Supplementary Information

Fluorous Biphase Drug Delivery System Triggered by Low Frequency Ultrasound: Controlled Release from Perfluorous Discoidal Porous Silicon Particles

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SUPPLEMENTARY EXPERIMENTAL SECTION

1. Methods

*Nuclear magnetic resonance (NMR)*. NMR spectra were recorded at 25 °C on a JEOL ECA-500 spectrometer. The chemical shifts were recorded in ppm relative to tetramethyilsilane (TMS) and with the solvent resonance as the internal standard. $^1$H, $^{13}$C, and $^{19}$F NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad), coupling constants ($J$) were reported in hertz (Hz), integration.

*Electrospray ionization liquid chromatography mass spectrometry (ESI-LC-MS)*. ESI-LC-MS analyses were performed on Thermo Finnigan LCQ Deca XP Plus LC/MS with a C18 column (Kinetex™ 5 µm XB-C18 100 Å, LC Column 50 × 4.6 mm, Phenomenex, USA) in gradient elution (10 – 95% Acetonitrile in H$_2$O, 12 min). The injection amount was 1 µL. Reactions requiring anhydrous conditions were performed under nitrogen or argon.

*Transmission electron microscopy (TEM)*. An aliquot of diluted particle suspension was applied to a carbon holey film on a copper grid. TEM analysis was performed with JEOL 2000 FX and JEOL 2010 FEG microscopes equipped with energy dispersive spectrometers (EDS) at accelerating voltage of 200 kV. Each sample was analyzed by conventional and high resolution (HR) TEM, selective area electron diffraction (SAED) and energy dispersive spectroscopy (EDS).

*Scanning electron microscopy (SEM)*. The scanning electron microscopy (SEM) images were taken by a SU8010 model instrument operated at an accelerating voltage of 10 kV. Samples for SEM imaging were prepared on a clean silicon wafer.

*X-ray photoelectron spectroscopy (XPS)*. The unmodified or modified fluorous particles were deposited on a 200 µm × 200 µm silicon substrate. A PHI 5700 X-ray
photoelectron spectrometer equipped with a monochromatic Al Kα X-ray source (1486.7 eV) at a takeoff angle (TOA) of 45° was used to analyze the sample. The spectrometer was operated at both high and low resolutions with pass energy of 23.5 and 187.85 eV, respectively. Electron binding energies were calibrated with respect to the O1s at 532.9 eV.

*Fourier transform infrared spectroscopy (FTIR).* The unmodified or modified fluorous particles were directly transferred on the sample stage. FTIR spectra were acquired using a Nicolet™ iST™10 FT-IR Spectrometer. The spectra were collected over 16 scans at a resolution of 2 cm\(^{-1}\).

*UV-Vis spectroscopy.* The UV-Vis absorption spectra were recorded UV-Vis spectrophotometer (Shimadzu UV-1800, Japan) in the range of 300 – 800 nm.

2. **Synthesis of fluorous-tagged compounds**

*Synthesis of perfluorooctanyl fluorescein isothiocyanate (FITC-C\(_{8}\)F\(_{15}\)).* FITC (15.6 mg, 0.04 mmol) was dissolved in ethanol (10 mL). 1H,1H-perfluorooctylamine (8 mg, 0.02 mmol) in ethanol (10 mL) was added dropwise into the FITC solution at room temperature under mild magnetic stirring and kept stirring for 24 hours. Afterwards, the solvent was evaporated and the residue was purified by flash column chromatography with 10% methanol in ethyl acetate as the eluent to give the product as a green powder, 22.0 mg, 93%. \(^1\)H NMR (500 MHz, Acetone-d6) \(\delta\) 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 (dt, \(J = 48.0, 16.4, 6.2\) Hz, 2H), 4.47 (q, \(J = 7.2\) Hz, 1H). \(^{13}\)C NMR (126 MHz, Acetone-d6) \(\delta\) 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, Acetone-d6) δ -117.45, -117.49, -117.51, -122.23, -122.46, -123.18, -123.75, -123.94, -126.59, -126.60, -126.62, -126.63, -126.65, -126.67. MS (ESI): [M+H] calcd for C$_{29}$H$_{18}$F$_{15}$N$_{2}$O$_{5}$S = 791.06; found 791.10.

3. **Bacterial cell culture**

*Pseudomonas aeruginosa* (PA) strain expressing green fluorescent protein and a carbenicillin resistance gene (PAO1-GFP) was purchased from ATCC. The bacteria were grown in LB media at 37 °C and harvested in the log growth phase. Cultures were centrifuged at 5000 rpm for 10 min to pellet cells.

4. **Plate counting assay**

The biofilm formation on the bottom of the 48-well plate was examined by the plate counting assay. Briefly, after 24-hour static incubation, the culture solutions in the 48-well plate were gently removed. 1 mL of 0.01% SDS solution was added into each well and the bacteria were detached by sonication at 25 °C for 1 min. Afterwards, the bacterial suspensions were serially diluted ($1, 10^{-2}, 10^{-4}, 10^{-6}$) and 100 µL of each aliquot was immediately plated on LB agars. The colonies formed were counted after 24-hour static incubation at 37 °C.

5. **Statistical analysis**

All data was obtained from at least three independent experiments and represented as mean ± standard deviation. The statistical significance of the data was determined by the Student’s $t$ test and the single factor one-way analysis of variance (ANOVA). A $P$ value < 0.05 was considered to be statistically significant.
Table S1. List of FDA Approved Fluorinated Pharmaceuticals.

| Uses          | Drug Name      | Brand Names          | Chemical Structure |
|---------------|----------------|----------------------|--------------------|
| Anesthetics   | Desflurane     | Suprane              | ![Desflurane_structure] |
|               | Droperidol     | Inapsine             | ![Droperidol_structure] |
|               | Enflurane      | Ethrane, Compound 347| ![Enflurane_structure] |
|               | Flumazenil     | Romazicon            | ![Flumazenil_structure] |
|               | Halothane      | Fluothane            | ![Halothane_structure] |
|               | Isoflurane     | Forane, Terrell      | ![Isoflurane_structure] |
|               | Methoxyflurane | Penthane             | ![Methoxyflurane_structure] |
|               | Midazolam      | Versed, Seizalam     | ![Midazolam_structure] |
|               | Sevoflurane    | Ultane, Sojourn      | ![Sevoflurane_structure] |
| Antacids      | Lansoprazole   | Prevacid             | ![Lansoprazole_structure] |
| Anti-anxiety  | Flurazepam     | Dalmane              | ![Flurazepam_structure] |
|               | Halazepam      | Paxipam              | ![Halazepam_structure] |
|               | Hydroflumethiazide | Saluron         | ![Hydroflumethiazide_structure] |
| Antibiotics   | Ciprofloxacin  | Cipro                | ![Ciprofloxacin_structure] |
| Antibiotics          | Brand Names                      | Structures            |
|----------------------|----------------------------------|-----------------------|
| Enoxacin             | Penetrex                         | ![Enoxacin Structure](image) |
| Flucloxacillin       | Floxapen                         | ![Flucloxacillin Structure](image) |
| Gatifloxacin         | Zymaxid, Zymar, Tequin           | ![Gatifloxacin Structure](image) |
| Gemifloxacin mesylate| Factive                          | ![Gemifloxacin Structure](image) |
| Grepafloxacin        | Raxar                            | ![Grepafloxacin Structure](image) |
| Levofloxacin         | Levaquin, Quixin, Iquix          | ![Levofloxacin Structure](image) |
| Linezolid            | Zyvox                            | ![Linezolid Structure](image) |
| Lomefloxacin         | Maxaquin                         | ![Lomefloxacin Structure](image) |
| Moxifloxacin         | Avelox                           | ![Moxifloxacin Structure](image) |
| Norfloxacin          | Noroxin                          | ![Norfloxacin Structure](image) |
| Ofloxacin            | Floxin                           | ![Ofloxacin Structure](image) |
| Sparfloxacin         | Zagam                            | ![Sparfloxacin Structure](image) |
| Temafloxacin         | Omniflox                         | ![Temafloxacin Structure](image) |
| Trovafloxacin mesylate| Trovan                          | ![Trovafloxacin Structure](image) |

**Antidepressants**
| Drug Name                  | Brand Name             |
|---------------------------|------------------------|
| Citalopram                | Celexa                 |
| Escitalopram              | Lexapro                |
| Fluoxetine                | Prozac, Sarafem, Rapiflux |
| Fluvoxamine maleate       | Luvox                  |
| Paroxetine                | Pexeva, Paxil, Brisdelle |

**Anti-fungal antibiotics**

| Drug Name                  | Brand Name             |
|---------------------------|------------------------|
| Fluconazole               | Diflucan               |
| Flucytosine               | Ancobon                |
| Voriconazole              | Vfend                  |

**Antihistamines**

| Drug Name                  | Brand Name             |
|---------------------------|------------------------|
| Levocabastine             | Livostin               |

**Antilipemics**

| Drug Name                  | Brand Name             |
|---------------------------|------------------------|
| Atorvastatin              | Lipitor                |
| Ezetimibe                 | Zetia                  |
| Fluvastatin sodium        | Lescol                 |
| **Anti-malarial**          |                  |                  |
|---------------------------|------------------|------------------|
| Halofantrine              | Halfan           | ![Chemical Structure](halofantrine.png) |
| Mefloquine                | Lariam           | ![Chemical Structure](mefloquine.png) |

| **Antimetabolites**       |                  |                  |
|---------------------------|------------------|------------------|
| Aprepitant                | Emend            | ![Chemical Structure](aprepitant.png) |
| Fluorouracil              | Adrucil          | ![Chemical Structure](fluorouracil.png) |

| **Arthritis**             |                  |                  |
|---------------------------|------------------|------------------|
| Celecoxib                 | Celebrex         | ![Chemical Structure](celecoxib.png) |
| Diflunisal                | Dolobid          | ![Chemical Structure](diflunisal.png) |
| Flurbiprofen              | Ansaid, Ocufen   | ![Chemical Structure](flurbiprofen.png) |
| Leflunomide               | Arava            | ![Chemical Structure](leflunomide.png) |
| Sulindac                  | Clinoril         | ![Chemical Structure](sulindac.png) |

| **Psychotropic**          |                  |                  |
|---------------------------|------------------|------------------|
| Fluphenazine              | Prolixin         | ![Chemical Structure](fluphenazine.png) |
| Haloperidol               | Haldol           | ![Chemical Structure](haloperidol.png) |
| Trifluoperazine           | Stelazine        | ![Chemical Structure](trifluoperazine.png) |

| **Steroids**              |                  |                  |
|---------------------------|------------------|------------------|
| Steroid            | Trade Name(s)                          |
|-------------------|----------------------------------------|
| Amcinonide        | Cyclocort                              |
| Clobetasol        | Temovate, Clobex, Dermovate, Impoyz    |
| Clocortolone      | Cloderm                                |
| Dexamethasone     | Baycadron, Decadron, Dexamethasone Intensol, DexPak, TaperDex, Zema-Pak, ZoDex, Zonacort |
| Diflorasone       | Psorcon, ApexiCon E, Apexicon, Maxiflor |
| Dutasteride       | Avodart                                |
| Flumethasone      | Flovent                                |
| Flunisolide       | Aerospan, Aerobid                      |
| Fluocinonide      | Lidex, Vanos                           |
| Fluorometholone   | FML, Flarex                            |
| Fluticasone propionate | Cutivate, Flonase, Flonase Allergy Relief |
| Flurandrenolide   | Cordran, Nolix                         |
| Hydroflumethiazide | Saluron |
|--------------------|--------|
| ![Chemical Structure](image_url) | ![Chemical Structure](image_url) |
**SUPPLEMENTARY DATA**

**Fig. S1** Calibration curve of the fluorescence intensity of emitted at 535 nm in function of the FITC-C₈F₁₅ concentration. Fluorescence Intensity = [FITC-C₈F₁₅] × 865850.7 + 985.6 ($R^2 = 0.9994$, red line is the corresponding linear fitting curve).
Fig. S2 Calibration curve for ciprofloxacin concentration vs. peak area. Each data point is the mean of four replicates and the error bar represents the standard deviation.

Calculation of the load rate of ciprofloxacin (Cip) on the fluorous particles:

(1) The Cip concentration in the solution was obtained from the mean detector signal (MDS) with reference to the standard curve in Fig. S2 using formula 1:

\[
[Cip] = \frac{MDS + 2.76 \times 10^6}{7.93 \times 10^6} \quad (1)
\]

(2) The load rate of Cip on the fluorous particles was calculated and the results were summarized in the Table S2.

Table S2. Calculation of the load rate of ciprofloxacin (Cip) on the fluorous particles.

| Sample ID | MDS     | Concentration (µM) | Load Rate |
|-----------|---------|--------------------|-----------|
| Sample 1  | 5273883 |                    |           |
| Sample 2  | 5040741 |                    |           |
| Sample 3  | 5370629 |                    |           |
| Avg       | 5228418 | 1.01               | 58%       |
| STD       | 169578  | 0.13               |           |
Fig. S3 High resolution F1s peak intensity of FAS17 functionalized porous silicon particles on a silicon wafer using perfluorosilane solution (0.1 – 10 mM).
Fig. S4 The pore size distribution of the release layer (A) and the device layer (B).
Fig. S5 Surface plot images at three different angles for the perfluorinated porous silicon particles after fluorinated FITC incorporation.
**Fig. S6** Fluorescence microscopy images of the fluorinated FITC molecules incorporated fluorous particles before (A) and after (B) LFUS application without $C_3F_{12}$ incorporation. The mean fluorescent intensity (MFI) was extracted from these images ($A = 642 \mu m^2$).
Copies of $^1$H, $^{13}$C, and $^{19}$F NMR spectra

Fig. S7 Copy of $^1$H NMR spectrum of perfluorooctanyl fluorescein isothiocyanate (FITC-C$_8$F$_{15}$).
Fig. S8 Copy of $^{13}$C NMR spectrum of perfluorooctanyl fluorescein isothiocyanate (FITC-C$_8$F$_{15}$).
Fig. S9 Copy of $^{19}$F NMR spectrum of perfluorooctanyl fluorescein isothiocyanate (FITC-$C_8F_{15}$).