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Perspective

Ion-Depleting Action of Perm-Selective Membranes for Enhancing Electrical Communication and Gated Ion Channel Activity in Cell Cultures

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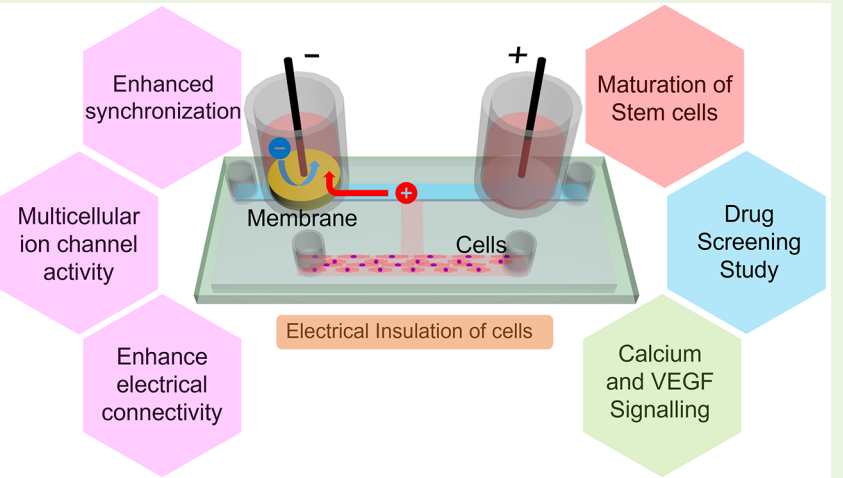

ABSTRACT: Ion-depletion action of an ion-selective membrane produces a moat channel that electrically insulates a cell colony and elevates the cell medium potential uniformly to synchronously activate and deactivate the voltage-gated ion channels of all cells. The result is robust synchronization with strong intercellular electrical communication and the discovery of ion channel deactivation that is only possible when the cells are in communication. The study suggests that the collective response of a cell colony to external stimuli is distinct from that of a single cell. Cell proliferation must hence be guided with strong intercellular communication and proper exogenous stimuli.

KEYWORDS: *ion depletion, perm-selective membranes, voltage-gated ion channels, cardiomyocytes*

SUMMARY

Patterned evolution of functional tissues from engineered stem cells to realize an OOC has been a long-lasting dream.[1−5] Once fully realized, they may replace animal models as the most convenient human surrogates for high-throughput pharmacological screening and fundamental research on interorgan and intercellular signaling driving tissue growth/regeneration and metabolic/immuno-system dynamics.[6−8] These miniaturized OOC platforms must closely mimic the cellular microenvironment with the proper extracellular matrix, the correct growth factor protocol, and the required chemical/mechanical/electrical stimuli that determine stem-cell fate.[9−12]

A key stimulus is the exogenous electric field that finds application in wound healing, patterned embryonic development, pluripotent stem cell maturation, and cancer metastasis.[13−18] Both AC (alternating current) and DC (direct current) fields have been used extensively to direct the differentiation of pluripotent stem cells to electrically excitable cardiac and nerve cells.[19,20] In heart-on-chip platforms, AC external electrical stimulations are routinely used to determine the cardiomyocytes' contractile properties, disease modeling and cytotoxicity studies,[21−25] as the exogenous field affects the intercellular electrical communication important for such colonies. More than any other signaling pathways, electrical intercellular communication and the proper exogenous electrical stimulation are difficult to mimic in an OOC. For example, stem cell maturation into heart and neuron cells is

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known to involve activities in voltage-gated ion channels,[26−28] and yet the optimum exogenous voltage protocol for these cells has yet to be developed.

The main culprit is that the cell media in OOC is highly conductive. The resulting field leakage does not permit robust intercellular electrical communication, which is required to study systemic response to exogenous stimuli. Moreover, the exogenous field lines will not penetrate the ion channels but bypass the cell as the resistance of the cellular membrane is much higher than the cell culture medium. Typically, the exogenous fields applied in a heart-on-a-chip device are low in amplitude (1−6 V/cm) and current (< 3 mA) to prevent any detrimental effects on cells due to joule healing, harmful faradaic reaction products, and air bubble formation.[29] The induced voltage drop across an individual cell can hence become less than the requisite 20−50 mV necessary to activate voltage gated ion channels. Interdigitated electrodes allow a higher voltage drop and are indeed able to pace cells, but they cannot maintain a uniform potential over a colony of communicating cells to study their collective response. This electrically lossy medium environment does not exist in a live organism with layers of insulating tissues.

Instead, the ion channel activities have mostly been studied at a single cell level using voltage clamp and patch clamp micromanipulators for studying ion channelopathies.[30,31] The intracellular electrode ensures field penetration through the membrane ion channels. However, such intracellular electrodes cannot be applied to a large colony of cells with strong intercellular communication. A detailed cytotoxicity study on ion channelopathies of a system of communicating cardiac cells must hence be studied with a different technique.

A novel flow-free and noninvasive method was developed recently using perm-selective membranes to apply a tunable, high, and constant potential over a colony of rCM cultured in a microfluidic OOC chip to effectively shield the colony electrically.[32] The rCM colony was grown in a cell channel connected to a parallel moat channel by an orthogonal side channel. The ion depletion action of an ion-selective membrane was used to deplete the ions in the moat channel with an electric field along the channel. The cell channel is electrically isolated by the near DI-water conductivity of the moat channel, thus allowing intense intercellular electrical communication within the colony. The high conductivity of the cell channel, however, permits significant field penetration from the moat channel. In fact, the high cell channel conductivity ensures that the entire cell electrolyte (which retains the original ionic strength) is at a constant potential. In these membrane microfluidics-based devices, the applied electric field can be as high as 100 V/cm, which is much higher than previously reported values, but a relatively low current (∼100 μA) minimizes Ohmic heating (∼sub-milliwatt) thanks to the low conductivity of the ion-depleted moat. This new technique can hence enhance both intercellular communication as well as allowing large but spatially uniform external medium potential for the entire colony.

With a 1000-cell cardiomyocyte culture, the authors demonstrated enhanced synchronization of cell beating with this technique. The imposed field is shown to increase the beating frequency of synchronized rCM cells approximately by a factor of 2. Moreover, the synchronization can be switched on and off reproducibly within a few milliseconds by activating and deactivating the depletion zone in the moat channel. Action potential waveform analysis indicates that the large exogenous potential has depolarized the communicating cells simultaneously by activating an HCN Na ion channel and deactivating all other calcium, sodium, and potassium channels. This is consistent with prior single-cell voltage clamp experiments that suggest that the long-polarized interval between the twin contraction/relaxation peaks, when the cell is in equilibrium and motionless, is reduced by activating HCN ion channels.[33,34] Immunostaining further confirmed the existence of HCN2 channels in neonatal rCM cells. Closer image analysis shows that the L-type Ca_v ion channels are deactivated more than the other channels by the positive extracellular potential, which has never been observed in single-cell voltage clamp experiments. These exploratory observations suggest that HCN and L-type Ca_v ion channels are more sensitive than others to extracellular voltage *in a synchronized colony with intercellular communication*. However, extensive pharmacologic and/or genetic experiments directly targeting HCN channels expressed by neonatal rat ventricular myocytes (i.e., HCN2 and HCN4) and L-type Ca_v needs to be performed to completely validate the suggested hypothesis. This OOC technique can hence mimic the conditions in the body, where an exogenous electrical stimulus from another organ or a pace-setter is imparted uniformly over a colony of cells simultaneously with intimate intercellular communication, with the collective response quite distinct from that of a single cell. We believe that this kind of device can direct differentiation/maturation of pluripotent stem cells with high electrical connectivity into neuronal and rCM colonies.

FUTURE DIRECTIONS

Low-cost membrane-based microfluidics devices have been extensively used for biosensing, sample pretreatment, analyte concentration, and pH actuation.[35−40] Under a voltage bias, an external depletion front develops across one side of a membrane and can be further propagated and controlled.[41,42] These devices can be integrated with cell culture to measure and control VEGF and calcium signaling between adjacent cells and globally over the entire tissue.

In the future, a perm-selective membrane-based constant potential environment setup can be used to test the controversial "ephaptic coupling" theory between neuron and cardiac cells. The theory is based on the hypothesis that long- and short-range synchronization of cells can be achieved by the extracellular charge generation due to ion channel activities of neighboring neuron or cardiac cells, respectively. The multi-scale coupling of long-range exogenous potential and intercellular action potential between neighboring cells is precisely what this technique can scrutinize.

So far, synthetic biology relies on genetic modification, without considering physiology and intercellular communication. Bioelectric intercellular communication during tissue growth is now attracting more attention not only for electrically active nerve and cardiac cells but also during angiogenesis. Recent work has shown that VEGF and calcium signaling is coupled differently for polarized and unpolarized cells, but the mechanisms coordinating multicellular behavior between communicating cells remain unknown, much less how exogenous fields and stimuli couple with this system dynamics.[43,44] Membrane-based microfluidic devices, in conjunction with a comprehensive synthetic biology toolkit that includes optogenetics, quantitative imaging, and molecular manipulation, may realize a next-generation OOC platform that allows us to probe and dynamically control these

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multiscale electrical and molecular communications between different cells and across different colonies.

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Notes

The authors declare no competing financial interest.

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GLOSSARY/DEFINITIONS

Ion-depletion, removing both cations and anions; ion-selective membrane, membranes that allow only counterions to pass through them by electromigration; OOC, organ-on-a-chip; rCM, neonatal rat cardiomyocytes; HCN, hyperpolarization-activated, cyclic nucleotide-gated (HCN) ion channels; VEGF, vascular endothelial growth factor is a protein that supports new blood vessel growth

REFERENCES

(1) Reardon, S. Biodefence Researchers Seek "Homo Chippiens. *Nature* **2015**, *518* (7539), 285−286.

(2) Palaninathan, V.; Kumar, V.; Maekawa, T.; Liepmann, D.; Paulmurugan, R.; Eswara, J. R.; Ajayan, P. M.; Augustine, S.; Malhotra, B. D.; Viswanathan, S.; Renugopalakrishnan, V.; Kumar, S. D. Multi-Organ on a Chip for Personalized Precision Medicine. *MRS Commun.* **2018**, *8* (3), 652−667.

(3) Lee, S. H.; Ha, S. K.; Choi, I.; Choi, N.; Park, T. H.; Sung, J. H. Microtechnology-Based Organ Systems and Whole-Body Models for Drug Screening. *Biotechnol. J.* **2016**, *11* (6), 746−756.

(4) Rogal, J.; Probst, C.; Loskill, P. Integration Concepts for Multi-Organ Chips: How to Maintain Flexibility?! *Future Sci. OA* **2017**, *3* (2), FSO180.

(5) Skardal, A.; Murphy, S. V.; Devarasetty, M.; Mead, I.; Kang, H.-W.; Seol, Y.-J.; Shrike Zhang, Y.; Shin, S.-R.; Zhao, L.; Aleman, J.; Hall, A. R.; Shupe, T. D.; Kleensang, A.; Dokmeci, M. R.; Jin Lee, S.; Jackson, J. D.; Yoo, J. J.; Hartung, T.; Khademhosseini, A.; Soker, S.; Bishop, C. E.; Atala, A. Multi-Tissue Interactions in an Integrated Three-Tissue Organ-on-a-Chip Platform. *Sci. Rep.* **2017**, *7* (1), 8837.

(6) Luni, C.; Serena, E.; Elvassore, N. Human-on-Chip for Therapy Development and Fundamental Science. *Curr. Opin. Biotechnol.* **2014**, *25*, 45−50.

(7) Cavero, I.; Guillon, J.-M.; Holzgrefe, H. H. Human Organotypic Bioconstructs from Organ-on-Chip Devices for Human-Predictive Biological Insights on Drug Candidates. *Expert Opin. Drug Saf.* **2019**, *18* (8), 651−677.

(8) Zhang, B.; Radisic, M. Organ-on-a-Chip Devices Advance to Market. *Lab Chip* **2017**, *17* (14), 2395−2420.

(9) Probst, C.; Schneider, S.; Loskill, P. High-Throughput Organ-on-a-Chip Systems: Current Status and Remaining Challenges. *Curr. Opin. Biomed. Eng.* **2018**, *6*, 33−41.

(10) Aziz, A. U. R.; Geng, C.; Fu, M.; Yu, X.; Qin, K.; Liu, B. The Role of Microfluidics for Organ on Chip Simulations. *Bioengineering* **2017**, *4* (2), 39.

(11) Geraili, A.; Jafari, P.; Hassani, M. S.; Araghi, B. H.; Mohammadi, M. H.; Ghafari, A. M.; Tamrin, S. H.; Modarres, H. P.; Kolahchi, A. R.; Ahadian, S.; Sanati-Nezhad, A. Controlling Differentiation of Stem Cells for Developing Personalized Organ-on-Chip Platforms. *Adv. Healthcare Mater.* **2018**, *7* (2), 1700426.

(12) Wang, L.; Li, Z.; Xu, C.; Qin, J. Bioinspired Engineering of Organ-on-Chip Devices. In *Biological and Bio-inspired Nanomaterials: Properties and Assembly Mechanisms*; Perrett, S., Buell, A. K., Knowles, T. P. J., Eds.; Advances in Experimental Medicine and Biology; Springer: Singapore, 2019; pp 401−440. DOI: 10.1007/978-981-13-9791-2_13.

(13) da Silva, L. P.; Kundu, S. C.; Reis, R. L.; Correlo, V. M. Electric Phenomenon: A Disregarded Tool in Tissue Engineering and Regenerative Medicine. *Trends Biotechnol.* **2020**, *38* (1), 24−49.

(14) Hunckler, J.; de Mel, A. A Current Affair: Electrotherapy in Wound Healing. *J. Multidiscip. Healthc.* **2017**, *10*, 179−194.

(15) Nunes, S. S.; Miklas, J. W.; Liu, J.; Aschar-Sobbi, R.; Xiao, Y.; Zhang, B.; Jiang, J.; Massé, S.; Gagliardi, M.; Hsieh, A.; Thavandiran, N.; Laflamme, M. A.; Nanthakumar, K.; Gross, G. J.; Backx, P. H.; Keller, G.; Radisic, M. Biowire: A Platform for Maturation of Human Pluripotent Stem Cell−Derived Cardiomyocytes. *Nat. Methods* **2013**, *10* (8), 781−787.

(16) Oliveira, K. M. C.; Barker, J. H.; Berezikov, E.; Pindur, L.; Kynigopoulos, S.; Eischen-Loges, M.; Han, Z.; Bhavsar, M. B.; Henrich, D.; Leppik, L. Electrical Stimulation Shifts Healing/Scarring towards Regeneration in a Rat Limb Amputation Model. *Sci. Rep.* **2019**, *9* (1), 11433.

(17) Feng, J.-F.; Liu, J.; Zhang, X.-Z.; Zhang, L.; Jiang, J.-Y.; Nolta, J.; Zhao, M. Guided Migration of Neural Stem Cells Derived from Human Embryonic Stem Cells by an Electric Field. *Stem Cells* **2012**, *30* (2), 349−355.

(18) Kang, D. K.; Hosseini, S. H. R.; Shiraishi, E.; Yamanaka, M.; Akiyama, H. Single Nanosecond Pulsed Electric Field Effects on Embryonic Development of the Medaka Fish. *IEEE Trans. Plasma Sci.* **2012**, *40* (10), 2379−2387.

(19) Jing, W.; Zhang, Y.; Cai, Q.; Chen, G.; Wang, L.; Yang, X.; Zhong, W. Study of Electrical Stimulation with Different Electric-Field Intensities in the Regulation of the Differentiation of PC12 Cells. *ACS Chem. Neurosci.* **2019**, *10* (1), 348−357.

(20) Hernández, D.; Millard, R.; Sivakumaran, P.; Wong, R. C. B.; Crombie, D. E.; Hewitt, A. W.; Liang, H.; Hung, S. S. C.; Pébay, A.; Shepherd, R. K.; Dusting, G. J.; Lim, S. Y. Electrical Stimulation Promotes Cardiac Differentiation of Human Induced Pluripotent Stem Cells. *Stem Cells Int.* **2015**, *2016*, e1718041.

(21) Tandon, N.; Cannizzaro, C.; Chao, P.-H. G.; Maidhof, R.; Marsano, A.; Au, H. T. H.; Radisic, M.; Vunjak-Novakovic, G. Electrical Stimulation Systems for Cardiac Tissue Engineering. *Nat. Protoc.* **2009**, *4* (2), 155−173.

(22) Holt, E.; Lunde, P. K.; Sejersted, O. M.; Christensen, G. Electrical Stimulation of Adult Rat Cardiomyocytes in Culture Improves Contractile Properties and Is Associated with Altered Calcium Handling. *Basic Res. Cardiol.* **1997**, *92* (5), 289−298.

(23) Berger, H. J.; Prasad, S. K.; Davidoff, A. J.; Pimental, D.; Ellingsen, O.; Marsh, J. D.; Smith, T. W.; Kelly, R. A. Continual

Electric Field Stimulation Preserves Contractile Function of Adult Ventricular Myocytes in Primary Culture. *Am. J. Physiol.-Heart Circ. Physiol.* **1994**, *266* (1), H341−H349.
(24) Tandon, N.; Marsano, A.; Maidhof, R.; Wan, L.; Park, H.; Vunjak-Novakovic, G. Optimization of Electrical Stimulation Parameters for Cardiac Tissue Engineering. *J. Tissue Eng. Regener. Med.* **2011**, *5* (6), e115−e125.
(25) Ronaldson-Bouchard, K.; Yeager, K.; Teles, D.; Chen, T.; Ma, S.; Song, L.; Morikawa, K.; Wobma, H. M.; Vasciaveo, A.; Ruiz, E. C.; Yazawa, M.; Vunjak-Novakovic, G. Engineering of Human Cardiac Muscle Electromechanically Matured to an Adult-like Phenotype. *Nat. Protoc.* **2019**, *14* (10), 2781−2817.
(26) Li, G.-R.; Deng, X.-L. Functional Ion Channels in Stem Cells. *World J. Stem Cells* **2011**, *3* (3), 19−24.
(27) Tan, Y.; Fei, D.; He, X.; Dai, J.; Xu, R.; Xu, X.; Wu, J.; Li, B. L-Type Voltage-Gated Calcium Channels in Stem Cells and Tissue Engineering. *Cell Proliferation* **2019**, *52* (4), e12623.
(28) Atsuta, Y.; Tomizawa, R. R.; Levin, M.; Tabin, C. J. L-Type Voltage-Gated Ca2+ Channel CaV1.2 Regulates Chondrogenesis during Limb Development. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (43), 21592−21601.
(29) Merrill, D. R.; Bikson, M.; Jefferys, J. G. R. Electrical Stimulation of Excitable Tissue: Design of Efficacious and Safe Protocols. *J. Neurosci. Methods* **2005**, *141* (2), 171−198.
(30) Lehmann-Horn, F.; Jurkat-Rott, K. Voltage-Gated Ion Channels and Hereditary Disease. *Physiol. Rev.* **1999**, *79* (4), 1317−1372.
(31) Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. Improved Patch-Clamp Techniques for High-Resolution Current Recording from Cells and Cell-Free Membrane Patches. *Pfluegers Arch.* **1981**, *391* (2), 85−100.
(32) Yadav, V.; Chong, N.; Ellis, B.; Ren, X.; Senapati, S.; Chang, H.-C.; Zorlutuna, P. Constant-Potential Environment for Activating and Synchronizing Cardiomyocyte Colonies with on-Chip Ion-Depleting Perm-Selective Membranes. *Lab Chip* **2020**, *20* (22), 4273−4284.
(33) Biel, M.; Schneider, A.; Wahl, C. Cardiac HCN Channels: Structure, Function, and Modulation. *Trends Cardiovasc. Med.* **2002**, *12* (5), 206−213.
(34) Er, F.; Larbig, R.; Ludwig, A.; Biel, M.; Hofmann, F.; Beuckelmann, D. J.; Hoppe, U. C. Dominant-Negative Suppression of HCN Channels Markedly Reduces the Native Pacemaker Current I_f and Undermines Spontaneous Beating of Neonatal Cardiomyocytes. *Circulation* **2003**, *107* (3), 485−489.
(35) Zhang, C.; Sun, G.; Senapati, S.; Chang, H.-C. A Bifurcated Continuous Field-Flow Fractionation (BCFFF) Chip for High-Yield and High-Throughput Nucleic Acid Extraction and Purification. *Lab Chip* **2019**, *19* (22), 3853−3861.
(36) Yin, Z.; Ramshani, Z.; Waggoner, J. J.; Pinsky, B. A.; Senapati, S.; Chang, H.-C. A Non-Optical Multiplexed PCR Diagnostic Platform for Serotype-Specific Detection of Dengue Virus. *Sens. Actuators, B* **2020**, *310*, 127854.
(37) Park, S.; Abu-Rjal, R.; Rosentsvit, L.; Yossifon, G. Novel Electrochemical Flow Sensor Based on Sensing the Convective-Diffusive Ionic Concentration Layer. *ACS Sens.* **2019**, *4* (7), 1806−1815.
(38) Sun, G.; Wan, J.; Lu, H. Rapid and Multi-Cycle SmFISH Enabled by Microfluidic Ion Concentration Polarization for in-Situ Profiling of Tissue-Specific Gene Expression in Whole C. Elegans. *Biomicrofluidics* **2019**, *13* (6), 064101.
(39) Berzina, B.; Anand, K. R. Continuous Micellar Electrokinetic Focusing of Neutral Species Driven by Ion Concentration Polarization. *Lab Chip* **2019**, *19* (13), 2233−2240.
(40) Egatz-Gomez, A.; Wang, C.; Klacsmann, F.; Pan, Z.; Marczak, S.; Wang, Y.; Sun, G.; Senapati, S.; Chang, H.-C. Future Microfluidic and Nanofluidic Modular Platforms for Nucleic Acid Liquid Biopsy in Precision Medicine. *Biomicrofluidics* **2016**, *10* (3), 032902.
(41) Slouka, Z.; Senapati, S.; Chang, H.-C. Microfluidic Systems with Ion-Selective Membranes. *Annu. Rev. Anal. Chem.* **2014**, *7* (1), 317−335.
(42) Sun, G.; Senapati, S.; Chang, H.-C. High-Flux Ionic Diodes, Ionic Transistors and Ionic Amplifiers Based on External Ion Concentration Polarization by an Ion Exchange Membrane: A New Scalable Ionic Circuit Platform. *Lab Chip* **2016**, *16* (7), 1171−1177.
(43) Noren, D. P.; Chou, W. H.; Lee, S. H.; Qutub, A. A.; Warmflash, A.; Wagner, D. S.; Popel, A. S.; Levchenko, A. Endothelial Cells Decode VEGF-Mediated Ca2+ Signaling Patterns to Produce Distinct Functional Responses. *Sci. Signaling* **2016**, *9* (416), ra20.
(44) Yokota, Y.; Nakajima, H.; Wakayama, Y.; Muto, A.; Kawakami, K.; Fukuhara, S.; Mochizuki, N. Endothelial Ca2+ Oscillations Reflect VEGFR Signaling-Regulated Angiogenic Capacity in Vivo. *eLife* **2015**, *4*, e08817.

https://doi.org/10.1021/acsbiomaterials.1c01384
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