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HIGHLIGHTS

Complex in vitro models: do not complicate it

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In vitro complex models

The physiologically relevant and mimetic in vitro 3D models of tissues and organs, the so-called complex in vitro models (CIVMs), can be grouped into extracellular matrix (ECM)-independent models and ECM-dependent models.

The holy grail of in vitro models is to achieve the same degree of complexity of the tissues to be mimicked to increase its relevance, which does not necessarily mean to involve the development of a complicated model. Important achievements in the field include the development of highly controlled in vitro models for studies of neurodegenerative diseases. Such complex microphysiological systems allow investigation of systemic interactions, pathology mechanisms associated with the microbiome, and how genetic and environmental factors can contribute to the appearance/progression of pathological states [1]. For several years now, CIVMs have been proposed for regenerative and personalized medicine [2]. Another important application of CIVMs includes drug development [3]. Several pharma companies are now increasingly investing in reliable technological platforms for boosting drug discovery and decreasing drug development costs.

CIVMs need to be properly designed and optimized for each specific application. As a starting point, the in vitro systems should fit a specific research question to be addressed. Other important "Do" and "Don't" tips and features should be also considered, as follows:

1. Do
   - Select the cell types to be used alone or in co-culture. A good way to generate the model is to start with two types of cells.
   - Select the adequate biomaterials and supportive artificial extracellular matrices that best mimic the target tissue/organ.
   - Select adequate processing and fabrication methods (e.g., conventional or advanced methods such as bioprinting) to decrease fabrication time and enhance the model's reproducibility.
   - Select and optimize your culture media and optimize your technologies for dynamic culturing (e.g., microfluidics and bioreactors).
   - Select the characterization techniques and make the model compatible with different scientific equipment for data acquisition, preferably for real-time monitoring and high-throughput analysis.
2. Don't
   - Complicate your model, i.e., a simple and reproducible model can make the adoption by other researchers easier.
   - Start your experiments without a prior pilot study, i.e., a prior optimization of the model is the key for the success.
   - Run experiments without a solid experimental design and planning, and select the best "gold" standard in order to properly validate your model.
   - Forget the statistical power of your experimental design.
   - Run experiments without considering the culturing time and costs of your model.

With respect to the type of cells to be chosen, induced Pluripotent Stem Cells (iPSCs) have attracted considerable attention in the field of in vitro modeling due to their ability to differentiate into almost every cell type,

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immunocompatibility, and ability to be reprogrammed from the patient's cells [4].

From the materials science and engineering point of view, the most promising technologies for the production of ECM-dependent CIVMs include advanced biomaterials and formulations [5, 6], and bioinks [7] to be used in biofabrication methods [8]. While hydrogels can potentially mimic the different tissue's ECM, bioinks can be used in bioprinted models for controlling the spatial distribution of cells [9]. These biomaterials can undergo different processing to best match the tissue architecture of interest. Thus, bioengineered models comprising multilayered and vascularized models can now be fabricated [10] with superior complexity, thus opening up new possibilities to address challenging research questions (e.g., unveiling biological mechanisms of disease).

Interestingly, the fluid dynamics principles and microfluidics technologies are also being applied to CIVMs aiming to reduce culture media volume requirements and apply different dynamic flow and shear stress. The microfluidics-based technologies are gaining such great importance that currently different organs- and organoids-on-a-chip have already been successfully translated into the clinics [11].

Bioreactors have been also exploited to develop CIVMs that enable to recapitulate of the features of mechanosensitive organs and induce different stimuli, including hydrostatic pressure [12, 13].

Interestingly, future directions in the field promise to develop models that can better mimic the stiffness of different tissues and organs. Thus, the development of soft and biodegradable hydrogel-based microfluidics as alternatives to hard polydimethylsiloxane (PDMS) microfluidics will offer countless possibilities in biomedical research.

In brief, bioengineered complex in vitro models are being successfully developed with superior physiological relevance. The possibility to personalize such types of models and associate extract-omics datasets is most advantageous for full validation and acceptance in both pre-clinical and clinical settings.

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References

1. Trapecar M, Wogram E, Svoboda D, Communal C, Omer A, Lungjangwa T, et al. Human physiomimetic model integrating microphysiological systems of the gut, liver, and brain for studies of neurodegenerative diseases. Sci Adv. 2021;7(5):eabd1707. Available from: http://advances.sciencemag.org/content/7/5/eabd1707.abstract.
2. Grassi L, Alfonsi R, Francescangeli F, Signore M, De Angelis ML, Addario A, et al. Organoids as a new model for improving regenerative medicine and cancer personalized therapy in renal diseases. Cell Death Dis. 2019;10(3):201. Available from: https://doi.org/10.1038/s41419-019-1453-0.
3. Loewa A, Feng JJ, Hedtrich S. Human disease models in drug development. Nat Rev Bioeng. 2023;1(8):545–59. Available from: https://doi.org/10.1038/s44222-023-00063-3.
4. Park S, Gwon Y, Khan SA, Jang K-J, Kim J. Engineering considerations of iPSC-based personalized medicine. Biomater Res. 2023;27(1):67. Available from: https://doi.org/10.1186/s40824-023-00382-x.
5. Lv W, Zhou H, Aazmi A, Yu M, Xu X, Yang H, et al. Constructing biomimetic liver models through biomaterials and vasculature engineering. Regen Biomater. 2022;9(file:///Users/miguelOliveira/Downloads/S2666534422000253.bib):rbac079. Available from: https://doi.org/10.1093/rb/rbac079.
6. Ahmed T. Biomaterial-based in vitro 3D modeling of glioblastoma multiforme. Cancer Pathog Ther. 2023;1(3):177–94. Available from: https://www.sciencedirect.com/science/article/pii/S2949713223000022.
7. Tian Jiao QL, Lian W, Wang Y, Li D, Reis RL, Oliveira JM. Properties of collagen/sodium alginate hydrogels for bioprinting of skin models. J Bionic Eng. 2022. Available from: https://link.springer.com/article/10.1007/s42235-022-00251-8.
8. Lawlor KT, Vanslambrouck JM, Higgins JW, Chambon A, Bishard K, Arndt D, et al. Cellular extrusion bioprinting improves kidney organoid reproducibility and conformation. Nat Mater. 2021;20(2):260–71. Available from: https://doi.org/10.1038/s41563-020-00853-9.
9. Mota C, Camarero-Espinosa S, Baker MB, Wieringa P, Moroni L. Bioprinting: from tissue and organ development to in vitro models. Chem Rev. 2020;120(19):10547–607. Available from: https://doi.org/10.1021/acs.chemrev.9b00789.
10 Hallfors N, Shanti A, Sapudom J, Teo JCM, Petroianu G, Lee S, et al. Multi-compartment lymph-node-on-a-chip enables measurement of immune cell motility in response to drugs. Bioengineering. 2021;8(2):19.
11. Carvalho MR, Yan L-P, Li B, Zhang C-H, He Y-L, Reis RL, et al. Gastrointestinal organs and organoids-on-a-chip: advances and translation into the clinics. Biofabrication. 2023;5(4):042004. Available from: http://iopscience.iop.org/article/10.1088/1758-5090/acf8fb.
12. Hoyle HW, Stenger CML, Przyborski SA. Design considerations of benchtop fluid flow bioreactors for bio-engineered tissue equivalents in vitro. Biomater Biosyst. 2022;8:100063. Available from: https://www.sciencedirect.com/science/article/pii/S2666534422000253.
13. Aw Yong KM, Horst E, Neale D, Royzenblat S, Lahann J, Greineder C, et al. A bioreactor for 3D in vitro modeling of the mechanical stimulation of osteocytes. Front Bioeng Biotechnol. 2022;10. Available from: https://www.frontiersin.org/articles/10.3389/fbioe.2022.797542.