Editorial

Recapitulation of microtissue models connected with real-time readout systems via 3D printing technology

Jinah Jang, Dong-Woo Cho

Department of Mechanical Engineering, Pohang University of Science and Technology (POSTECH), Nam-gu, Pohang, Kyungbuk 37673, Korea
Correspondence to: Dong-Woo Cho. Department of Mechanical Engineering, Pohang University of Science and Technology (POSTECH), 77 Cheongam-ro, Nam-gu, Pohang, Kyungbuk 37673, Korea. Email: dwcho@postech.ac.kr.
Provenance: This is an invited Editorial commissioned by the Section Editor Kai Zhu (Department of Cardiac Surgery, Zhongshan Hospital Fudan University, Shanghai, China).
Comment on: Lind JU, Busbee TA, Valentine AD, *et al.* Instrumented cardiac microphysiological devices via multimaterial three-dimensional printing. Nat Mater 2017;16:303-8.

Submitted Jan 07, 2017. Accepted for publication Jan 07, 2017.
doi: 10.21037/jtd.2017.02.33
View this article at: http://dx.doi.org/10.21037/jtd.2017.02.33

The ultimate goal of biomedical research is to understand human diseases and to determine improved therapeutic approaches based on scientific evidence. So far, many researchers have suggested new therapeutic targets and drugs by examining cells or genetically modified transgenic animals as candidates. However, recent studies have found that these models fail to mimic the human condition, and this is probably the reason why many drugs are withdrawn after clinical trials (1). Currently, *in vitro* microtissue devices are emerging as alternatives to animal or conventional cell culture studies (2). Such devices can recapitulate human tissue microenvironments and their fundamental functional units, including the contractile laminar tissue layers of cardiac and skeletal muscles (2-5), alveolar-capillary interface of a lung (6), villi of intestines (7), lobules of a liver (8), or proximal tubules of the kidney (9). These devices are developed based on attempts to mimic native cellular arrangements and original mechanical movements, and the fabrication of the devices is carried out through microfabrication and microfluidic technologies. Although it is not possible to recreate the entire complexity of the human condition, these models enable a study of drug efficacy, safety, and mode of action via the testing of physiological functions *in vitro*.

These simplified systems comprehensively validate the cell phenotypes, such as the gene expression, structure, metabolism, and function of cells seeded on microenvironments by mathematical modeling and imaging analysis. The systems also recapitulate healthy or diseased conditions and facilitate tissue-specific functional evaluation. In this regard, 2D muscular tissue devices have received particular attention (10). These devices use the concept of tissue engineering to generate the laminar tissue layers of native myocardium and function better than traditional cardiomyocytes cultures. Further, geometrical, physical, or chemical cues can be built on the surface of devices by micro-molding or microcontact printing of biological materials (3,5,10-12). This process promotes maturation and the directional propagation of action potentials along the engineered patterns and enhances cell–cell interaction and stress generation, which is similar in value to that of *ex vivo* preparation (10,12-14). For example, the cells on a line-patterned substrate have been made to orient along a preferential alignment along the direction of the lines, which can direct cardiac myofiber anisotropy as observed in native tissue (15-17). Further, a high-aspect-ratio polydimethylsiloxane (PDMS) mold, comprising arrays of micro-posts with defined size, elongation, and spacing, has been fabricated by soft lithography technology to facilitate casting of a cell/hydrogel mixture for generating 3D cardiac muscle architectures (e.g., several square centimeters large, with a thickness of a few hundred microns) (13,18,19). This mold guides local 3D cell alignment and provides the spatial pattern of the mechanical tension governing highly organized and uniformly differentiated cardiac muscle fibers. These 2D or 3D tissue models have become popular

in assessing electrophysiology and rhythm disorder due to the spatial heterogeneity of the tissue, and further, their conduction velocity is comparable with those obtained from native cardiac tissue. Furthermore, the models are capable of assessing contractile function by microscopy coupled with visual tracking analysis that can provide real-time readouts while the microtissue devices are culturing.

Current advancements in engineered tissue devices provide reasonable modeling and small-scale drug screening applications, but they are not well suited for high-throughput studies because such studies require multiple steps and are expensive and laborious processes, which hinder rapid prototyping and customization. In contrast, multi-material 3D printing technology enables the utilization of a wide range of functional, structural, and biological materials (20,21). Further, the components can all be integrated in a single programmable manufacturing step. Importantly, a higher-order assembly of physiologically relevant 3D human tissue models can be constructed by utilizing medical images (e.g., CT, MRI). Moreover, 3D-printed *in vitro* tissues in conjunction with microfluidic systems can provide an ideal test platform for the analysis of chemical, biological, and toxicological agents along with enabling basic research via offering design and system flexibilities (22-24).

In a recent issue of Nature Materials, Lind *et al.* have introduced a novel fabrication process for instrumented microphysiological devices via 3D printing technology (25). The primary benefit of this system is highly integrated sensors that facilitate electronic readout of the contractile function of the generated tissues continuously, and further, the all-in-one fabrication with multi-material 3D printing process utilized in their approach reduces the currently used multistep procedures. In addition, a soft-material-based strain gauge is embedded in the micropatterned architecture that guides the self-assembly of physio-mimetic laminar cardiac tissues via utilizing cardiomyocytes from rats (neonatal ventricular rat myocytes, NRVM) and humans (human-induced pluripotent stem-cell-derived cardiomyocytes, hiPS-CM).

In their study, Lind *et al.* integrated four individually controllable printing heads into an automated calibration process (25). This allows continuous printing with the sharing of coordinates among the print heads. First, they printed dextran films to provide sacrificial release layers that allow the detachment of cantilevers whose bending due to tissue contraction from the substrate. To match the range of intrinsic cardiac muscle stiffness (1–15 kPa), they applied a highly diluted thermoplastic polyurethane (TPU) ink for cantilever fabrication. This facilitates the patterning of thin layers (0.5–6.5 μm in thickness) because the low viscous ink easily spreads. The rate of solvent evaporation was increased to achieve the required lateral dimensions. Above the TPU layer, strain gauge wires were printed with a TPU ink reinforced with carbon black nanoparticles (CB: TPU), followed by a second layer of TPU printing. Although they examined several alternatives, they finally chose CB as filler from the perspectives of printability, stiffness, and electrical signal responsiveness (hysteresis).

To guide tissue self-assembly, 60-μm-wide filaments with tissue were patterned using viscous PDMS ink on top of each cantilever. Next, a highly conductive ink was designed to print the electrical leads and contact pads by blending silver particles and polyamide polymer (Ag: PA). Eight individual wells were generated per device with rigid biopolymers, and the whole device was cured at 100 ℃. Finally, the device was sterilized and subsequently seeded with cardiomyocytes. Although most of the steps were automated, there is a limit to achieving an all-in-one process of fabricating whole tissue devices containing cells due to the polymerization characteristic of the applied inks. Further development of functional inks is required to realize either rapid sol-gel transition or fast polymerization after the printing process, which is one of the most important factors in developing 3D printing technology for biomedical applications.

Lind *et al*. also investigated the influence of the microgroove spacing on muscular tissue maturation, and they found that a 60-μm filament spacing yielded the highest degree of sarcomere orientational order parameter (OOP) (25). For the 60-μm groove spacing, the action potential propagation was faster parallel to the grooves as compared with the transverse by a factor of 2.7, which is similar to the longitudinal-to-transverse velocity ratio of 2.1 observed in the native ventricle. The alignment of cardiomyocytes in an anisotropic fashion with the use of various biophysical cues including microgrooves and nanogrooves, substrate stiffness, and patterning of the extracellular matrix component indicated the promotion of homogeneous cell alignment and elongation parallel to the grooves. These features resulted in unidirectional cantilever deflection that employed the contractile force enhancement. Notably, these devices exhibited electrophysiological properties that closely matched with those of the devices fabricated by molding or microcontact printing. This result indicates that the automatic

fabrication process is a reliable manufacturing method.

Moreover, Lind *et al.* acquired a direct non-invasive electronic readout of the contractile stresses when the printed cardiac devices were culturing (25). Dose-response studies of drugs were performed for direct assessing of the contraction strength and beating rate via real-time monitoring, which is an ideal *in vitro* testing platform. These models exhibited a negative inotropic response to verapamil and a positive chronotropic response to isoproterenol, resembling conventional cell cultures and 2D microtissue models. This robust drug response and contraction function continued over a period of 28 days and even improved in terms of strength and spontaneous contraction stresses. In particular, hiPS-CMs underwent structural maturity and exhibited an increase in the twitch force over time, thereby suggesting that these models facilitate not only acute drug investigation but also chronic response studies.

Although their study demonstrated a mono-layered or a four-layered microtissue device similar to the function of the 2D muscle tissue that has been developed thus far, it is to be noted that 3D printing technology can potentially shift the paradigm toward creating a thick and complex tissue device that can recapitulate the micro-arrangement of cardiomyocytes, endothelial cells, and fibroblasts according to the physiologically relevant organization. Furthermore, 3D printing technology can enable the development of an all-in-one manufacturing method including cells via the design of biologically favorable materials and its crosslinking chemistry for rapid polymerization. The integration of sensors and the tissue device usually requires simplified data acquisition and analysis, and the application of a long-term monitoring system can facilitate real-time digital imaging processing, which can give new insights into tissue morphogenesis, pathogenesis, and drug-responsive remodeling processes.

In summary, the recently published research article by Lind *et al.* demonstrated an automated fabrication process for instrumented microphysiological devices via 3D printing technology for the first time. These developments have significant implications for advanced manufacturing methods. Notably, multi-material-based 3D printing processes can generate functional cardiac tissue models for *in vitro* drug testing platforms connected with real-time readouts. Although the use of 3D printing technology for *in vitro* tissue model fabrication has received considerable attention, the technical and functional capabilities of the models in resembling native tissue remains to be overcome. The abovementioned devices used microscopic observation and simplified strain gauge sensors to measure contractile forces, and the presence of a reliable readout can be beneficial when the device is culturing in the incubator. The authors have also completed a valuable study promoting tissue assembly on printed micro-physiological devices by exploiting the versatility of 3D printing technology. To mimic the innate tissue responses under *in vitro* conditions, further investigations on the precise replication of the tissue microenvironment including cellular organization, composition, and extracellular matrix environments are required.

Acknowledgements

Funding: This work was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education (No. 2015R1A6A3A04059015). And by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through Agri-Bio industry Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (No. 316031-3).

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Seok J, Warren HS, Cuenca AG, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. Proc Natl Acad Sci U S A 2013;110:3507-12.
2. Benam KH, Dauth S, Hassell B, et al. Engineered in vitro disease models. Annu Rev Pathol 2015;10:195-262.
3. Uzel SG, Pavesi A, Kamm RD. Microfabrication and microfluidics for muscle tissue models. Prog Biophys Mol Biol 2014;115:279-93.
4. Park JW, Vahidi B, Taylor AM, et al. Microfluidic culture platform for neuroscience research. Nat Protoc 2006;1:2128-36.
5. Feinberg AW, Feigel A, Shevkoplyas SS, et al. Muscular thin films for building actuators and powering devices. Science 2007;317:1366-70.
6. Huh D, Matthews BD, Mammoto A, et al. Reconstituting organ-level lung functions on a chip. Science

2010;328:1662-8.
7. Kim HJ, Huh D, Hamilton G, et al. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab Chip 2012;12:2165-74.
8. Ho CT, Lin RZ, Chen RJ, et al. Liver-cell patterning lab chip: mimicking the morphology of liver lobule tissue. Lab Chip 2013;13:3578-87.
9. Jang KJ, Mehr AP, Hamilton GA, et al. Human kidney proximal tubule-on-a-chip for drug transport and nephrotoxicity assessment. Integr Biol (Camb) 2013;5:1119-29.
10. Ma Z, Liu Q, Liu H, et al. Laser-patterned stem-cell bridges in a cardiac muscle model for on-chip electrical conductivity analyses. Lab Chip 2012;12:566-73.
11. Nagamine K, Kawashima T, Sekine S, et al. Spatiotemporally controlled contraction of micropatterned skeletal muscle cells on a hydrogel sheet. Lab Chip 2011;11:513-7.
12. Bursac N, Papadaki M, Cohen RJ, et al. Cardiac muscle tissue engineering: toward an in vitro model for electrophysiological studies. Am J Physiol 1999;277:H433-44.
13. Agarwal A, Farouz Y, Nesmith AP, et al. Micropatterning Alginate Substrates for in vitro Cardiovascular Muscle on a Chip. Adv Funct Mater 2013;23:3738-46.
14. Thavandiran N, Dubois N, Mikryukov A, et al. Design and formulation of functional pluripotent stem cell-derived cardiac microtissues. Proc Natl Acad Sci U S A 2013;110:E4698-707.
15. Pong T, Adams WJ, Bray MA, et al. Hierarchical architecture influences calcium dynamics in engineered cardiac muscle. Exp Biol Med (Maywood) 2011;236:366-73.
16. Kim DH, Lipke EA, Kim P, et al. Nanoscale cues regulate the structure and function of macroscopic cardiac tissue constructs. Proc Natl Acad Sci U S A 2010;107:565-70.
17. Chung CY, Bien H, Sobie EA, et al. Hypertrophic phenotype in cardiac cell assemblies solely by structural cues and ensuing self-organization. FASEB J 2011;25:851-62.
18. Hansen A, Eder A, Bönstrup M, et al. Development of a drug screening platform based on engineered heart tissue. Circ Res 2010;107:35-44.
19. Bielawski KS, Leonard A, Bhandari S, et al. Real-Time Force and Frequency Analysis of Engineered Human Heart Tissue Derived from Induced Pluripotent Stem Cells Using Magnetic Sensing. Tissue Eng Part C Methods 2016;22:932-40.
20. Zhang X, Zhang Y. Tissue Engineering Applications of Three-Dimensional Bioprinting. Cell Biochem Biophys 2015;72:777-82.
21. Park JY, Gao G, Jang J, et al. 3D printed structures for delivery of biomolecules and cells: tissue repair and regeneration. J Mater Chem B 2016;4:7521-39.
22. Lee H, Cho DW. One-step fabrication of an organ-on-a-chip with spatial heterogeneity using a 3D bioprinting technology. Lab Chip 2016;16:2618-25.
23. Miller JS, Stevens KR, Yang MT, et al. Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. Nat Mater 2012;11:768-74.
24. Ma X, Qu X, Zhu W, et al. Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting. Proc Natl Acad Sci U S A 2016;113:2206-11.
25. Lind JU, Busbee TA, Valentine AD, et al. Instrumented cardiac microphysiological devices via multimaterial three-dimensional printing. Nat Mater 2017;16:303-8.

Cite this article as: Jang J, Cho DW. Recapitulation of microtissue models connected with real-time readout systems via 3D printing technology. J Thorac Dis 2017;9(2):233-236. doi: 10.21037/jtd.2017.02.33

