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Ching-Te Kuo,[1,2] Hsinyu Lee,[2,a)] and Si-Chen Lee[1,3,a)]

AFFILIATIONS

[1]TSMC-NTU Joint Research Center, National Taiwan University, Taipei, Taiwan 10676, R.O.C.
[2]Department of Life Science, National Taiwan University, Taipei, Taiwan 10676, R.O.C.
[3]Department of Electrical Engineering, Graduate Institute of Electronics Engineering, National Taiwan University, Taipei, Taiwan 10676, R.O.C.

[a)]**E-mail:** hsinyu@ntu.edu.tw (H. Lee), sclee@ntu.edu.tw (S.-C. Lee)

ABSTRACT

Treatments designed to intervene in conventional direct-drug-to-cell-binding communication have been hallmark approaches in designing new drug candidates. Given the random collision of molecules in living systems, this binding is laborious and too ineffective to precisely match the binding site. Therefore, it has been proposed that another non-chemical and non-electrical drug-cell communication channel, termed "bindingless" or "wired", could exist to impact signal transduction. Here we present the use of a micro-barrier well-array device to explore the unidentified bindingless anti-cancer mechanism. The device involves a 95-μm polydimethylsiloxane barrier to compartmentalize cancer cells and chemotherapeutic drugs. The lack of permeation of solutions across the device barrier was validated. Paclitaxel diluted in medium significantly inhibited the growth of prostate PC-3 cancer cells over a 95-μm barrier instead of cisplatin. The inhibition was attenuated by diluting drugs in other solvents, such as deionized water and phosphate-buffered saline. In addition, the orientation of magnetic field could partially dominate the "wired" communication. The collective data provide the experimental evidence of the postulated "wired" drug-cell communication as a potent in anti-cancer mechanism. These findings may inform cell biology investigations and stimulate studies of new physical and chemical phenomena.

I. INTRODUCTION

Studies of strategies to treat cancer typically focus on the conventional drug-cell communication (CDCC), which involves the direct contact and binding of participating DNAs or proteins to cells. Based on CDCC, several approaches to optimize the cancer treatments have been developed, including large scale simulations,[1,2] feedback system control algorithms[3,4] and cell co-cultures.[5] Given that the distance needed for binding is less than few nanometers, it is extremely difficult to coincide the random binding interaction of matched molecules. Therefore, a mystery second channel that influences non-chemical and non-electrical drug-cell communication, i.e. bindingless, could arise to facilitate the anti-cancer treatment.

The hallmark of the bindingless signal, e.g. *X* signal, is mechanically separate and cannot communicate through chemical and electrical reactions among matched molecules and cells. In Chinese medicine, for example, "Qi-energy" (life-energy) has been demonstrated to benefit cancer patients, leading to the attenuation of cancer cell proliferation as well as the stimulation of immune function in patients.[6] In addition, synchronous behavior in neighboring cells separated mechanically has been revealed in a study of the activation of nuclear factor-kappa B (NFκB) by hydrogen peroxide (H_2O_2).[7] Our previous studies experimentally demonstrated a bindingless signal (*X* information) delivery in human cells through the examination of calcium mobilization or tetracycline inducible green fluorescence protein (GFP) expression.[8,9] It is proposed that *X* information is the residual space-time spin structure of antibodies. The *X* information can move freely and penetrate the blocking polymer wall.

Figure 1(a) displays the CDCC process in the left panel and the "wired" drug-cell communication (WDCC) in the right panel. Whether the bindingless drug-cell communication affects

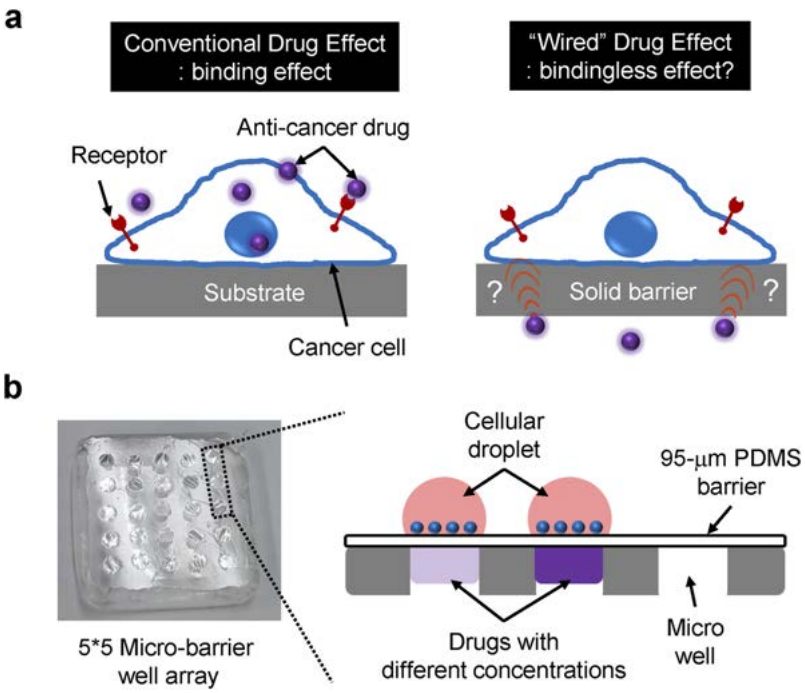

FIG. 1. Design of micro-barrier well-array devices. (a) Schematic diagram showing the conventional drug-cell communication (CDCC), i.e. binding effect, and our proposed "wired" signal transduction through a solid barrier (WDCC), i.e. bindingless effect. (b) Photography of the fabricated device. The right panel illustrated the cross section and the setup of the device. The barrier utilized in this paper was a PDMS membrane with a thickness of 95 μm.

anti-cancer mechanisms is unclear so far. Herein, we present a facile micro-barrier well-array device that was used to examine the issue. The device involves a 95-μm polydimethylsiloxane (PDMS) barrier to mechanically separate the prostate PC-3 cancer cells and two anti-cancer drugs (cisplatin and paclitaxel) (Figure 1(b)). Through the evaluation of cell viability, the bindingless anti-cancer mechanism was explored experimentally in a high-throughput manner. The outcomes of magnetic orientation were also examined. Given that such communication mainly relies on the barrier of the device, it was designated WDCC. The proposed approach allows arrayed cell cultures and high-throughput drug screening. These advantages could be exploited in the development of novel WDCC-based drugs and relevant biological assays.

II. MATERIALS AND METHODS

A. Fabrication of micro-barrier well-array devices

A polydimethylsiloxane (PDMS) prepolymer (Sylgard 184; Dow Corning, Midland, MI, USA) was customizedly cast as a square blank with an area of 22 × 22 mm^2 and an approximately thickness of 4.5 mm. A 5 × 5 well-array was generated using a steel tube to make 1.2 mm-diameter holes in the PDMS blank. The holes were spaced 3 mm apart. A 95 μm-thickness PDMS barrier was pre-fabricated using spin-coating of PDMS prepolymer (10:1 base:cure) by spinning at 2500 rpm for 10 seconds and curing at 70 °C for 30 minutes. The perforated PDMS blank was aligned and bonded with the barrier by oxygen plasma treatment (15 sccm, 60 W, 60 seconds). After peeling off the PDMS set, the micro-barrier well-array device was sterilized using ultraviolet (UV) light for 30 minutes and stored in a sealed dish until use. The device is shown in Figure 1(b).

B. Cell culture

Prostate PC-3 cancer cells (CRL-1435; ATCC, Manassas, VA, USA) were maintained in RPMI-1640 medium (23400-021; GIBCO, Franklin Lakes, NJ, USA) supplemented with 10% fetal bovine serum (FBS, SV30014; Hyclone, Logan, UT, USA) and 1% penicillin/streptomycin (15140; GIBCO) in a humidified 5% CO2 incubator at 37°C.

C. Permeation testing

1. Hoechst staining

To confirm the absence of permeation of small molecules across the fabricated barrier, the Hoechst 33342 (which dyes cell nuclei and which has a molecule weight of 616 g/mol) was prepared as an 8-μM solution. Aliquots of 1.5 μl were added to the bottom wells of the device (Figure 2(a)). Then 1.5 μl of a cell suspension contained 150 cells was added to the corresponding top area of the barrier. The device was surrounded with cell culture medium, placed in a Parafilm-sealed dish to prevent evaporation, and transferred to an incubator. Following a 24-hour incubation, the Hoechst signal was observed by upright fluorescence microscopy and evaluated using ImageJ software (NIH). The positive control consisted of direct loading of the cell droplet in a device lacking the barrier.

2. Anti-cancer drug

To confirm the lack of permeation of the anti-cancer drug (cisplatin) across the barrier, the procedure illustrated in Figure 2(c)

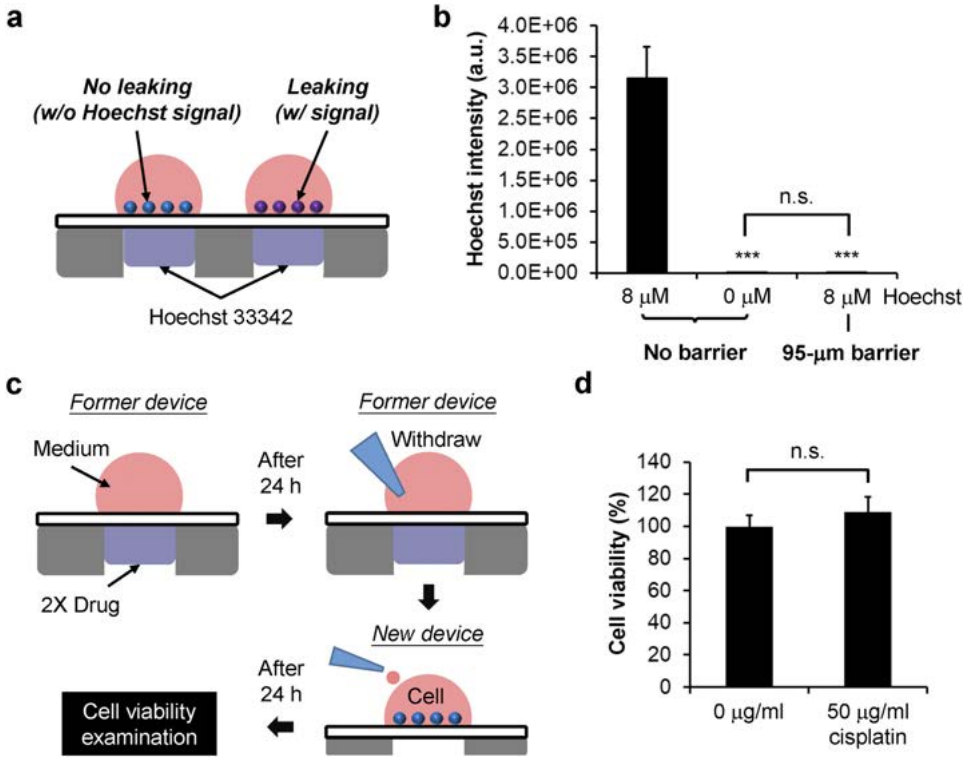

FIG. 2. Permeation test of the fabricated device. (a) Schematic diagram showing the validation of the lack of permeation of small MW compound (Hoechst 33342) across the fabricated barrier. The absence of a nuclear signal in cells indicated the lack of permeation. (b) Comparison of Hoechst intensities in the absence of the barrier and with the 95-μm barrier fabricated in the device. Each bar represents the mean ± SEM (n = 4 ~ 10). ***p < 0.001 was compared with the 8 μM in no barrier. a.u., arbitrary unit. (c) Schematic diagram showing the validation involving the absence of chemotherapeutic drug diffusing across the barrier. (d) Comparison of cell viabilities evaluated from (c) using cisplatin. Each bar represented the mean ± SEM (n = 12 from three independent devices).

was utilized. Cisplatin drug (100 μg/ml, 1.5 μl) was added to the bottom well. A droplet (1.5 μl) of cell-free medium was dispensed onto the top surface of the barrier. After incubation for 24 hours, the former droplet was transferred to a new device, in which the cell droplet was pre-dispensed with a 1.5-μl volume containing 150 cells. Following another 24-hour incubation, the cell viability was then observed by staining viable cells with Calcein AM dye and evaluated suing ImageJ software. If cisplatin permeation occurred,

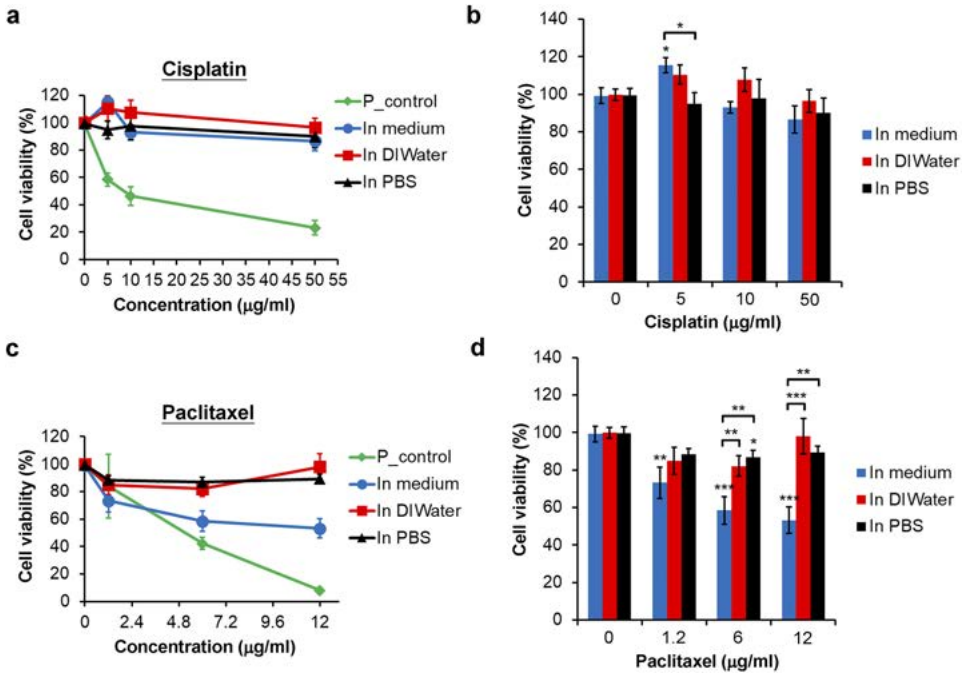

FIG. 3. Characteristics of anti-cancer drug profiles from CDCC and WDCC effects. (a) Comparison of cisplatin dose responses from CDCC (P_control) and WDCC (cisplatin was diluted in cell culture medium, DI water, or PBS). The effects of WDCC from different solutions were further compared in (b). Similarly, paclitaxel dose responses and effects from different solutions were compared in (c) and (d), respectively. For CDCC, P_control indicated that the drugs are loaded directly into the cell droplets. For WDCC, in contrast, the drugs were loaded into the device wells beneath the 95-μm PDMS barrier, following the illustration shown in Fig. 1(b). Each bar represented the mean ± SEM (n = 6 ~ 13 from three independent devices). *p < 0.05, **p < 0.01 and ***p < 0.001 were compared with the individual control solvent (i.e. 0 μg/ml) except for the indicator.

the final concentration of the transferred drug was suggested to be 50 μg/ml.

D. WDCC experimental procedure

Cisplatin (MW 300 g/mol; Fresenius kabi, Solan, Himachal Pradesh, India) and paclitaxel (MW 854 g/mol; Phyxol; Sinphar Pharmaceutical, Taiwan) were used at various concentrations in the device. The drugs were diluted in cell culture medium, deionized (DI) water or phosphate-buffered saline (PBS). Each concentration (1.5 μl) was added to the bottom well of the device. The same volume of a droplet containing 150 PC-3 cells was added to the relevant top portion of the barrier. The device was incubated for 24 hours. Cell viability and the effect of the WDCC were determined as detailed in Section III B.

For the positive control, i.e. no barrier, 2× concentration of cisplatin or paclitaxel was directly added to the previously dispensed cell droplets, resulting in a final 1× concentration suitable for the comparison with the WDCC results.

To assess the impact of magnetic orientation, a pair of magnets were placed on the top and bottom surfaces of the sealed dish in which the device was located in. The distance between the magnets was 13 mm, and the distance from the cell culture area to the bottom magnet was 4.5 mm. The magnetic flux density of the setup was approximately 750 gauss (75 mT) to cells according the manufacture's literature. Cell viability was determined as described above.

E. Effectivity of cancer cell inhibition (Eff)

It was inherently difficult to characterize the drug dose responses of WDCC (Figures 3 and 4) from a commonly-used four-parameter logistic equation.[10] To simplify such comparisons, instead, we adopted a linear regression equation ($y = ax + b$) to

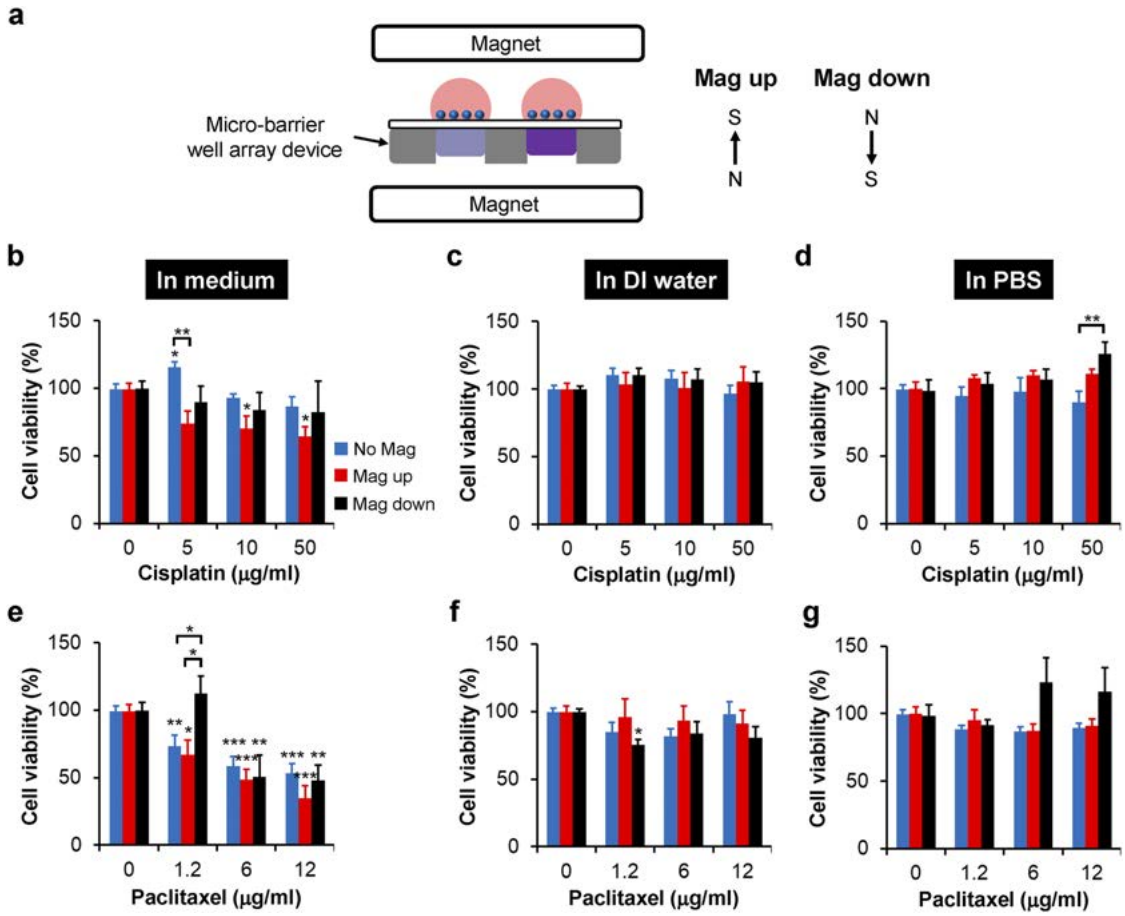

FIG. 4. Orientation of magnetic field partially dominates the WDCC effect. (a) Schematic showing the validation setup. "Mag up" and "Mag down" indicated the magnetic fields (from N to S poles) initialized upward and downward, respectively. (b)-(d) Comparison of cisplatin dose responses using the different magnetic orientations, in which cisplatin was diluted in medium, DI water, or PBS, respectively. Similarly, paclitaxel was examined following the above procedures as shown in (e) to (g). Each bar represented the mean ± SEM (n = 9 ~ 13 from three independent devices). *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were compared with the individual control concentration (i.e. 0 μg/ml) except for the indicator.

model these responses and then extracted their characteristics. We defined Eff as the negative derivative of this linear regression, i.e. Eff = $-a$. Positive or higher Eff indicated that the WDCC would effectively attenuate the cancer cell growth. In contrast, negative or lower Eff indicated the lack of an impact.

F. Statistical analyses

Student's t test was used to compare data from two groups. One-way ANOVA test was used to compare data from more than two groups. A p-value < 0.05 was considered to be statistically significant.

III. RESULTS AND DISCUSSION

The assessments of the lack of permeation of molecules or drugs in the micro-barrier well-array device, validation of WDCC using the device, examination of drug solvents (including cell culture medium, DI water, and PBS), and the influence of magnetic orientation on WDCC are described below. Additional information is also available in the supplementary material.

A. Permeation of the micro-barrier well-array device

PDMS preferentially allows the adhesion of small MW molecules (100 to approximately 300 g/mol) or proteins because of its hydrophobic nature.[11–13] Since we adopt a 95-μm PDMS barrier to separate cells and drugs, it is important to confirm whether the anti-cancer drugs we used could permeate the PDMS barrier. Two approaches was used to examine this issue (Figures 2(a) and 2(c), with the details in the Materials and Methods). The lack of binding of the Hoechst dye to cell nuclei was evident in the experimental set (8 μM separated from cell by the 95-μm barrier) as compared with the background signal (no dye, no barrier) and the positive experiment (8 μM dye, no barrier) (Figure 2(b)). The MW of Hoechst (616 g/mol) was similar to that of paclitaxel (854 g/mol). No significant permeation of 50-μg/ml cisplatin drug across the 95-μm barrier was evident (Figure 2(d)). As well, no significant permeation of 6-μg/ml paclitaxel was observed in the device, even when a thinner barrier of 20 μm was used (Figure S1). The collective findings suggested that the fabricated device incorporating a 95-μm PDMS barrier avoids the pitfalls of drug permeation.

B. Validation of WDCC in the micro-barrier well-array device

To explore whether our postulated WDCC process is realistic as an anti-cancer mechanism, we utilized the commonly-used anti-cancer drugs, cisplatin and paclitaxel. They were each diluted in cell culture medium, DI water, or PBS, and tested on PC-3 cells. WDCC effect was not evident in the inhibition of PC-3 cell growth under all cisplatin treatments, as compared with positive control (P_control in Figure 3(a)) and with each blank solvent (0 μg/ml cisplatin in Figure 3(b)). However, cell viability was significantly increased in 5 μg/ml cisplatin diluted in medium (Figure 3(b)). Interestingly, the effect of WDCC in inhibiting PC-3 cell growth could be attenuated when the solvent used as the diluent was PBS, rather than DI water. When paclitaxel was tested, a significant WDCC-mediated inhibition of cell growth was evident in the experimental set using medium as the diluent, even though the P_control remained the most powerful effector (Figures 3(c) and 3(d)). The inhibitory ability of WDCC could be significantly attenuated when the diluent was DI water or PBS (Figure 3(d)).

C. Orientation of magnetic field partially dominates the WDCC effect

To assess whether the nature of the WDCC could be altered by an external fields, we used paired magnets to examine the combined effect of magnetic field and WDCC (Figure 4(a)). The procedure was similar to that described in Figure 3. Combination of cisplatin and the upward direction of the magnetic field could improve the WDCC-mediated inhibition of cell growth (Figure 4(b)). No significant improvement of WDCC was evident when DI water or PBS was used as the drug diluent (Figures 4(c) and 4(d)). Similarly, paclitaxel diluted in DI water or PBS did not significantly improve the effect of WDCC, while some improvements were observed using medium diluent (Figures Figures 4(e) to 4(g)). A combined improvement of WDCC inhibition of PC-3 cell growth resulted from the use of medium as the drug diluent along with the upward magnetic field (Figures 4(b) and 4(e)). Use of a downward magnetic field did not enhance the inhibition of cell growth. Interestingly, applying cisplatin or paclitaxel diluted in PBS combined with a downward magnetic field could primarily attenuate the WDCC effects (Figures 4 (d) and (g)).

Since the cell inhibition rate due to WDCC did not exceed 50% (Figures 3 and 4), it is difficult to characterize the dose responses from a commonly-used four-parameter logistic regression. Thus, we define a new parameter, Eff, to address the issue. Figure 5 shows the comparisons of Eff derived from cisplatin and paclitaxel treatments. The highest Eff resulted when either drug was diluted in medium combined with an upward magnetic field (0.84 for cisplatin and 6.24 for paclitaxel). Use of cisplatin or paclitaxel diluted in PBS combined with a downward field resulted in the lowest Eff (-0.52 and -1.78, respectively). Relatively less effect of WDCC, i.e. Eff ~ 0, was found when the drugs were diluted in DI water or in partial PBS sets, suggesting that DI water could be a potential inhibitor targeting WDCC.

For CDCC treatment, higher concentrations of drugs can potently inhibit cell proliferation,[14] as demonstrated in our P_control in Figures 3(a) and 3(c). For WDCC, in contrast, it could not follow the standard. For example, the cell viability using 5 μg/ml cisplatin was significantly higher than that of the control (blue bars in Figure 3(b)). A similar result was found using paclitaxel treatment, in which cell viability was greater using the highest concentration of paclitaxel compared with the lower concentration (red bars in Figure 3(d)). Interestingly, the effect of WDCC from cisplatin was less pronounced than that of paclitaxel, especially when the drugs were diluted in medium. Given the distinctly different MWs of cisplatin (300 g/mol) and paclitaxel (854 g/mol) used, we suggest that anti-cancer drugs with a higher MW may more readily achieve an effective WDCC. This suggestion is reinforced by our previous demonstration that endothelin-1 that has a markedly higher MW 2492 g/mol can generate bindingless signal across the 95-μm PDMS membrane.[8] In contrast, tetracycline (lower MW 444 g/mol) generates a signal across a membrane only up to a 20 μm membrane.[9]

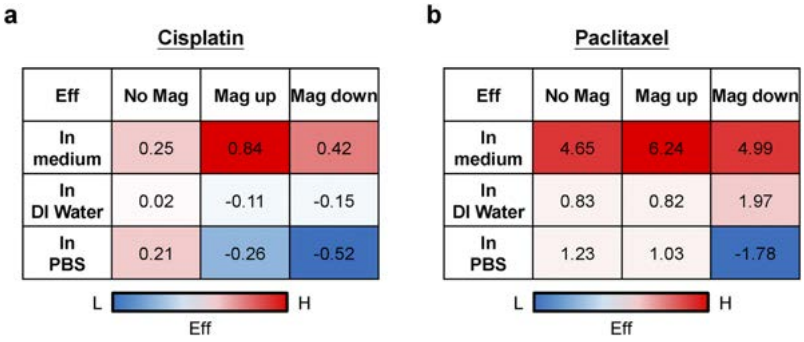

FIG. 5. Effectivity of cancer cell inhibition (Eff) with (a) cisplatin and (b) paclitaxel treatments using different setups.

Humans or cells exposed to static magnetic fields below 0.5 T are not adversely affected, with no effect on cell proliferation and cell damage.[15–17] During our experiments, no significant decrease in cell proliferation was observed using the 75-mT magnetic flux (data not shown). Thus, the orientation of magnetic field could mainly affect the WDCC, instead of damaging cells and adversely affecting proliferations.

It is possible that other mechanisms are involved in WDCC. For example, phonon signal transport in PDMS could be another potential effector by inter-chain phonon scattering in long-chained crystalline structures.[18] The physical and chemical mechanism of WDCC might be attributed to the residual space-time spin structure of the antibody molecule. We continue to examine critical parameters involved in WDCC, such as various anti-cancer drugs, drug solvents, cancer cell lines, barrier thicknesses, magnetic amplitudes, electric field, electromagnetic wave, temperature, and time. However, for now we are confident that the current findings using our novel micro-barrier well-array device provide the experimental evidence of WDCC.

IV. CONCLUSIONS

A micro-barrier well-array device was used to demonstrate WDCC through a mechanically separated PDMS barrier. WDCC may be involved in anti-cancer treatments and may enhance the bindingless drug-cell signal transduction. The device is free from permeations of cisplatin and paclitaxel across the barriers. WDCC resulted from paclitaxel was superior than that of cisplatin drug. In addition, the orientation of the magnetic field was influential in the effectiveness of the WDCC. Notably, a combined effect could be achieved by combining dilute cisplatin or paclitaxel in medium and applying an upward magnetic field. In contrast, drugs diluted in PBS combined with a downward field attenuated the WDCC. DI water was also a potential inhibitor of WDCC. The present findings should inform the development of WDCC as an anti-cancer mechanism in future physical and chemical studies.

SUPPLEMENTARY MATERIAL

See supplementary material for the permeation test of the fabricated devices.

ACKNOWLEDGMENTS

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REFERENCES

[1] S. Iadevaia, Y. Lu, F. C. Morales, G. B. Mills, and P. T. Ram, Cancer Res. **70**, 6704 (2010).
[2] B. Zhao, J. R. Pritchard, D. A. Lauffenburger, and M. T. Hemann, Cancer Discov. **4**, 166 (2014).
[3] A. Weiss, R. H. Berndsen, X. Ding, C. M. Ho, P. J. Dyson, H. van den Bergh, A. W. Griffioen, and P. Nowak-Sliwinska, Sci. Rep. **5**, 14508 (2015).
[4] M. Dallinga, L. Shen, R. O. Schlingemann, R. Pili, C. M. Ho, P. J. Dyson, H. van den Bergh, A. W. Griffioen, and P. Nowak-Sliwinska, Angiogenesis **18**, 233 (2015).
[5] H. A. Kenny, M. Lai-Nag, E. A. White, M. Shen, C. Y. Chiang, A. K. Mitra, Y. Zhang, M. Curtis, E. M. Schryver, S. Bettis, A. Jadhav, M. B. Boxer, Z. Li, M. Ferrer, and E. Lengyel, Nat. Commun. **6**, 6220 (2015).
[6] S. T. Ohnishi, T. Ohnishi, K. Nichino, Y. Tsurusaki, and M. Yamaguchi, eCAM **2**, 387 (2005).
[7] A. Farhadi, C. Forsyth, A. Banan, M. Shaikh, P. Engen, J. Z. Fields, and A. Keshavarzian, Bioelectrochemistry **71**, 142 (2007).
[8] C. T. Kuo, F. T. Chuang, P. Y. Wu, Y. C. Lin, H. K. Liu, G. S. Huang, T. C. Tsai, C. Y. Chi, A. M. Wo, H. Lee, and S. C. Lee, J. Appl. Phys. **116**, 044702 (2014).
[9] C. T. Kuo, C. Y. Chi, P. Y. Wu, F. T. Chuang, Y. C. Lin, H. K. Liu, G. S. Huang, T. C. Tsai, A. M. Wo, H. Lee, and S. C. Lee, J. Appl. Phys. **119**, 024702 (2016).
[10] L.-C. Hsiung, C.-L. Chiang, C.-H. Wang, Y.-H. Huang, C.-T. Kuo, J.-Y. Cheng, C.-H. Lin, V. Wu, H.-Y. Chou, D.-S. Jong, H. Lee, and A. M. Wo, Lab Chip **11**, 2333 (2011).
[11] L.-C. Hsiung, C.-L. Chiang, C.-H. Wang, Y.-H. Huang, C.-T. Kuo, J.-Y. Cheng, C.-H. Lin, V. Wu, H.-Y. Chou, D.-S. Jong, H. Lee, and A. M. Wo, Lab Chip **11**, 2333 (2011).
[12] K. Ozasa, J. Lee, S. Song, M. Hara, and M. M. aeda, Lab Chip **13**, 4033 (2013).
[13] M. W. Toepke and D. J. Beebe, Lab Chip **6**, 1484 (2006).
[14] C. T. Kuo, C. L. Chiang, C. H. Chang, H. K. Liu, G. S. Huang, R. Y. J. Huang, H. Lee, C. S. Huang, and A. M. Wo, Biomaterials **35**, 1562 (2014).
[15] L. Potenza, L. Ubaldi, R. D. Sanctis, R. D. Bellis, L. Cucchiarini, and M. Dacha, Mutation Research **561**, 53 (2004).
[16] J. Miyakoshi, Prog Biophys Mol Biol. **87**, 213 (2005).
[17] V. Zablotskii, T. Polyakova, O. Lunov, and A. Dejneka, Sci. Rep. **6**, 37407 (2016).
[18] T. Luo, K. Esfarjani, J. Shiomi, A. Henry, and G. Chen, J. Appl. Phys. **109**, 074321 (2011).