exponent $b$ of the Weibull function amounted to 0.54–0.60, which is regarded as diffusion in fractal or disordered substrates. The diffusional exponent (n) in the Korsmeyer–Peppas model was 0.69 (artificial urine) or 0.62 (HEPES/PLU) and suggests an anomalous, non-Fickian release mechanism at 4 °C.

PLGA 2300 microparticles correlated well with Korsmeyer–Peppas (adjusted $R^2 = 0.929$) and Weibull (adjusted $R^2 = 0.902$) in artificial urine and, in HEPES/PLU, with Korsmeyer–Peppas (adjusted $R^2 = 0.992$), Weibull (adjusted $R^2 = 0.989$), first order and zero order (adjusted $R^2 = 0.982$ for both), and Higuchi (adjusted $R^2 = 0.911$). The Weibull function suggests a complex release mechanism ($b = 1.05$) in artificial urine and diffusion in a normal Euclidian space ($b = 0.74$) in buffer, while the Korsmeyer–Peppas model suggests a zero-order release ($n = 1.23$) in artificial urine, which is in line with the high correlation to the zero-order fit, and an anomalous, non-Fickian release in HEPES/PLU ($n = 0.86$).

Since the drug loss from both types of microparticles in HEPES/PLU was smaller than 3%, the particles are expected to be safely stored at 4 °C without significant loss of efficacy for up to 2 days. For longer storage, however, the formulation should be kept lyophilized to prevent loss of trimethoprim.

2.2.2. Release Profile at Room Temperature. The release profiles from PLGA 503H microparticles in HEPES/PLU at room temperature and 4 °C were comparable (Figure 6A). Thus, storage for up to 2 days should be possible in buffer at room temperature. In comparison to pH 7.4, the 1.5-fold release at pH 5.0 (Figure 5A) indicates the contribution of acidic microclimate to random hydrolysis and, thus, accelerated degradation of the polyester. Release from PLGA 503H microparticles correlated well with Higuchi in artificial urine (adjusted $R^2 = 0.976$) and, in HEPES/PLU (adjusted $R^2 = 0.987$), with Weibull (adjusted $R^2 = 0.986$ and 0.983) and Korsmeyer–Peppas (adjusted $R^2 = 0.983$ and 0.987). Both the Weibull function ($b = 0.40$ in artificial urine and 0.47 in buffer) and the Korsmeyer–Peppas model ($n = 0.46$ and 0.50) suggest a Fickian release.

Particles prepared from PLGA 2300 complied best the Weibull (adjusted $R^2 = 0.996$) and Korsmeyer–Peppas model (0.995) as well as zero order and first order (0.970) and Higuchi (0.935). In both artificial urine and buffer, an anomalous, non-Fickian release ($n = 0.81$ and 0.85) and diffusion in a normal Euclidian space ($b = 0.69$ and 0.73) was calculated.

The faster drug release from PLGA 2300 microparticles at room temperature in HEPES/PLU as well as in artificial urine

Figure 4. Overview and detailed scanning electron microscopic images of microparticles prepared from PLGA 503H with different trimethoprim amounts added during preparation (see Table 2).

Barthelmes et al. faced similar issues when trying to encapsulate trimethoprim into chitosan-based nanoparticles, only being able to achieve a payload of under 5%. When coupled with starch microparticles, the drug load was capped at 5% as well. Due to the hydrophilic nature of trimethoprim and further functionalization, PLGA with free carboxylic groups were chosen over their end-capped counterparts. The former choice also proved advantageous for the encapsulation of the hydrophilic antibiotic levofloxacin.

PLGA 2300, however, proved to be vastly superior for encapsulation of this particular antibiotic with a 2-fold increase in capacity over PLGA 503H and over 20% increase over previous studies about trimethoprim encapsulation.

2.2. Release Profile of Trimethoprim from PLGA Microparticles. The release profile from PLGA is triggered by two key parameters: temperature and pH. Raising the temperature increases diffusivity of degradative protons as well as flexibility of the polymer chains. As PLGA is also known to be preferably degraded by acid-driven hydrolysis, the acidity of the medium promotes release of the drug.
indicates higher pH and also thermosensitivity of this polymer type. Due to the lower molecular weight of the polymer backbone, it is more likely that the influence of pH on hydrolytic scission of the polymer leads to more easy and premature formation of water-soluble oligomers and, thus, accelerated release of trimethoprim. Within 2 days, more than 20% of the entrapped trimethoprim was released in both media. Keeping the PLGA 2300 microparticles at room temperature for a longer time after reconstitution would not be advisable.

2.2.3. Release Profile at 37 °C. At 37 °C (Figures 5 and 6), an initial burst of about 30% was observed after 24 h and the whole drug content was released after 2 weeks, independent of the type of PLGA and the medium applied. In artificial urine, PLGA 503H (Figure 5A) microspheres released more than 50% of the encapsulated antibiotic after 4 days and reached a plateau after 10 days. In the case of PLGA 2300 (Figure 5B) microspheres, 50% of trimethoprim was liberated even after 2 days and a steady concentration was reached after 7 days.

Calculating the best fit, the adjusted $R^2$ of PLGA 503H particles in artificial urine and buffer suggested the highest correlation to Higuchi (0.967 and 0.917), Weibull (0.950 and 0.968), and Korsmeyer–Peppas models (0.962 and 0.959), while the exponents suggest a Fickian diffusion ($b = 0.41$ and 0.35; $n = 0.47$ and 0.40). PLGA 2300 microparticles correlated well with Weibull and Korsmeyer–Peppas in artificial urine (0.933 and 0.923) and HEPES/PLU (0.976 and 0.968). In buffer, high correlation was also observed with the zero-order (0.942), first-order (0.943), and Higuchi (0.928) models. Both Weibull and Korsmeyer–Peppers indicate a Fickian release regardless of suspension medium ($n = 0.44$ in artificial urine and 0.43 in buffer; $b = 0.38$ for both).

While the initial burst release was comparable in both PLGA types, PLGA 2300 proved advantageous regarding the subsequent higher drug release rate. Karp et al. have observed an even higher initial burst release from florfenicol-loaded microparticles, prepared with another low-molecular-weight PLGA. Trimethoprim is diffusely distributed throughout the matrix, but trimethoprim located on the surface is released, first provoking a strong burst effect. Both bulk erosion of PLGA and diffusional processes facilitate the release of the antibiotic. The release pattern consisting of a burst release followed by a plateau phase is in agreement with previous findings.

Thus, PLGA 2300 is more suitable for instillitative therapy, considering the limited time span the drug carrier can reside inside the bladder before being washed out.

Figure 5. Amount of trimethoprim released from microparticles prepared from (A) PLGA 503H and (B) PLGA 2300 in artificial urine (pH 5) at 4 °C, room temperature, and 37 °C.
2.3. Binding of Lectin Coupled Microparticles on SV-HUC Cell Monolayer. Constant urine production in the bladder impedes direct drug application due to washing out and removal via micturition after a short time. A strategy to delay this process is to enhance the binding of the microparticles to the bladder cells by means of a lectin corona on the particle surface. To approach this aim, the surface of the microparticles was modified with fluorescein-labeled wheat germ agglutinin (WGA).

To get an idea of the adhesion capability of lectin-functionalized PLGA microparticles on bladder cells, an in vitro assay using a monolayer of SV-HUC cells was performed. SV-HUC cells are immortalized, healthy human uroepithelial cells that display no tumorigenic characteristics. To assess the bioadhesion of the microparticles, suspensions of unmodified and WGA-modified microparticles were incubated with the cell monolayer and nonbound particles were removed by subsequent washings. After both steps, microscopic images were acquired. The microscopic evaluation displayed in Figure 7 (PLGA 2300 particles) and Figure 8 (WGA-PLGA 2300 particles) indicates a higher cell binding rate of the WGA-modified microparticles as compared to plain microparticles after washing. Due to lectin-mediated, improved adhesion, intravesically applied particles might remain longer in the bladder, surpassing even the dwell time of the instilled media. The prolonged residence time together with prolonged release of the antibiotic and shortened diffusional pathway of the released drug might improve the efficacy of this therapeutic approach. Nevertheless, further assays are necessary to confirm this observation, considering that fluorescein-labeled WGA might not exhibit the same binding potential as the nonlabeled WGA.

3. CONCLUSIONS

Loading PLGA 2300 microparticles with trimethoprim yielded a better result than encapsulation in PLGA 503H, increasing the loading capacity 2-fold while retaining similar particle sizes. The lower-molecular-weight PLGA 2300 facilitates a more rapid degradation of the polyester even in an acidic environment. While release profiles were similar initially, an overall faster liberation of trimethoprim from the low-molecular-weight PLGA furthers the advantages of this novel carrier. As preliminary studies with bioadhesive-coated microspheres yielded extremely promising results, the gap in therapeutic efficacy will widen even further. All in all, PLGA 2300 proved to be a vastly superior matrix for the intended...
application than the commonly used PLGA 503H. Due to faster drug release and higher drug loading capacity, this novel polymer seems to be better suited for use in intravesical therapy when treating urinary tract infections.

4. MATERIALS AND METHODS

4.1. Materials. PLGA (Resomer RG 503 H, poly(D,L-lactide-co-glycolide)) was obtained from Boehringer Ingelheim (Ingelheim, Germany), and PLGA Resomer Sample CR Type RG 50:50 Mn2300 (PLGA 2300) was kindly provided by Evonik Nutrition & Care GmbH (Darmstadt, Germany). Trimethoprim, Kolliphor P 188 (Pluronic F68), Kolliphor P 407 (Pluronic F127), N-hydroxy succinimide (NHS), ethylenediaminetetraacetic acid (EDTA), creatinine, trisodium citrate, and sodium phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fluorescein-labeled wheat germ agglutinin (f-WGA) was provided by Vector Laboratories, Inc. (Burlingame, CA, USA). Chloroform (≥98%) was obtained from VWR International (Pennsylvania, USA), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC), ethyl acetate (≥99.5%), sodium chloride, disodium oxalate, HEPES (Pufferan, ≥99.5% buffer grade), and glycerine were purchased from Carl-Roth GmbH & Co.KG (Karlsruhe, Germany).

4.2. Preparation of Trimethoprim-Loaded PLGA Microspheres. The microspheres were prepared from an oil-in-water emulsion following a solvent evaporation protocol. For that purpose, 400 mg of either PLGA 503H or PLGA 2300 was added to a solution of 40 mg of trimethoprim in 3.68 mL chloroform and dissolved at 4 °C. This organic phase was quickly poured into 8 mL of an aqueous solution of Pluronic F68 (1%, w/v) and dispersed for 5 min at level 4 with an ULTRA-TURRAX T8 homogenizer (IKA Labortechnik, Staufen, Germany) (emulsion 1). To harden the droplets, this emulsion was diluted with 100 mL of an aqueous solution of Pluronic F68 (3%, w/v) (emulsion 2), and solvent evaporation was facilitated by stirring with an OMNI 5000 Homogenizer (Omni International, Kennesaw, GA, US) for 60 min at speed level 2. Residual amounts of chloroform were removed by rotary evaporation considering the distinct glass-transition temperatures of the PLGA types in use. In the case of PLGA 503H, the vacuum was set at 130 mbar for 30 min followed by a pressure of <10 mbar for another 30 min. In the case of PLGA 2300, evaporation started at 300 mbar for 30 min followed by lowering of the pressure every 10 min by 50 mbar and finally keeping it at <10 mbar for 5 min.

The particle suspension was centrifuged at 3200 rpm and 4 °C for either 10 min (PLGA 503H) or 15 min (PLGA 2300). The pellet was resuspended in 20 mM HEPES/NaOH (pH...
4.3. Characterization of the Microspheres. 4.3.1. Determination of the Particle Size Distribution. The particle size distribution was analyzed by laser diffraction (Mastersizer 3000, Malvern Instruments, Malvern, U.K.). To guarantee reliable results, the microparticle suspension in HEPES/PLU was dispersed in a 0.1% (w/v) aqueous solution of Pluronic. Measurements were performed at 5–10% laser obscuration while being stirred at 1700 rpm.

4.3.2. Trimethoprim Content of the Microparticles. The amount of encapsulated trimethoprim was determined by high-performance liquid chromatography (HPLC) using an Agilent 1100 Series system (Agilent Technologies, Santa Clara, CA, USA) equipped with an UV diode array detector.

4.3.2.1. Sample Preparation. Lyophilized microparticles (2.0–8.0 mg) were dissolved in 2 mL of ethyl acetate. By adding 1 mL of 0.1% (v/v) aqueous formic acid solution and thoroughly mixing, trimethoprim was accumulated in the aqueous phase. The extraction step was repeated twice, the collected aqueous layers were lyophilized, and the lyophilate was dissolved in 0.1 mL of 0.1% (v/v) aqueous formic acid solution for analysis.

4.3.2.2. Quantification with HPLC. After injection of a 5 μL sample, separation was achieved on an analytical RP-18e column (Acclaim 120, Thermo Scientific, Waltham, MA, USA) by applying a linear gradient with 0.1% aqueous formic acid solution and 0.1% (v/v) formic acid in acetonitrile from 1 + 99 to 95 + 5 within 10 min as a mobile phase. The flow rate was set to 0.5 mL/min and trimethoprim was detected at 280 nm.

The limit of detection based on the calibration curve and calculated with the standard deviation of the regression line is the number of dissolution data points (M/t), and p is the number of parameters in the model.

4.3.4. Scanning Electron Microscopy (SEM). Microparticles were suspended in 20 mM HEPES/NaOH (pH 7.4) with 0.1% (w/v) Pluronic F68 and a drop was placed on a 0.1 μm polycarbonate membrane filter. After drying in vacuo, the samples were sputter-coated with gold and examined in a FlexSEM 1000 (Hitachi High-Technologies Corporation, Tokyo, Japan) scanning electron microscope at 20 kV (accelerating voltage).

4.4. Surface Modification of PLGA Microparticles with WGA. The surface of PLGA microparticles was modified by covalently binding wheat germ agglutinin (WGA) to freely accessible carboxylates. For this purpose, 5 mg of lyophilized microparticles was suspended in 500 μL of 20 mM HEPES/NaOH (pH 8, HEPES-8). Free carboxyl groups of PLGA were activated by incubation with a solution containing 281 mg of EDAC and 11.74 mg of NHS in 500 μL of HEPES-8 for 30 min at 4 °C. Excessive coupling reagents were removed by washing twice with 1.5 mL of HEPES-8 and centrifugation at 14,000 rpm for 3 min at 4 °C. Finally, the suspension volume was adjusted to 150 μL.

For the coupling reaction, 50 μL of F-WGA (5 mg/mL HEPES-8) was added and the solution was stirred at 4 °C under light protection for 2 h. To block remaining active binding sites, 242 μL of glycine solution (100 mg/mL HEPES-8) was added and the solution was stirred for another 30 min.

To remove excessive coupling agents, the microparticles were centrifuged (4 °C, 14000 rpm, 5 min) and the supernatant was discarded. HEPES-8 (1.5 mL) was added and this washing step was repeated once. Three more washing steps were performed in the same way but using distilled water instead of HEPES-8 to purify the microparticles. Finally, the WGA-functionalized PLGA microparticles were lyophilized.

4.5. Cell Culture. 4.5.1. Cultivation of SV-HUC Cells. SV-HUC is a cell line originating from human uroepithelial cells and immortalized by transfection with Simian Virus 40. The cells were obtained from American Type Culture Collection (Rockville, USA) and used between passages 35 and 48. SV-HUC cells were cultivated in Ham's F12 medium at 37 °C in 5% CO₂/95% air atmosphere and 95% relative humidity. The cells were subcultivated with trypsin/EDTA at approximately 80% confluence and seeded at a density of 3,260,000 cells/mL into 75 cm² cell culture flasks for further cultivation.

4.5.2. Cultivating Monolayers. SV-HUC cell monolayers were cultivated in 96-well microplates as well as in flexiPERM micro12 mounted glass coverslips after seeding 17,000 cells/well. To avoid contamination, the flexiPERM and the glass coverslips were disinfected with 70% ethanol before use. For binding assays, cells were used after reaching 100% confluence, typically achieved after 7 to 8 days of incubation.
4.6. Binding Assay of WGA-PLGA Microparticles to SV-HUC Monolayers. After removal of the supernatant, the SV-HUC monolayers, cultivated either in HEPES/NaOH buffer (pH 7.4) (HEPES-isoo). A homogeneous dispersion of 40 μL of WGA-PLGA microparticles (1 mg/mL in HEPES-isoo) in 100 μL of HEPES-isoo was added followed by incubation for 30 min at 4 °C. PLGA microparticles without any surface modification served as a negative control. Particle loading was observed microscopically using a Zeiss EpiFluorescence AxioObserver.Z1 deconvolution microscopy system (Carl Zeiss, Oberkochen, Germany). After washing the monolayers twice with 100 μL of HEPES-isoo and adding 150 μL of the same buffer, the amount of cell-bound microparticles was examined microscopically.

4.7. Statistical Analysis. Statistical analysis was performed with SigmaPlot 13 software (Systat Software, Inc., San Jose, CA, USA). All data are presented as mean ± standard deviation (SD). Comparisons between groups were evaluated using t-test and one-way ANOVA. P values of ≤0.05 were considered statistically significant.

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Notes
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ABBREVIATIONS
HEPES/PLUTab20 mM HEPES/NaOH (pH 7.4) containing 1% (w/v) Pluronic F68; HEPES-8tab20 mM HEPES/NaOH (pH 8); HEPES-isotabisotone 20 mM HEPES/NaOH buffer (pH 7.4)

REFERENCES
(1) Kennedy, J. L.; Haberling, D. L.; Huang, C. C.; Lessa, F. C.; Lucero, D. E.; Daskalakis, D. C.; Vora, N. M. Infectious Disease Hospitalizations: United States, 2001 to 2014. Chest 2019, 156, 255–268.
(2) Harding, G. K. M.; Ronald, A. R. The Management of Urinary Infections; What Have We Learned in the Past Decade? Int. J. Antimicrob. Agents 1994, 4, 83–88.
(3) Flores-Mireles, A. L.; Walker, J. N.; Caparon, M.; Hultgren, S. J. Urinary Tract Infections: Epidemiology, Mechanisms of Infection and Treatment Options. Nat. Rev. Microbiol. 2015, 13, 269–284.
(4) Hooton, T. M. Uncomplicated Urinary Tract Infection. N. Engl. J. Med. 2012, 366, 1028–1037.
(5) McEllan, L. K.; Hunstad, D. A. Urinary Tract Infection: Pathogenesis and Outlook. Trends Mol. Med. 2016, 22, 946–957.
(6) Naber, K. G.; Bergman, B.; Bishop, M. C.; Bjerklund-Johansen, T. E.; Botto, H.; Lobel, B.; Jinenez Cruz, F.; Selvaggi, F. P.; Urinary Tract Infection (UTI) Working Group of the Health Care Office (HCO) of the European Association of Urology (EAU). EAU Guidelines for the Management of Urinary and Male Genital Tract Infections. Eur. Urol. 2001, 40, 576–588.
(7) Foxman, B. Urinary Tract Infection Syndromes. Occurrence, Recurrence, Bacteriology, Risk Factors, and Disease Burden. Infect. Dis. Clin. North Am. 2014, 28, 1–13.
(8) Naber, K. G.; Schito, G.; Botto, H.; Palou, J.; Mazzei, T. Surveillance Study in Europe and Brazil on Clinical Aspects and Antimicrobial Resistance: Epidemiology in Females with Cystitis (ARESC): Implications for Empiric Therapy. Eur. Urol. 2008, 54, 1164–1178.
(9) Neuhaus, J.; Schwalenberg, T. Intravesical Treatments of Bladder Pain Syndrome/Interstitial Cystitis. Nat. Rev. Urol. 2012, 9, 707–720.
(10) Khandelwal, P.; Abraham, S. N.; Apodaca, G. Cell Biology and Physiology of the Uroepithelium. Am. J. Physiol.-Renal Physiol. 2009, F1477–F1501.
(11) Mulvey, M. A.; Schilling, J. D.; Hultgren, S. J. Establishment of a Persistent Escherichia Coli Reservoir during the Acute Phase of a Bladder Infection. Infect. Immun. 2001, 69, 4572–4579.
(12) Bonkat, G.; Pickard, R.; Bartoletti, R.; Cai, T.; Bruyère, F.; Geerlings, S. E.; Köves, B.; Wagenlehner, F. EAU Guidelines on Urological Infections 2018. In European Association of Urology Guidelines, 2018 Edition.; European Association of Urology Guidelines Office: Arnhem: The Netherlands, 2018; Vol. presented.
(13) Pietropaolo, A.; Jones, P.; Moors, M.; Birch, B.; Somani, B. K. Use and Effectiveness of Antimicrobial Intravesical Treatment for Prophylaxis and Treatment of Recurrent Urinary Tract Infections (UTIs): A Systematic Review. Curr. Urol. Rep. 2018, 19, 78.
(14) GuhaSarkar, S.; Banerjee, R. Intravesical Drug Delivery: Challenges, Current Status, Opportunities and Novel Strategies. J. Controlled Release 2010, 148, 147–159.
(15) Neutsch, L.; Wambacher, M.; Wirth, E. M.; Sipjker, S.; Kählig, H.; Wirth, M.; Gabor, F. UPEC Biomimicry at the Urothehal Barrier: Lecin-Functionalized PLGA Microparticles for Improved Intravesical Chemotherapy. Int. J. Pharm. 2013, 450, 163–176.
(16) Zacche, M. F.; Sinkina, S.; Cardozo, L. Novel Targeted Bladder Drug-Delivery Systems: A Review. Res. Rep. Urol. 2015, 7, 169–178.
(17) Jain, R. A. The Manufacturing Techniques of Various Drug Loaded Biodegradable Poly(Lactide-Co-Glycolide) (PLGA) Devices. Biomaterials 2000, 21, 2475–2490.
(18) Prajapati, V. D.; Jani, G. K.; Kapadia, J. R. Current Knowledge on Biodegradable Microspheres in Drug Delivery. Expert Opin. Drug Deliv. 2015, 12, 1283–1299.
(19) Makadia, H. K.; Siegel, S. J. Poly Lactic-Co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. Polymers 2011, 3, 1377–1397.
(20) Wang, X. Y.; Koller, R.; Wirth, M.; Gabor, F. Lectin-Coated PLGA Microparticles: Thermoresponsive Release and in Vitro
Evidence for Enhanced Cell Interaction. *Int. J. Pharm.* 2012, 436, 738–743.

(21) Abelha, T. F.; Neumann, P. R.; Holthof, J.; Dreiss, C. A.; Alexander, C.; Green, M.; Dailey, L. A. Low Molecular Weight PEG–PLGA Polymers Provide a Superior Matrix for Conjugated Polymer Nanoparticles in Terms of Physicochemical Properties, Biocompatibility and Optical/Photoacoustic Performance. *J. Mater. Chem. B* 2019, 7, 5115–5124.

(22) Zhao, X.; Gao, Y.; Tang, X.; Lei, W.; Yang, Y.; Yu, F.; Liu, Y.; Yang, M.; Wang, Y.; Gong, W.; Li, Z.; Gao, C.; Mei, X. Development and Evaluation of Ropivacaine Loaded Poly(Lactic-Co-Glycolic Acid) Microspheres with Low Burst Release. *Current Drug Delivery* 2019, 16, 490–499.

(23) Druglib.com. Trimethoprim - Description and Clinical Pharmacology http://www.druglib.com/druginfo/trimethoprim/description_pharmacology/ (accessed Mar 17, 2020).

(24) Barthelmes, J.; Perera, G.; Hombach, J.; Dünnhaupt, S.; Bernkop-Schnürch, A. Development of a Mucosally Adhesive Nanoparticulate Drug Delivery System for a Targeted Drug Release in the Bladder. *Int. J. Pharm.* 2011, 416, 339–345.

(25) Laakso, T.; Stjärnvik, P.; Sjöholm, I. Biodegradable Microspheres VI: Lysosomal Release of Covalently Bound Antiparasitic Drugs from Starch Microspheres. *J. Pharm. Sci.* 1987, 76, 134–140.

(26) Gaspar, M. C.; Pais, A. A. C. C.; Sousa, J. J. S.; Brillaut, J.; Olivier, J. C. Development of Levofloxacin-Loaded PLGA Microspheres of Suitable Properties for Sustained Pulmonary Release. *Int. J. Pharm.* 2019, 556, 117–124.

(27) Keles, H.; Naylor, A.; Clegg, F.; Sammon, C. Investigation of Factors Influencing the Hydrolytic Degradation of Single PLGA Microparticles. *Polym. Degrad. Stab.* 2015, 119, 228–241.

(28) Crotts, G.; Park, T. G. Protein Delivery from Poly(Lactic-Co-Glycolic Acid) Biodegradable Microspheres: Release Kinetics and Stability Issues. *J. Microencapsulation* 1998, 15, 699–713.

(29) Park, T. G.; Lu, W.; Crotts, G. Importance of in Vitro Experimental Conditions on Protein Release Kinetics, Stability and Polymer Degradation in Protein Encapsulated Poly (dl-Lactic Acid-Co-Glycolic Acid) Microspheres. *J. Controlled Release* 1995, 33, 211–222.

(30) Hines, D. J.; Kaplan, D. L. Poly(Lactic-Co-Glycolic Acid)-Controlled Release Systems: Experimental and Modeling Insights. *Crit. Rev. Ther. Drug Carrier Syst.* 2013, 30, 257–276.

(31) Papadopoulou, V.; Kosmidis, K.; Vlachou, M.; Macheras, P. On the Use of the Weibull Function for the Discernment of Drug Release Mechanisms. *Int. J. Pharm.* 2006, 309, 44–50.

(32) Ritger, P. L.; Peppas, N. A. A Simple Equation for Description of Solute Release I. Fickian and Non-Fickian Release from Non-Swellable Devices in the Form of Slabs, Spheres, Cylinders or Discs. *J. Controlled Release* 1987, 5, 23–36.

(33) Karp, F.; Busatto, C.; Turino, L.; Luna, J.; Estenoz, D. PLGA Nano- and Microparticles for the Controlled Release of Florfenicol: Experimental and Theoretical Study. *J. Appl. Polym. Sci.* 2019, 136, 47248–47210.

(34) Faisant, N.; Siepmann, J.; Benoit, J. P. PLGA-Based Microparticles: Elucidation of Mechanisms and a New, Simple Mathematical Model Quantifying Drug Release. *Eur. J. Pharm. Sci.* 2002, 15, 355–366.

(35) Christian, B. J.; Loretz, L. J.; Oberley, T. D.; Reznikoff, C. A. Characterization of Human Uroepithelial Cells Immortalized in Vitro by Simian Virus 40. *Cancer Res.* 1987, 47, 6066–6073.

(36) Siepmann, J.; Siepmann, F. Mathematical Modeling of Drug Delivery. *Int. J. Pharm.* 2008, 364, 328–343.

(37) Costa, P.; Sousa Lobo, J. M. Modeling and Comparison of Dissolution Profiles. *Eur. J. Pharm. Sci.* 2001, 13, 123–133.