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Observation of “wired” cell communication over 10-μm and 20-μm poly(dimethylsiloxane) barriers in tetracycline inducible expression systems

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Communication between cells and extracellular environments is of interest because of its critical roles in cell development and differentiation. Particularly, this signal transduction is commonly believed to rely on the contact and binding of the participating molecules/proteins, suggesting that the binding distance needed is less than a few nanometers. However, it is difficult to precisely match the rapidly binding interaction which depends on the probability of molecular collision in living systems, raising a hypothesis that another mechanism exists, could promote this signal communication, and remains unknown. Here we report that a long-range signal delivery over 10-μm and 20-μm polydimethylsiloxane (PDMS) barriers can be observed in microfluidically tetracycline (Tet) inducible expression systems. Results show that a significant increment of the long-range induced green fluorescent protein in human embryonic kidney 293T (HEK 293T) cells by the stimulation of Tet is demonstrated, and that such a signal induction is not dominated by Tet diffusion and displays a specific bindingless property. In addition, our experimental results, combined with theoretical modeling, suggest that this communication exhibits a bump-shaped characteristic depending on barrier thickness, materially structural property, surface roughness, and agonist concentration. It strongly relies on the PDMS barrier to delivery signal; therefore, we call such a mechanism as “wired” cell communication instead of wireless. These results could ignite interests in the novel and “wired” cell communication, which we call it X-signal, and in the use of such systems for the study of cellular biology and development of new drug. © 2016 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution 3.0 Unported License. [http://dx.doi.org/10.1063/1.4939677]

I. INTRODUCTION

There are growing interests in the study of cell communication regarding how cells receive and give messages with their surrounding environments and with themselves, thereby controlling cell fate changes. For example, somatic cells can be reprogrammed to induced pluripotent stem (iPS) cells by defined factors,1 and 3D cultures can generate cells with stem-like properties in vitro.2 In addition, external acting fields (e.g., electromagnetic fields3 and infrared radiation4) can affect cell development and differentiation as well, thanking the rapid and precise responding to signals from environments. In living systems, however, a perfect match between ligands/agonists and receptors may be questioned owing to the relatively low binding probability dominated by molecule collision.5 Therefore, we hypothesize that another mechanism exists, cooperatively contributes to this interaction, and behaves as bindingless and “wired” signal communication.

Our recent work has demonstrated a long-range stimulation of calcium mobilization in human prostate cancer cells, persisting over PDMS barriers of 95 μm.5 To further examine our hypothesis described above, we utilize a microfluidic approach combined with a green fluorescent protein (GFP) inducible expression HEK 293T cell line by using a T-REx™ system. It is a Tet-regulated mammalian expression system that uses regulatory elements from E. coli Tn10-encoded Tet resistance operon, which contains two expression plasmids—pcDNA5/TO and pcDNA6/TR.6,7 pcDNA5/TO is an inducible expression plasmid for expression of GFP, under the control of the strong human CMV promoter and two Tet operator 2 (TetO2) sites. pcDNA6/TR encodes the Tet repressor (TetR) under the control of the CMV promoter. The expression of GFP can be regulated by Tet treatment if cells stably express with pcDNA5/TO-GFP and pcDNA6/TR. That is, the GFP expression would be repressed by TetR in the absence of Tet treatment. In contrast, following the treatment with Tet, TetR would bind with Tet and dissociate from the TetO2 site, resulting in a highly expressed GFP in cells. Utilizing the microfluidically Tet inducible expression system (Figure 1(a)), the long-range and bindingless interaction between TetR and Tet can be observed by the detection of GFP induced. Taken together, the proposed approach has advantages of viable culture of cellular microenvironment and study of the novel “wired” signal delivery between cells and...
II. MATERIALS AND METHODS

A. Fabrication of the microfluidic device

The microfluidic device was prepared as described. Briefly, two fabricated PDMS microchannels and a customized membrane with various thicknesses (10 μm, 20 μm, and 35 μm) were aligned and then permanently bounded together (Figure 1(b)). The size was 600-μm width × 85-μm height for the top channel and 8000-μm width × 40-μm height for the bottom channel. Polymethylmethacrylate (PMMA) nuts with an inner volume of 100 μl were used as solution reservoirs and adhered to the inlet/outlet of the device. To further explore the relationship between the surface roughness of the membrane and the induced signal, the PDMS membranes coated with 60-nm silicon dioxide (SiO2) by double-site-deposition using electron beam evaporation (ULVAC) were utilized.

B. Cell culture

HEK 293T cell line (CRL-11268, ATCC) was maintained in Dulbecco’s modified Eagle medium (DMEM, 31600-034, GIBCO), supplemented with 10% fetal bovine serum (FBS, SV30014, Hyclone) and 1% penicillin/streptomycin (15140, GIBCO) in a humidified 5% CO2 incubator at 37°C.

C. Generation of GFP inducible expression cell line

To set up a Tet-inducible GFP system, HEK 293T cells were first transfected with the pcDNA6/TR vector, encoding molecules, which may be further applicable for new drug developments and biological assays.
were achieved and amplified for the following testing.

determined by using an imaging software (ImageJ, 1.42q).

experiments. The induced GFP expression was captured by a

tion level height as described,8 and the device was placed in

dium were perfused into the top channel by tuning the solu-

incubator at 37

cally significant.

more than two groups.

ANOVA test was used to compare data from

Additional information is also

between Tet antibiotic and TetR protein in cells (Figures

1(d)), and the demonstration of "wired" signal delivery

and permeation testing of the fabricated membrane (Figure

1(a) to examine this phenomenon (for details, refer

Section II). The bindingless means that the interaction

between Tet and TetR does not result from the molecular col-

lision and binding process. Results show that (1) the time-

sequenced images reveal that HEK 293T cells cultured on the

PDMS membranes, under the long-range Tet (11.2 μM) stimu-

lation for 24 h, were induced with GFP (for 10-μm, 20-μm

PDMS, and P-control) but not observed in 35-μm PDMS and

N-control (Figure 2(a) and Figure S119). P-control and

N-control indicate that Tet was loaded into the top channel

and no Tet was introduced into either top or bottom channels,

respectively. Others mean Tet was loaded into the bottom

channel. Cells were cultured in the top channel; (2) the corre-

spondingly time-lapse intensity of the induced fluorescence

was measured as shown in Figures 2(b) (membranes w/o

SiO2–coated) and 2(d) (w/ SiO2–coated), revealing that the
time required to a saturation induction was longer than 24 h

(P-control) and the response time to a saturation state, of

10-μm and 20-μm membranes (w/ SiO2–coated), was quicker

than the P-control and the bare membranes (10μm and

20μm), whereas the induced intensity was less than the P-

control. Where the saturation induction and the time required
to the saturation were defined as that the trend of the induced
intensities has no significant difference compared with the
signal at 24 h and time required to reach the first time-point in
the saturation state, respectively. For example, the response
time to a saturation state in 10-μm PDMS (w/o SiO2) set was
18 h (Figure 2(b)), and the time required in 10-μm/20-μm

PDMS (w/ SiO2) set was 4 h (Figure 2(d)). GFP intensities
induced in cells were measured and averaged at each time
point, and the relative intensity was defined as that each aver-
ged intensity was normalized by the initial intensity, i.e.,
t=0 h before adding Tet antibiotics. Above results suggest
that the surface modification by SiO2 could promote and
speed up such a signal delivery; and (3) cells cultured on the
10-μm and 20-μm membranes (either with or without SiO2
modification) all acquired a significant upregulation of fluo-
rescent intensity compared to the N-control, whereas these
upregulations were lower than that of the P-control at the 24-h
time point (Figures 2(c) and 2(e)). In addition, there was no
significant regulation in the two 35-μm membranes, indicating
that such a long-range signal could transport over up to a
20-μm PDMS barrier.

The hydrophobic property of PDMS tends to adsorb small
hydrophobic molecules and proteins, although the limited
molecule permeation into PDMS is in the 100–300 molecular
weight (MW) range.9–11 To further confirm whether the Tet
antibiotic (molecular weight, MW = 444 g/mol) would perme-
ate into the PDMS membrane thereby questioning our results,
we conducted a permeation testing by using a nuclei dye
(Hoechst 33342; MW = 616 g/mol; 800 μM), which contains
a resembling MW but a higher concentration compared with
the tetracycline used above. Figure 1(d) reveals that the P-
control acquired an apparent nuclei signal after the 10-min

treatment of Hoechst, whereas the experimental sets (includ-
ing 10-μm, 20-μm, and 35-μm membranes) had no signifi-
cantly increased intensity compared with the background
signal, even up to the 24-h treatment. Where the P-control rep-
resents that Hoechst dye was directly loaded into the top chan-
nel of the microdevice. These findings suggest that Tet

the tetracycline/doxycycline repressor behind a constitutive
CMV promoter by Lipofectamine 2000, following the manu-
facturer’s protocol. The drug resistant clones of HEK 293T-

pcDNA6 were obtained by Blasticidin-S selection (5 μg/ml).
The GFP genes were cloned into the pcDNA5/TO vectors and
then transfected into the HEK 293T-pcDNA6 cells by

Lipofectamine 2000. After around 2-week Hygomycin B

(200 μg/ml) selection, the antibiotic-resistant single clones
were achieved and amplified for the following testing.

D. Operational procedures for the microfluidically Tet
induction system

Before loading of cells, the microfluidic device was first
sterilized by UV light in a laminar-flow hood for at least 1 h.
The device was then placed in the vacuum chamber to degas
for 10 min, followed by filling with 70% ethanol.

Afterwards, the device was washed twice with phosphate
buffered saline (PBS) and stored in a sterilized dish at 4
°C until needed.

To enhance the cell attachment on PDMS membrane,
type I collagen solution (BD Biosciences; 100 μg/ml in PBS)
was loaded into the top channel and placed in a humidified
incubator at 37 °C for 1 h to allow and enhance binding to
the surface of the membrane. Afterwards, PBS and culture
medium were introduced to wash away unbound collagen.

100 μl of HEK 293T cells (~1.5 × 10⁵ cells) in culture me-

dium were perfused into the top channel by tuning the solu-
tion level height as described,9 and the device was placed in
a 5% CO₂ incubator at 37 °C overnight to enable cell attach-
ment and spread on the membrane. Subsequently, Tet was
introduced into the bottom channel at t = 0 h, as on-device

experiments. The induced GFP expression was captured by a

charge-coupled device (CCD) camera (DP-70, Olympus) on

an inverted microscopy and the fluorescent intensity was
determined by using an imaging software (ImageJ, 1.42q).

E. Statistical analysis

Student’s t test was used to compare data from two

groups, and the ANOVA test was used to compare data from
more than two groups. p < 0.05 was considered to be statisti-
cally significant.

III. RESULTS AND DISCUSSION

The efficacy of the microdevice in enabling in-vitro Tet
inducible GFP expression (Figure 1(c)), the characterization
and permeation testing of the fabricated membrane (Figure
1(d)), and the demonstration of “wired” signal delivery
between Tet antibiotic and TetR protein in cells (Figures
2–5) will be presented below. Additional information is also
available in the supplementary material.

A. Demonstration of the “wired” Tet-induced GFP in
cells

To explore whether a “wired” communication exists in
the Tet and TetR interaction in cells afforded by bindingless
signal delivery,9 we followed the procedure illustrated in
Figure 1(a) to examine this phenomenon (for details, refer
antibiotic could not permeate across the PDMS membrane from the bottom channel to the top, indicating that the proposed system could be adopted for the demonstration of our hypothesis herein. However, diffusion of molecules in polymeric materials could not be determined by molecular weight only, but the structure of molecules plays an important role as well. The molecular structure of Tet may be different from Hoechst used, thus questioning our hypothesis. Therefore, another assay for the test of Tet permeation in PDMS needs to be conducted and will be discussed below.

B. The “wired” signal induction was not dominated by diffusion and non-specific binding

To answer the question—this “wired” communication between Tet and TetR protein in cells may be possible mainly due to the diffusion and non-specific binding or not—we treated the cells with two concentrations of Tet (11.2 μM and 112 μM) and another antibiotic—zeocin, which were utilized to elucidate this question (Figure 3). Several results are noteworthy. First, the time-lapse measurements of

FIG. 2. Demonstration of the “wired” Tet-induced GFP expression in cells. (a) Time sequence of the top-viewed photographs showing GFP-induced HEK 293T cells, which was cultured in the top channel of the microfluidic device and under the 11.2 μM Tet stimulation from the bottom channel (for 10, 20, and 35 μm PDMS sets w/o SiO2 coated). P-control and N-control indicate that Tet was applied and no Tet was introduced, respectively, and cells were cultured on-device. Scale bars, 100 μm. (b) Averaged GFP intensities induced in cells were measured by ImageJ software at each time point from (a). The relative intensity was normalized by the initial value, i.e., t = 0h. In addition, (c) the relative intensities were compared at t = 24h. The membranes used were bare PDMS for (a), (b), and (c). (d) and (e) show the corresponding measurements as the membranes were replaced by SiO2–coated PDMS. Each bar represents the mean ± SEM from 2–3 independent experiments (n = 40–150 cells). *p < 0.05, **p < 0.01, and ***p < 0.001 were compared to the N-control.
the induced fluorescence were shown in Figures 3(a) (bare membranes) and 3(b) (membranes coated with SiO₂), revealing that the GFP induction was positively dependent on the concentration in the P-control sets; however, it was not consistent with the experimental sets. Where the P-control means that Tet was applied directly on cells in the top channel of the microdevice. Second, the resulted intensity, at the 24-h time point, was dependent on the concentration of Tet adapted (Figure 3(c)). Notably, with a higher Tet concentration (10⁻⁶M) used, the intensity was significantly downregulated as compared with the treatment with 1⁻⁶M-Tet (for 10⁻⁷M-bare and 20⁻⁷M-SiO₂-coated membranes). In contrast, the P-control and the 20⁻⁷M bare membrane set acquired a significant upregulation of induced intensity, positively depending on the Tet concentration used. Third, the long-range signal induction was not mediated with the non-specific binding, which was confirmed by using another antibiotic (zeocin) and demonstrated that such a signal cannot be induced by zeocin (Figure 3(d)).

Most the effects are dependent on the concentration of active ingredient in cells for conventional dose-response treatments. For example, drugs with higher concentrations would cause more profound effects on cells than using lower concentrations.²,⁹,¹² Moreover, other research groups had revealed that the induced fluorescence of GFP is positively dependent on the corresponding Tet/Dox concentration,¹³,¹⁴ which is consistent with our P-control results (Figure 3(c)). In micro/nano scales, however, particles coated with drugs

FIG. 3. The “wired” signal induction was not mediated with diffusion and non-specific binding. (a) and (b) show the time-sequenced measurements of induced GFP intensities under the 11.2μM (1×) Tet and 112μM (10×) Tet stimulations, in which cells were cultured on the bare PDMS membrane (a) and SiO₂-coated PDMS (b), respectively. P-control and N-control indicate that cells were cultured on-dish but not on-device as shown in Figure 2. (c) The relative intensities induced were compared at t = 24 h from (a) and (b). Each bar represents the mean ± SEM from 2–3 independent experiments (n = 40–150 cells). *p < 0.05, **p < 0.01, and ***p < 0.001 were compared to the N-control, except for the indicators. (d) shows the “wired” Tet inducible expression system was specific while compared with the on-device N-control and the stimulation by zeocin antibiotic, in which Tet and zeocin were applied from the bottom channel of the microfluidic device. Each bar represents the mean ± SD from n = 30 cells. ***p < 0.001 was compared to the N-control, except for the indicator. Scale bar, 100 μm.

FIG. 4. Fit of the experimental data with the “wired” signal induction model. (a) and (b) show the experimental results from Figures 2(c) and 2(e), respectively, and the correspondingly fitted curves from Eq. (1). The signal intensity was defined as the relative intensity from Figures 2(c) and 2(e) subtracted the background signal (N-control), in which we assumed that the “wired” inducible signal would approach zero while the PDMS barrier was removed, i.e., d = 0.
of a higher concentration may not achieve a more effective tumor targeting than coating with a low concentration, suggesting that another mechanism may exist and dominate in this scale. Importantly, our results (Figure 3(c)) show that the induced signal achieved a significant increment under the low Tet concentration (1×) treatment rather than using the high concentration (10×), for 10-μm (w/o SiO2) and 20-μm (w/ SiO2) membranes, which behave oppositely compared with the molecular diffusion dominated model. The Tet antibiotic size is approximately in the range of tens nanometer, suggesting that the “wired” cell communication observed in this work would cooperatively contribute in these scales. Moreover, with the higher Tet concentration (10×) treatment, the induced intensities between the 10-μm (w/o SiO2) and 20-μm PDMS (w/o SiO2) sets (Figure 3(c)) exhibit a significant difference and reveal an opposite result if the molecule diffusion dominates in this work. That is, the stimulated intensity through a 10-μm barrier should be larger than that through a 20-μm distance while the diffusion controls such a signal delivery. Although the results are opposite between the 10-μm and 20-μm PDMS (w/o SiO2) sets (under the 1× Tet treatment shown in Figure 3(c)), these suggest that the “wired” signal induction may be concentration dependent. In addition, the response time to a saturation state in 10-μm PDMS (w/o SiO2 coated) set is 18 h (Figure 2(b)), which is slower than the membrane of the 20-μm thickness but coated with 60-nm SiO2 (for 4 h in Figure 2(d)). It indicates that the response time in a thinner distance should be quicker than that in a thicker distance if the diffusion of molecules dominates, which is conflicting with our observation. Due to the discussion above, the permeation and diffusion of Tet antibiotics through the PDMS membrane to the top cell-supporting region may not exist, thus confirming our results and hypothesis in this work.

C. Physical characteristics of the “wired” signal induction

Our previous work has presented a physical model to describe a bindingless signal delivery between ligands and receptors using the equation

\[
f = Cx e^{-\frac{x}{d}} ,
\]

where \( f \) is the signal intensity, \( C \) is a scaling factor determined by surface roughness and materially structural property of the membrane, \( x = d/l \cos \theta \), \( d \) is the thickness of the membrane, \( l \) is the mean free path required for signal delivery, and \( \theta \) is the tilted angle of the signal delivering cone. Here we define the mean free path as the average distance travelled by the “wired” signal within the membrane before it is scattered by material structure or other adjacent “wired” signals. The tilted angle represents the coverage area of this signal and behaves inversely to the mean free path. If the “wired” signal acquires a smaller tilted angle, for example, it would activate more concentratedly, thus transmitting with a longer mean free path. To compare the theoretical model and the results presented in this work, we assumed that the “wired” signal induction would approach zero while the PDMS membrane is removed. That is, the contact and binding interaction between Tet and TetR in cells would dominate the signal transduction without any barrier. To determine scaling factor, tilted angle, and mean free path in this work, the thickness-response data (10 μm, 20 μm, and 35 μm in Figures 2(c) and 2(e)) were fitted by the three-parameter \( (C, \theta, \text{ and } l) \) equation (Eq. (1)) using commercial software (Origin, OriginLab Corp.). The signal intensity \( f \) was defined as the relative intensity from Figures 2(c) and 2(e) subtracted the background signal (N-control), in which we assumed that the “wired” inducible signal would approach zero while the PDMS barrier was removed, i.e., \( f = 0 \) at \( d = 0 \). Figures 4(a) and 4(b) show the comparisons of the theoretical model, fitted from Eq. (1), with the experimental results from Figures 2(c) and 2(e), respectively. The fitted scaling factor \( C \), tilted angle \( \theta \), and mean free path \( l \) were 23.1, 2.0°, and 4.2 μm for bare PDMS and 3.8, 1.7°, and 6.6 μm for SiO2-coated PDMS. The fitting trend was similar between the two PDMS membranes, whereas the scaling factor of SiO2-coated PDMS was relatively less than that of the bare membrane. In addition, for 10-μm bare membrane, the signal abruptly rises at 16 h and quickly saturates.
in 2 to 8 h as shown in Fig. 2(b). This suggests that Tet molecules may permeate into the rough surface of a bare PDMS membrane to the sealed channels in the membrane but cannot diffuse through them (details will be discussed below), resulting in accumulating more Tet molecules there. It took Tet molecules 16 h to accumulate to a specific concentration near the sealed channels at which the “wired” signal can reach the top surface of the 10-μm bare membrane. Once the “wired” signal reached the top surface, it took 4 to 8 h to fully react with the cells similar to the P-control. These indicate that the surface modification by SiO2 might retard this permeation process and lead to smaller Tet molecule density at the bottom surface and smaller scaling factor on the signal induction (see also Figure S219) although this modification can concentrate the tilted angle by 0.3° and increase the mean free path by 2.4 μm.

Our previous results have revealed that the surface treatment by SiO2 onto the PDMS membrane can promote the signal delivery and achieve a ballistic-like signal transduction by using a thinner membrane, due to the curvilinear micro/nano structures by SiO2 coating. In this work, however, we did not observe the similar property by using the Tet inducible expression system, in which the estimated mean free path is less than 10 μm (Figure 4). The minimum and optimized membrane thickness we can fabricate was 10 μm that is uniform and compatible for cell culture in this system. To enhance the reliability of the fitting equation, more data points (i.e., thickness is less than 10 μm) would be needed. Although this drawback can be overcome in the future, the results presented in this work should enable demonstrating the “wired” signal induction. Note that the response time to a steady state (10-μm and 20-μm SiO2–coated membranes shown in Figure 2(d)) was quicker than those of the P-control and the 10-μm/20-μm bare membrane (Figure 2(b)), suggesting that the SiO2 modified membrane could prevent the Tet molecules from permeating into the rough surface layer of PDMS membranes, which speeds up the signal transport by the concentrated tilt angle of cone (from 2.0° to 1.7°) and the enhanced mean free path of the signal delivery (from 4.2 μm to 6.6 μm). Surprisingly, these findings resemble the phonon conduction in micro/nano scales and in ordered polymer structures, which revealed the unconventional signal transduction. In addition, there is no significant difference in the induced GFP signal by using different thicknesses of SiO2 modification (30 nm and 60 nm), as shown in Figure S3.19 It suggests that this induced signal could be independent on the thickness of SiO2 modification in the nanometer scales but depends on the resulted micro/nano structure on the surface of membrane by SiO2 modification.5 If the thickness of SiO2 modification increases to the micrometer scales, it would suggest that the modification will dominate the interaction as the results shown in Figure 2(e). These hypotheses need to be further confirmed in the future. Taken together, the model presented herein, however, is not optimized but suitable to partially explain the findings.

To further confirm no Tet diffused or permeated across the PDMS membranes, we conducted another experiment to validate it, as shown in Figure 5(a). The schematic shows the validation procedure. Step 1: Tet is perfused into the bottom channel of the microdevice, in which no cell is cultured in the top channel that contains culture medium only, and then incubated for 24 h. Step 2: After 24 h, Tet in the bottom channel is replaced by fresh culture medium without Tet molecules. The former medium is retained in the top channel. Step 3: Afterwards, cells are seeded into the top channel and adhere onto the former membrane. Step 4: Fluorescence examination is utilized to validate no Tet diffusion after 24 h. The thickness of PDMS membrane used is 10 μm without SiO2 coated. Results show that the measured fluorescence signal from Figure 5(a) has no significant difference compared to the N-control; however, the P-controls are significantly larger than the others (Figure 5(b)). Where the P-control and N-control indicate that Tet was applied directly on cells in the top channel and Tet not acting on cells, respectively. These imply that the former medium in the top channel contains no or un-detectable Tet concentration to induce GFP signal in HEK 293T cells. The minimal concentration of Tet required to induce GFP is around 112 nM (Figure S4).19 In addition, no Tet molecule was detectable to accumulate onto the top surface of the membrane, thereby inducing GFP in cells. Thus, there should be no Tet diffusion across the membrane into the medium in the top channel. Note that the whole time for replacing Tet solutions and loading cells both in step 2 and step 3 was around 30 min, which is significantly less than the time required for Tet degradation (around 2 weeks according to the manufacturer’s documentation). Moreover, the induced signal measured from Figure 3(c) is significantly larger than the N-control. If the Tet diffusion dominates our hypothesis herein, the measured signal from Figure 5 would be larger than the N-control. Based on the above results, therefore, the diffusion of Tet across the PDMS membranes should be ignorable, thus further confirming our hypothesis and experimental observations.

Although our results demonstrate that Tet cannot diffuse across PDMS membranes of 10 μm–35 μm, one research group had revealed that Tet would diffuse through the PDMS membrane of around 145 μm and then induce GFP in HeLa cells. This divergence may question our results above. In their system, Tet was diluted and prepared in water, and the PDMS membrane was not treated with any bio-materials. In contrast, the PDMS membrane in our system was coated with collagen and pre-soaked with cell culture medium overnight, and Tet was prepared in medium. The membrane would tend to adsorb small hydrophobic molecules and proteins due to PDMS’s hydrophobic nature, which may contribute to sealing off the leaking channels in the membrane. Therefore, we hypothesize that the pre-treatment of PDMS membrane (i.e., both coating with collagen and pre-soaking with medium) and Tet in medium would play a crucial role in our system, resulting in that Tet may partially diffuse into membrane but cannot diffuse through because all leaking channels are sealed off. It may cause opposite results between ours and other research group. To verify our hypothesis herein, we conducted two further experiments as shown in Figure S5 and Figure S6.19 Figure S5 shows that the results of permeation testing in PDMS membrane by
loading Hoechst nuclei dye into the bottom channel of the microfluidic device. Cells were cultured in the top channel. The PDMS membrane was not coated with collagen and not pre-soaked with medium. Hoechst dye of 40 μM was prepared in DI water. The thickness of membrane was 10 μm. Results show that Hoechst (in water) would diffuse and permeate across the bare membrane, thus acquiring a significant nuclei signal in cells (Figure S6), whereas no nuclei signal was detected in the cases of membrane with pre-treatments and Hoechst prepared in medium (Figure 1(d)). In addition, Figure S6 shows the comparison of the validation of no Tet diffusion across PDMS membranes. All the experimental procedures were corresponding to those illustrated in Fig. 5(a). Tet of 11.2 μM in DI water was utilized, and the thickness of membrane was 10 μm. Notably, Tet diluted in DI water would induce a significant GFP signal compared to the N-control, in which the membrane was not pre-treated by both collagen coating and medium pre-soaking. N-control represents no Tet treatment. In contrast, Tet prepared in medium [see also Fig. 5(b)] cannot induce a significant GFP signal, suggesting that the PDMS membrane pre-soaking with medium would prevent Tet diffusion into itself. Taken together, the diffusion of Tet across PDMS membranes can be blocked via the pre-soaking with medium, thus demonstrating our hypothesis above and the results in this work. What is the physical nature of this “wired” signal, we really do not know and would rather call it X-signal which might travel along with the long-chained molecular structure such as the PDMS membrane.

Are there other mechanisms involved in the “wired” signal induction? For example, the exchange of charges or transduction of phonon within the PDMS membrane could be an alternative mechanism. The nature of PDMS is hydrophobic and electrically isolated,11,21 and the Tet antibiotics used is dissolved in an aqueous solution; therefore, the possibility of charge exchange within the membrane could be negligible. Phonon energy transport in PDMS membrane could be another possible mechanism due to the vibration of molecules on the membrane surface, resulting in signal transport by inter-chain phonon scatterings in crystalline structures of PDMS.22 In the future, therefore, other parameters (including molecule/cell/membrane properties, molecular concentration, temperature, time, etc.) and the possible mechanism should be compared and considered into the model and the experiments.

IV. CONCLUSIONS

Together, we have presented a microfluidically Tet inducible platform for the demonstration of “wired” signal induction in cells. This platform is valuable in isolation of Tet and TetR in cells and can be utilized for the real-time observation of the long-range induced signal. Key results demonstrated that the wired cell communication could transport signals over 10-μm and 20-μm PDMS barriers, and the response time of the induced signal could be speeded up by the surface modification by SiO2. It is proposed that the spin-field space-time structure of the Tet molecules is responsible for the wired communication. Thus, the present system not only provides a useful platform for the study of the wired signal delivery in living systems, but potentially will be of great interest to understand the molecular mechanism underlying the effects of “wired” cell communication in the future.

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19See supplementary material at http://dx.doi.org/10.1063/1.4893577 for six images showing the time-sequenced GFP induced images (Figure S1), the relative induced intensity versus different. PDMS membrane thickness (Figure S2), the induced GFP intensity versus different, thicknesses of SiO2 deposition (Figure S3), the minimal Tet concentration required for GFP induction (Figure S4), the permeation testing of PDMS (Figure S5), and the validation of no. Tet diffusion across PDMS membranes (Figure S6).