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# Supplementary Materials for

## Treatment of otitis media by transtympanic delivery of antibiotics

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Published 14 September 2016, *Sci. Transl. Med.* **8**, 356ra120 (2016)
DOI: 10.1126/scitranslmed.aaf4363

**The PDF file includes:**



**Other Supplementary Material for this manuscript includes the following:**
(available at
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## SUPPLEMENTARY MATERIALS AND METHODS

### Apparent diffusion coefficient calculation

The apparent diffusion coefficients (D) were determined by the equation (*37*):

$$D = \frac{h^2}{6 \times x_0} \text{ (Eq. 1)}$$

where $x_0$ is the absolute value of the x-intercept determined by a linear fit of the data in Fig. 3B, and $h$ denotes the thickness of the stratum corneum layer alone in chinchilla TMs (table S3). The stratum corneum is ~4.7 μm thick in healthy TMs and ~8.2 μm in TMs with OM, as measured from Fig. 5A.

The apparent diffusion coefficients (table S3) for Cip-3CPE-18%[P407-PBP] across healthy TMs ($D_B$) and TMs with OM ($D_C$) allowed the calculation of the factor by which OM increased flux across the TM ($K_{OM}$). From this, and the apparent diffusion coefficient of Cip across healthy TMs ($D_A$), we could calculate the diffusion coefficient of Cip across TMs with OM ($D_{A\text{-}OM}$):

$$D_{A-OM} = D_A \cdot K_{OM} = D_A \frac{D_C}{D_B} = 8.67 \times 10^{-17} \times \frac{2.11 \times 10^{-15}}{1.32 \times 10^{-16}} m^2 s^{-1} = 1.38 \times 10^{-15} m^2 s^{-1} \text{ (Eq. 2)}$$

assuming the ratio of diffusion coefficients for infected TM and healthy TM is identical for all formulations. (This coefficient may overestimate flux across the human TM from ciprofloxacin solution, since it is derived from formulations that do not contain CPEs. In this case, however, that strengthens the resulting conclusion, which is that it is unlikely that ciprofloxacin solution would cross the human TM even in OM.)

In order to assess whether flux across human TMs with OM was possible with these coefficients ($D_{A\text{-}OM}$ and $D_C$), we used them in an established transient model (*41*) to predict the cumulative mass of ciprofloxacin that would permeate across a human TM with OM over a 7-day treatment. The non–steady-state transport of ciprofloxacin within the stratum corneum can be described by Fick's 2nd law:

$$\frac{\delta C}{\delta t} = D\left[\frac{\delta^2 C}{\delta x^2}\right] \text{ for } 0 \prec x \prec h \text{ and } 0 \prec t \prec \infty \qquad \text{(Eq. 3)}$$

where $C$ is the concentration of ciprofloxacin in a 1-D space (mass per length [across the thickness of the TM]), $x$ is the distance from the drug-stratum corneum interface, and $t$ is time. The equation could be solved using the method of separation of variables and L'Hospital's Rule to determine the concentration at any point within the stratum corneum:

$$C(x,t) = \sum_{k=1}^{\infty} \frac{2A}{h} Cos(x\sqrt{\mu_k}) e^{(-Dt\mu_k)} \text{ (Eq. 4)}$$

where $A$ is the mass of ciprofloxacin administered and $\mu_k$ is the constant of integration generated by separation of variables $\mu_k = (2k-1)^2\left[\frac{\Pi}{2h}\right]^2$.

Equation 4 was integrated from $x = 0$ to $x = h$ = 82 μm (the thickness of the stratum corneum in a TM ten times the thickness of the chinchilla stratum corneum with OM) at 20 equally spaced time points from day 0 to 7 to give rise to the curves in fig. S3 B:

$$b = \int_0^t \int_{x=0}^{x=h} C(x,t)dxdt = \int_0^t \int_{x=0}^{x=h} \sum_{k=1}^{\infty} \frac{2A}{h} Cos(x\sqrt{\mu_k}) e^{(-Dt\mu_k)} dxdt \text{ (Eq. 5)}$$

where *b* is the cumulative mass of ciprofloxacin delivered across human TM with OM.

**Auditory brainstem response (ABR) measurements**

ABR experiments were conducted with a custom-designed stimulus generation and measurement system built around National Instruments software (Lab View) and hardware. The hardware included a GPIB controller and an ADC board. A custom LabView program computed the stimuli, and downloaded the stimuli to a programmable stimulus generator (Hewlett Packard 33120A). The stimulus was then filtered by an antialiasing filter (KrohnHite 3901) and attenuated (Tucker-Davis Technologies). The filter and the attenuator were controlled by the LabView software. Simultaneous with stimulus output, the 2 ADC channels sampled the amplified ABR signal and the output of a microphone sealed in the ear canal of the animal.

The attenuated stimulus was played through a hearing-aid earphone placed within the intact ear canal of adult female chinchillas (400-600 g) anesthetized by IP administration of sodium pentobarbital (60 mg per kg). The earphone coupler included a microphone that monitored the sound stimulus level. ABRs, obtained in a sound-attenuating booth, were measured with a differential amplifier with a gain of 10,000 and a measurement bandwidth of 100 Hz to 3 kHz. The measurements were obtained from the positive electrode in the muscle behind the measured ear; the negative electrode was at the cranial vertex, and the ground electrode behind the contralateral ear. After obtaining baseline measurements, 200 μl of Cip-3CPE-18%[P407-PBP] gel was applied to the ear canal and the same measurements immediately performed to examine the resultant changes, if any, in auditory sensitivity thresholds.

The acoustic stimuli were pairs of 20-ms tone bursts of opposite polarity. The frequency of the bursts increased from 500 Hz to 18 kHz in octave steps. Each burst was sine windowed, with 40 ms between the two bursts. ABR responses to 250 pairs of stimuli were averaged at each stimulus level. The ABR response was computed from the sum of the averaged response to the two different polarities. Stimulus level was varied in 10-dB steps. A visual judgment of threshold at each stimulus frequency was determined post-measurement in a blinded fashion. After ABR measurements, the animals were monitored till they recovered from anesthesia and were returned to the colony.

**Histopathology**

Formulations were administered to the ear canals of live healthy/OM chinchillas. Seven days later, they were euthanized as described elsewhere (*10*). After sacrifice, the TMs were excised and immediately fixed in 10% neutral buffered formalin overnight, then decalcified, embedded

in paraffin, sectioned (5 μm thick) and stained with hematoxylin and eosin by the Department of Pathology at Boston Children's Hospital (fee for service), using standard techniques. All stained specimens were evaluated under light microscopy (Olympus FSX-100).

**SUPPLEMENTARY FIGURES**

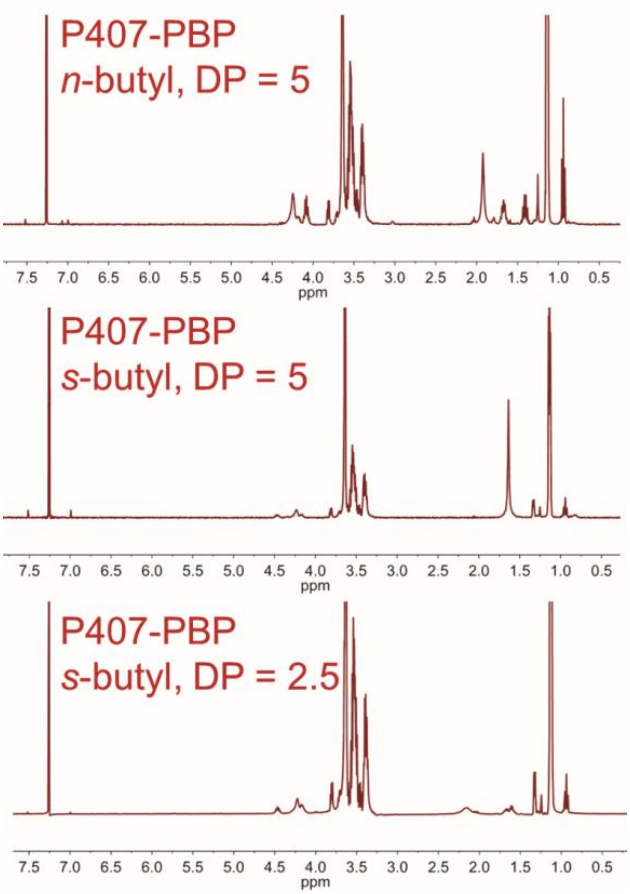


**Fig. S1**. **NMR of pentablock copolymers.** DP was calculated using the ratio of peak areas at 0.90 ppm (monomer side chain) and 1.14 ppm (P407 backbone). The chemical shifts (δ, in ppm) for the peaks corresponding to the hydrogens in italics in the following list of polymers are provided. t/m/broad indicate the shape of a peak (i.e., triplet, multiple, broad). $CDCl_3$ was the solvent. For P407-PBP with *n*-butyl groups, $^1$H NMR ($CDCl_3$, ppm): δ 0.90-0.96 (t, 3H, $CH_2CH_2CH_2\mathit{CH_3}$), 1.14 (m, 3H, $CH_2CH(\mathit{CH_3})O$), 1.36-1.46 (m, 2H, $CH_2CH_2\mathit{CH_2}CH_3$), 1.62-1.72 (m, 2H, $CH_2\mathit{CH_2}CH_2CH_3$), 3.36-3.42 (m, $CH_2\mathit{CH}(CH_3)O$), 3.48-3.58 (m, 2H, $\mathit{CH_2}CH(CH_3)O$), 3.65 (m, 4H, $O\mathit{CH_2CH_2}O$), 4.04-4.14 (m, 2H, $P\mathit{CH_2}CH_2CH_2CH_3$), 4.16-4.30 (broad, 4H, $PO\mathit{CH_2CH_2}O$). For P407-PBP with *s*-butyl groups, $^1$H NMR ($CDCl_3$, ppm): δ 0.90-0.96 (t, 3H, $CH_3CHCH_2\mathit{CH_3}$), 1.13 (m, 3H, $CH_2CH(\mathit{CH_3})O$), 1.30-1.34 (b, 3H, $\mathit{CH_3}CHCH_2CH_3$), 1.55-1.73 (m, 2H, $CH_3CH\mathit{CH_2}CH_3$), 3.35-3.42 (m, $CH_2\mathit{CH}(CH_3)O$), 3.48-3.58 (m, 2H, $\mathit{CH_2}CH(CH_3)O$), 3.65 (m, 4H, $O\mathit{CH_2CH_2}O$), 4.11-4.19 (m, 1H, $CH_3\mathit{CH}CH_2CH_3$), 4.19-4.35 (broad, 4H, $PO\mathit{CH_2CH_2}O$).

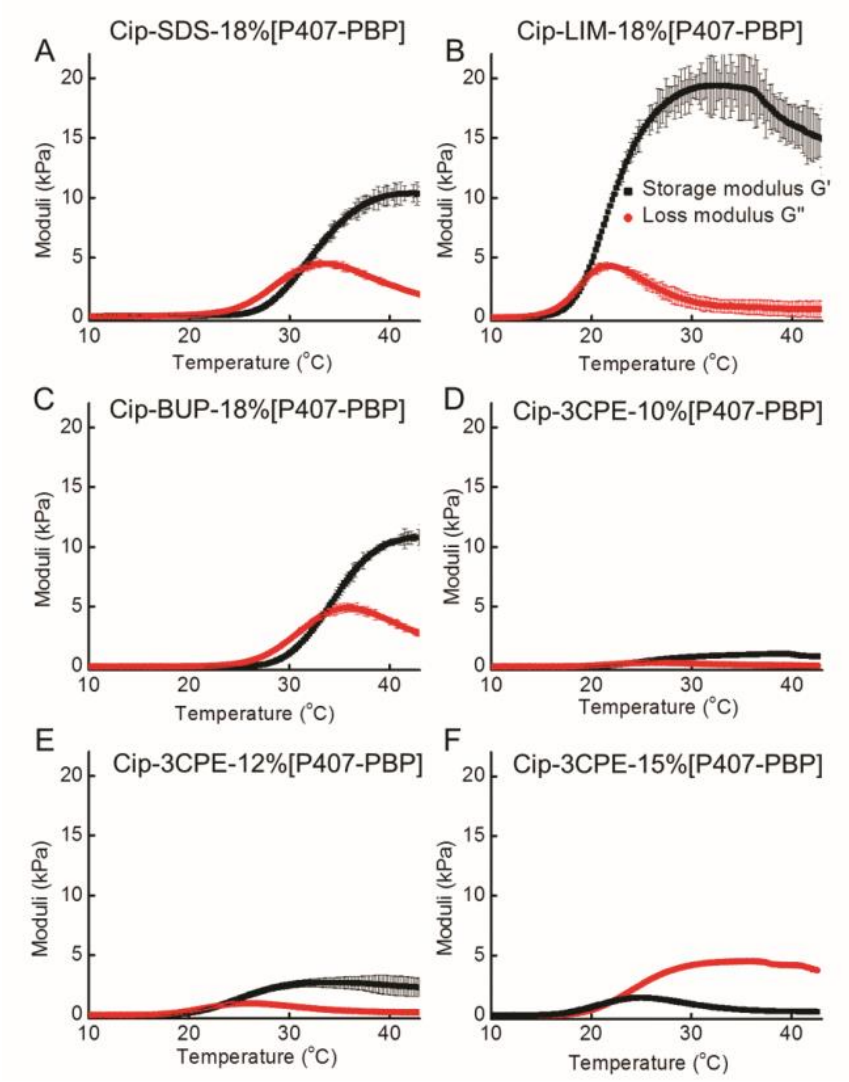


**Fig. S2**. **Modulation of gelation of aqueous solutions of Cip-[P407-PBP].** (**A** to **C**) Individual effects of 1% SDS, 2% limonene (LIM), or 0.5% bupivacaine (BUP). (**D** to **F**) Effect of varying the concentration of polymer in the formulation Cip-3CPE-P407-PBP. Data are means ± SD of the storage and loss shear moduli ($n$ = 4).

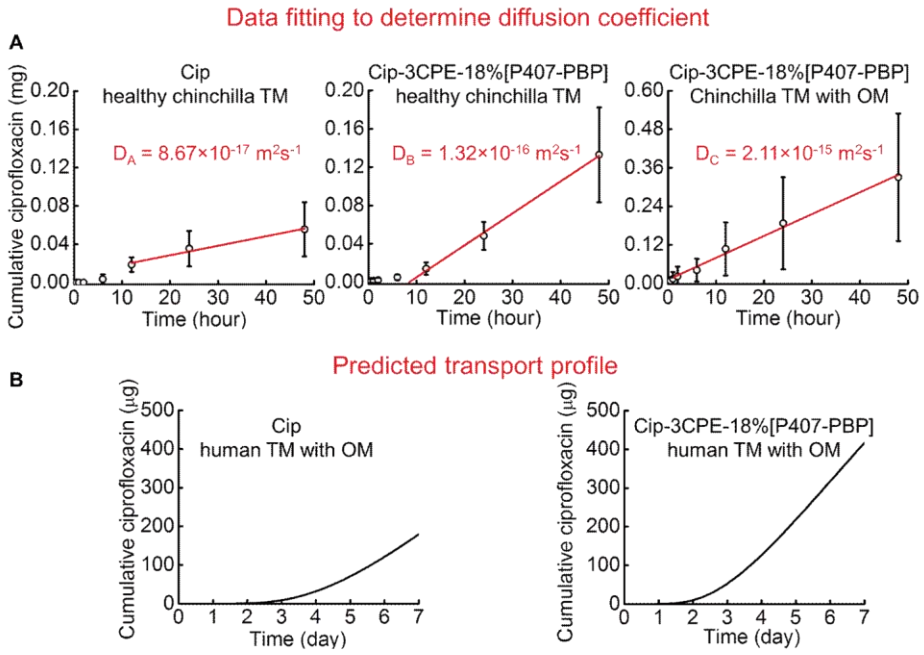


**Fig. S3**. **Simulation of drug transport across human TMs.** (**A**) Data for the determination of the apparent diffusion coefficients for chinchilla TMs exposed to Cip or Cip-3CPE-18%[P407-PBP]. $D_{A\text{-}C}$ are the apparent diffusion coefficients for panels A-C. (**B**) Prediction of the profiles of cumulative ciprofloxacin transport across human TMs with OM.

## SUPPLEMENTARY TABLES

**Table S1**. **Molecular weight and polydispersity indices of P407 and pentablock copolymers P407-PBP with *n*- or *s*-butyl groups.** MW was measured by GPC and NMR (spectra in fig. S1). The DP of PBP blocks was calculated by taking the ratio of the peaks corresponding to PEO and PBP (fig. S1). $M_n$ of P407-PBP by NMR was calculated by adding the molecular weight of PBP blocks, calculated using the DP value, to the reported P407 molar mass (12.1 kDa, provided by the manufacturer).

| | **P407** | **P407-PBP *n*-butyl DP=5** | **P407-PBP *s*-butyl DP=5** | **P407-PBP *s*-butyl DP=2.5** |
|---|---|---|---|---|
| Weight average molecular weight, $M_w$ (kDa) | 18.0 | 23.7 | 23.5 | 21.2 |
| Number average molecular weight, $M_n$ (kDa) | 15.1 | 19.1 | 18.6 | 17.3 |
| $M_n$ calculated using NMR (kDa) | n.a. | 13.9 | 13.9 | 13.4 |
| Polydispersity index, $M_w/M_n$ | 1.2 | 1.2 | 1.3 | 1.2 |

**Table S2**. **Systemic exposure to ciprofloxacin by Cip and Cip-3CPE-12%[P407-PBP].** Blood samples were analyzed for ciprofloxacin content at predetermined intervals after treatment. Data are means (SD was 0) ($n$ = 10 for each group).

| **Time** | **Concentration of ciprofloxacin in plasma (μg)** | |
|---|---|---|
| | Cip | Cip-3CPE-12%[P407-PBP] |
| **0** | 0 | 0 |
| **30 minutes** | 0 | 0 |
| **5 hours** | 0 | 0 |
| **1 day** | 0 | 0 |
| **3 days** | 0 | 0 |
| **5 days** | 0 | 0 |
| **7 days** | 0 | 0 |

**Table S3**. **Apparent diffusion coefficients of Cip and Cip-3CPE-18%[P407-PBP] across healthy and infected human TM.** $D_A$, $D_B$, $D_{A\text{-}OM}$, and $D_C$, are the apparent diffusion coefficients of ciprofloxacin from the formulation Cip into in healthy human TM, from Cip-3CPE-18%[P407-PBP] into healthy human TM, from Cip into TM with OM, and from Cip-3CPE-18%[P407-PBP] into TM with OM, respectively. Data are derived from Eq 1-5, Fig. 3B, Fig. 5A, and fig. S3.

| **Formulation** | **Apparent diffusion coefficient ($m^2/s$)** | |
|---|---|---|
| | Healthy TM | TM with OM |
| Cip | $8.67 \times 10^{-17}$ ($D_A$) | $1.38 \times 10^{-15}$ ($D_{A\text{-}OM}$) |
| Cip-3CPE-18%[P407-PBP] | $1.32 \times 10^{-16}$ ($D_B$) | $2.11 \times 10^{-15}$ ($D_C$) |

**Table S4. Tabulated data for Figs. 4 and 5 (provided as an Excel file).**

## SUPPLEMENTARY MOVIES

**Movie S1**. **Gelation of Cip-3CPE-18%[P407] and Cip-3CPE-18%[P407-PBP].** Cip-3CPE-18%[P407-PBP] formed a solid gel within 7 seconds, whereas Cip-3CPE-18%[P407] remained liquid and flowed down the wall of the vial when inverted.

**Movie S2**. **Gelation of Cip-18%[P407] and Cip-18%[P407-PBP].** Cip-18%[P407-PBP] formed a solid gel within 7 seconds, whereas Cip-18%[P407] remained liquid and flowed down the wall of the vial when inverted.

**Movie S3. Extrusion of Cip-3CPE-18%[P407-PBP].** Cip-3CPE-18%[P407-PBP] extruded as a gel from a 20-gauge, 1.8-inch catheter.

**Move S4. Extrusion of Cip-3CPE-15%[P407-PBP].** Cip-3CPE-15%[P407-PBP] extruded as a gel from a 20-gauge, 1.8-inch catheter.

**Movie S5. Extrusion of Cip-3CPE-12%[P407-PBP].** Cip-3CPE-12%[P407-PBP] remained liquid after extrusion from a 20-gauge, 1.8-inch catheter.