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Development of ciprofloxacin-loaded contact lenses using fluorous chemistry

Guoting Qin [a, *], Zhiling Zhu [b], Siheng Li [b], Alison M. McDermott [a], Chengzhi Cai [b, **]

[a] College of Optometry, University of Houston, Houston, TX, 77204, USA
[b] Department of Chemistry, University of Houston, Houston, TX, 77204, USA

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ABSTRACT

In this work, we developed a simple method to load drugs into commercially available contact lenses utilizing fluorous chemistry. We demonstrated this method using model compounds including fluorous-tagged fluorescein and antibiotic ciprofloxacin. We showed that fluorous interactions facilitated the loading of model molecules into fluorocarbon-containing contact lenses, and that the release profiles exhibited sustained release. Contact lenses loaded with fluorous-tagged ciprofloxacin exhibited antimicrobial activity against *Pseudomonas aeruginosa* in vitro, while no cytotoxicity towards human corneal epithelial cells was observed. To mimic the tear turnover, we designed a porcine eye infection model under flow conditions. Significantly, the modified lenses also exhibited antimicrobial efficacy against *Pseudomonas aeruginosa* in the ex vivo infection model. Overall, utilizing fluorous chemistry, we can construct a drug delivery system that exhibits high drug loading capacity, sustained drug release, and robust biological activity.

1. Introduction

Currently more than 90% of ophthalmic drugs are delivered in the form of eye drop solutions [1]. However, only ~ 1–7% of the administered dose is actively absorbed [2]. Therefore, high drug dosage and frequent administration are necessary in order to have a therapeutic effect, which results in undesired toxicity and low patient compliance. In the search for alternative approaches, contact lenses as ocular drug delivery systems have attracted tremendous attention due to high ocular drug availability, less frequent administration, and low drug toxicity, which could potentially provide a more convenient treatment regime and better patient compliance [3–8].

The approaches to incorporate drugs into contact lenses have evolved in recent years. Initially, simple immersion of contact lenses in drug solution was tested and found to be insufficient due to its low loading efficiency and fast burst release within the first few minutes, which does not offer significant advantages compared to eye drops [9–17]. In some cases, lens transparency was largely decreased due to drug precipitation [14]. In attempts to overcome these issues, several strategies have emerged, such as molecular imprinting, covalent attachment of drug molecules to contact lenses, plasma treatment of the contact lenses, and delivery of drugs using nanomaterials. Molecular imprinting, a method that creates binding sites within contact lenses with high affinity for specific drugs, greatly improved loading and reproducibility [18–28]. A "layer-by-layer" design of contact lenses [29,30], in which a layer of drugs was sandwiched between two polymeric layers, may offer extended drug release. However, this method is difficult to be incorporated in to the contact lens manufacturing process, and some drugs may lose their biological activity during the process. Plasma treatment, a technology that has been long used to improve the wettability of contact lenses, has shown to desirably slow down the initial burst release of drugs from lenses [31,32]. However, the plasma conditions have to be carefully controlled as insufficient and aggressive treatments either do not have any effect or have the potential to result in the loss of the drugs' bioactivity. Recently contact lenses have been combined with nano-/micro- scale drug delivery systems such as liposomes [33], polymeric materials [15,27,30,34], and micelles [35]. These nano-/micro- scale drug delivery systems offer high drug loading capacity and suitability for nearly any kind of drug by rational design of the system. One recent approach described a polymeric "nanowafer" disk that was fabricated using lithography and loaded with drug into nano-/micro-

* Corresponding author.
** Corresponding author.
E-mail addresses: gqin@central.uh.edu (G. Qin), cai@uh.edu (C. Cai).

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sized holes, which greatly increased drug loading [36]. Each method has its own advantages in one or two areas, but unfortunately, none to date has provided simple manufacture process, high drug loading efficacy, satisfactory drug release kinetics, and high optical quality after drug incorporation. Furthermore, contact lens wear is associated with high risk of complications such as potentially vision threatening infection and inflammation [37–42]. With the widespread and rapid growth of contact lens wear not only for optical correction but also for purposes such as cosmesis and smart lenses with biosensors [43–51], it is essential to make contact lenses safer to wear by equipping them with antimicrobial and anti-inflammatory abilities. To this end, a recent design for safer contact lenses with covalently attached antimicrobial peptide has been tested in laboratory animals and humans [52]. Overall, novel designs of contact lens drug delivery systems are needed to address all the above issues. In this work, we developed a simple yet effective method based on fluorous chemistry to incorporate drugs into contact lenses that has great potential to provide the desirable characteristics of an ideal contact lens drug delivery system.

Fluorous chemistry is based on the unique fluorous interactions with which fluorocarbons are attracted to each other much more than to other media [53–55]. Fluorous interactions have been widely used for anti-fouling coatings [56–58]. Furthermore, perfluorocarbons are used to enhance oxygen delivery in contact lenses, ultrasound imaging and therapy [59–64]. We have used fluorous interactions for incorporating active groups such as alkynes or carboxylic acids onto various substrate surfaces including contact lens surfaces pre-modified with fluorous chains [65]. The functional molecules such as antimicrobial peptides are then covalently attached to the surface via click chemistry. However, this approach has its limitations. In particular, this approach is a 2-step procedure, and during the covalent attachment step, fluorous molecules are susceptible to dissociation from the surface. To keep the molecules on the surface requires a very strong fluorous interaction, thus limiting its application to contact lenses with a high content of fluorocarbon chains, which is not commonly found in commercial contact lenses. Furthermore, the toxic copper catalysts used for covalent modification of contact lenses may be difficult to remove.

In this work, we overcome the above limitations by attaching a short fluorous tag to the drug molecule. We show that the fluorous-tagged drugs can be easily loaded to common fluorine-containing contact lenses and the system exhibits a desired release profile.

2. Methods and materials

2.1. Materials

Ciprofloxacin, trimethylamine, methylene chloride, heptafluorobutyric acid, perfluoropentanoic acid, fluorescein isothiocyanate (FITC) and perfluoroheptanoic acid were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. *Pseudomonas aeruginosa* strain 19660 was purchased from ATCC. A telomerase transformed human corneal epithelial cell line (hTCEpi) [66] was used for in vitro studies.

2.2. Synthesis of fluorous-tagged FITC and ciprofloxacin derivatives

2.2.1. Synthesis of fluorous-tagged FITC (FITC-F)

A solution of 1H, 1H-perfluorooctylamine (2 mM, 10 mL) in ethanol was added dropwise to a stirred solution of Fluorescein isothiocyanate (FITC, 15.57 mg) in 10 mL of ethanol at room temperature. The solution was stirred for 24 h, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography eluted with ethyl acetate/MeOH 9/1 to give **FITC-F** (22 mg, 93%) as a green powder. ^{1}H NMR (500 MHz, acetone-d6) δ 9.05 (s, 2H), 7.98–7.89 (m, 2H), 7.26 (dd, J = 8.1, 7.0 Hz, 1H), 6.75 (d, J = 2.5 Hz, 2H), 6.72 (d, J = 4.0 Hz, 1H), 6.70 (d, J = 3.5 Hz, 1H), 6.65–6.64 (m, 1H), 6.63 (dt, J = 4.9, 1.8 Hz, 1H), 4.75 (dtd, J = 48.0, 16.4, 6.2 Hz, 2H), 4.47 (q, J = 7.2 Hz, 1H). ^{13}C NMR (126 MHz, Acetone-d6) δ 184.39, 169.11, 169.07, 160.30, 153.35, 153.32, 153.28, 149.98, 146.63, 141.81, 131.30, 130.17, 130.13, 130.09, 128.40, 125.88, 125.17, 125.12, 124.75, 119.22, 119.07, 117.36, 116.63, 113.30, 111.65, 111.58, 111.55, 103.33, 55.44. ^{19}F NMR (471 MHz, $CDCl_3$) δ −81.44 − 81.64 (m, 3F), −117.09 − 117.92 (m, 2F), −122.23 (s, 2F), −122.46 (s, 2F), −123.18 (s, 2F), −123.94 (s, 2F), −126.63 (td, J = 14.6, 6.9 Hz, 2F). MALDI-TOF-MS m/z: $[M]^+$ calcd for $C_{29}H_{17}F_{15}N_2O_5S$: 790.06; found: 790.10.

2.2.2. Synthesis of fluorous-tagged ciprofloxacin derivatives compound ***1–4***

Ciprofloxacin (250 mg, 0.75 mmol) and triethylamine (139 μL, 1 mmol) were stirred in anhydrous methylene chloride (5 mL) at 0 °C for 15 min. Heptafluorobutyric acyl chloride (259.8 mg, 1.12 mmol) was added dropwise into the mixture under nitrogen atmosphere. The suspension was stirred at room temperature for 12 h, and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography eluted with ethyl acetate/acetone/dichloromethane (v:v:v = 10:1:20) to give **1** (173.9 mg, 33%) as a white powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.73 (s, 1H), 8.01 (d, J = 12.7 Hz, 1H), 7.38 (d, J = 7.0 Hz, 1H), 3.96 (dd, J = 8.9, 4.7 Hz, 4H), 3.56 (s, 1H), 3.45–3.36 (m, 4H), 1.42 (q, J = 6.6 Hz, 2H), 1.21 (t, J = 5.0 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.15, 166.83, 156.47, 154.70, 152.70, 147.81, 145.03, 139.05, 120.82, 112.95, 112.77, 108.40, 105.50, 50.17, 49.42, 45.95, 43.55, 35.49, 8.43. ^{19}F NMR (470 MHz, $CDCl_3$) δ −79.58 (t, J = 9.5 Hz, 3F), −111.46 − 111.53 (m, 2F), −121.14 − 121.21 (m, 2F), −125.51 − 125.57 (m, 2F). MS (ESI) m/z: $[M+H]^+$ calcd for $C_{21}H_{18}F_8N_3O_4$ = 528.12; found 528.09.

Compound **2** was similarly obtained in 178.9 mg, 31% as a yellow powder. ^{1}H NMR (500 MHz, Acetone-d6) δ 8.70 (s, 1H), 7.93 (d, J = 13.1 Hz, 1H), 7.79 (d, J = 7.4 Hz, 1H), 4.06–3.94 (m, 4H), 3.88 (dd, J = 7.1, 3.5 Hz, 1H), 3.61–3.52 (m, 4H), 1.48 (d, J = 6.0 Hz, 2H), 1.39–1.30 (m, 2H). ^{13}C NMR (126 MHz, Acetone-d6) δ 177.89, 166.64, 156.59, 155.42, 153.44, 149.00, 145.87, 145.79, 140.35, 130.20, 129.37, 127.17, 120.96, 112.31, 112.12, 108.59, 107.80, 50.73, 50.05, 46.41, 44.04, 36.61, 8.49. ^{19}F NMR (471 MHz, $CDCl_3$) δ −80.86 (t, J = 9.8 Hz, 3F), −110.94 (t, J = 12.2 Hz, 2F), −121.09 (dd, J = 12.7, 6.9 Hz, 2F), −121.86 (dddd, J = 13.4, 10.0, 6.7, 3.4 Hz, 2F), −124.50 − 124.62 (m, 2F). MS (ESI) m/z: $[M+H]^+$ calcd for $C_{22}H_{18}F_{10}N_3O_4$ = 578.11; found 578.08.

Compound **3** was similarly obtained in 202 mg, 40% yield as a pale yellow powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 (s, 1H), 7.86 (d, J = 12.7 Hz, 1H), 7.34 (d, J = 7.0 Hz, 1H), 3.93–3.71 (m, 4H), 3.61–3.51 (m, 1H), 3.47–3.28 (m, 4H), 2.68 (dd, J = 9.6, 6.2 Hz, 2H), 2.49 (ddd, J = 17.6, 14.4, 7.8 Hz, 2H), 1.40 (q, J = 6.7 Hz, 2H), 1.20 (q, J = 6.5 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 176.91, 168.77, 167.31, 154.64, 152.64, 147.63, 145.46, 145.37, 139.06, 120.04, 119.98, 112.50, 112.31, 107.84, 105.24, 49.89, 49.36, 45.23, 41.67, 35.53, 26.57, 26.40, 26.23, 24.38, 8.32. ^{19}F NMR (470 MHz, $CDCl_3$) δ −80.62 (t, J = 9.9 Hz, 3F), −110.68 (t, J = 13.4 Hz, 2F), −120.60 (s, 2F), −121.00 (dd, J = 15.2, 8.0 Hz, 2F), −121.14 (dd, J = 12.7, 6.9 Hz, 2F), −122.67 (s, 2F), −125.81 − 125.92 (m, 2F). MS (ESI): $[M+H]^+$ calcd for $C_{22}H_{22}F_6N_3O_4$ = 506.1, Found 506.2.

Compound **4** was similarly obtained in 203 mg, 30% yield as a yellow powder. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.05 (d, J = 12.7 Hz, 1H), 7.38 (d, J = 6.6 Hz, 1H), 3.96 (d, J = 11.8 Hz, 4H), 3.55 (s, 1H), 3.40 (s, 4H), 1.42 (d, J = 5.9 Hz, 2H), 1.22 (s, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.23, 166.85, 156.57, 154.73, 152.73, 147.87, 144.95, 139.08, 121.04, 120.98, 113.10, 112.92, 111.37, 110.84, 110.62,