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Review Article

New methodologies for old problems: tridimensional gastrointestinal organoids and guts-on-a-chip

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ABSTRACT

Objectives: The present review intended to present a critical overview of the methodological and experimental advances concerning tridimensional cell culture models within the scope of gastrointestinal research.
Methods: A literature review was performed and some of the main published articles in the area were mentioned.
Main results: Classic studies and high impact results were presented, starting from the pioneer works with gastrointestinal organoids, with a small gut organoid, to the achievement of guts-on-a-chip and multi-organ-chips. It was also discussed which implications the construction of such co-cultures bring, as well as future applications arising from these new methodologies.
Conclusions: Despite the still discrete number of publications, in quantitative terms, there are qualitative promising and consistent results addressing physiopathological aspects and new therapeutic perspectives of tridimensional in vitro cultures in the gastroenterology field. It is expected, thus, that such new methodological approaches, including organoids and guts-on-a-chip, may contribute decisively to the advance in knowledge on basic aspects, as well as on the translation to new therapeutic approaches in gastrointestinal diseases.

Novas metodologias para velhos problemas: organoides gastrointestinais e guts-on-a-chip tridimensionais

Palavras-chave:
Organoides
Organs-on-a-chip
Gut-on-a-chip

RESUMO

Objetivos: A presente revisão visou apresentar uma abordagem crítica dos avanços metodológicos e experimentais referentes a modelos de cultura celular tridimensionais no âmbito do sistema gastrintestinal.

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Modelos in vitro tridimensionais Gastrintestinal

Métodos: Foi realizada revisão da literatura com ênfase nos principais artigos publicados na área.

Resultados principais: São apresentados trabalhos clássicos e resultados de maior impacto, desde os trabalhos pioneiros com organoides do sistema gastrintestinal, com intestino delgado, até a obtenção de *guts-on-a-chip* e *multi-organ-chips*. Discutiu-se, ainda, as implicações decorrentes da elaboração de tais co-culturas, bem como as futuras aplicações decorrentes dessas novas metodologias.

Conclusões: Apesar do número ainda discreto de publicações, em termos quantitativos, há, qualitativamente, resultados promissores e consistentes abordando aspectos fisiopatológicos e de novas perspectivas terapêuticas em gastrenterologia decorrentes das culturas tridimensionais in vitro. É esperado, portanto, que essas novas abordagens metodológicas incluindo organoides e *guts-on-a-chip* possam contribuir decisivamente para o avanço no conhecimento sobre de aspectos básicos, bem como para a translação do conhecimento para novas abordagens terapêuticas em doenças gastrintestinais.

Preclinical studies, which involve both animal and in vitro research, have enabled a vast part of what is known in life sciences today, and have significantly contributed to the comprehension of human anatomophysiology and pathology, as well as for the development of new drugs and therapeutic approaches in different branches of medical specialties. Such investigations are crucial to achieve a better understanding of the development and triggering of diverse diseases, as well as to test efficacy and safety of new therapeutic approaches, which can be further brought to clinical experimentation and, then, as consequence, could be applied in human patients.

Animal models are the most common tools in health-related investigations and have been attributed to more than 70% of biomedical advances.[1] Amongst the advantages of animal experimentation, which rely mostly on rodent models, are the ease to breed animals under laboratorial conditions, obtain large numbers of individuals in a relatively short time, and the possibility of developing transgenic lineages for specific purposes.[2] In the spectrum of gastrointestinal diseases, the use of animal research is valuable due to the similarity between rodent and human gastrointestinal systems, and thus allows the investigation of different stages of intestinal diseases, from the development of the first symptoms to the complete establishment of the pathology.[3] On the other hand, there are important divergences between model and human reality, such as differences in the intestinal microbiota composition,[4] alongside with the ethic concern on the use of animals for research purposes, what evidences the urge for suitable experimental alternatives.

The search for methodologies that represent a better translation between basic research and therapeutic application, at the same time that substitute methods for animal models are highly desired, has led to the development of new experimental in vitro approaches. The classical bidimensional cell cultures have been an important tool for the analysis of molecular and biochemical aspects in life sciences, also as a complement to the researches using animal experimentation. The uniformity of the cell types composing the bidimensional (2D) cell cultures allow an advantageous standardization for morphological and functional studies; nevertheless, this very same standardization represents a limitation of such methods in relation to the representation of physiological and pathological aspects as a whole, meaning they fail to support the different cell types and structure of an organ.[5] Therefore, tridimensional (3D) cell cultures have gained increased attention in the last decade.[6]

The 3D models are mostly composed by different cell types of the desired organ or tissue that self-organize into a tissue architecture, and thus allow a more specific and more realistic representation of a live tissue, what could not be achieved using monotype 2D cultures. The most recent investigations and protocols using three-dimensional cultures already show models which simulate several organs/systems interconnected, representing, at least partially, a human being in an in vitro assay ('human-on-a-chip' or 'lab-on-a-chip', see Marx et al.[7] and Abaci et al.[8] for review).

The most known tridimensional culture method is the organoid, named due to its capacity to mimic, at least partially, structure and function of a live organ, although its size rarely surpasses some millimeters; 'oid' comes from the Latin 'oides', and means resemblance.[9] Either starting from adult or pluripotent stem cells, the formation of the organoid can be achieved using two main principles: the multi-type cell culture is either set up by the experimenter, or the stem cells are orchestrated into the desired tissue by inducing factors and build the three-dimensional structure spontaneously, as they differentiate. For the first approach, the different cell types are differentiated from stem cells or obtained separately, cultivated in different flasks and then set together in the same environment along with an extracellular matrix, and aggregate in form of pellets or spheroids[10–12]; this aggregation can be performed, for example, using magnetic levitation to handle each cell culture separately, and further unite them in a desired order in the same flask.[12,13] For the spontaneous formation, the stem cells are grown on proteins gels or synthetic polymers, or even in regular dishes with culture medium, and spontaneously form the different layers and structure of the desired tissue or organ as they differentiate, responding to the environmental, in vitro, cues.[14] Either way, the cell aggregation leads to a 3D structure that allows the reproduction of

in vivo characteristics in an in vitro model.[12] Because of the more accurate representation of a live tissue, organoids can be used in assays for investigation of basic genetic functions as well as cellular processes and different pathologies,[15] offering, thus, a potent substitute for conventional methods such as 2D cultures and animal models.

Although the term "organoid" has been recently referred to the 3D modeling of organs, and the boom of researches in the field date from the last 10–20 years, the very first three-dimensional cultures in the aim of reproducing a tissue were performed in the 70s. James Rheinwald and Howard Green, who developed human skin 3D cultures starting from small amounts of skin from donor patients who had suffered from third-degree burn, managed to obtain large numbers of cultures which were later successfully transplanted into the patients.[16] Despite the more simple organization of the skin in comparison to other organs, composed by different tissues and more cell types, the findings by these researchers with other organs besides skin are of utmost importance. A beautiful retrospective of the advances in the development of organoids from the early discoveries can be seen in 15.

Considering the resurface of organoids, the intestinal organoids, usually referred to as mini-guts, were among the first models described. These models are usually generated either using adult stem cells (intestinal tissue stem cells) or pluripotent stem cells (embryonic or induced pluripotent stem cells). In 2009, Sato and co-workers could establish a mini-gut from the small intestine using different mouse-derived intestinal cell lines,[17] and more recently, mini-guts based on human intestinal cells have also been achieved.[18,19] The tridimensional structure holds an intestinal crypt-villus and presents gastric, intestinal or colonic characteristics.[17] The organoid technology can also be used to repair damaged intestinal tissue, since transplantation of mini-guts into sick hosts was already performed and showed to allow the formation of a functional and histologically normal intestinal epithelium.[20,21] In addition, due to their capacity of delivering intestinal stem cells at long-term, the transplantation of organoids into individuals affected by gastrointestinal pathologies represent a promising therapeutic intervention in cases where medication is only palliative or insufficient to ameliorate the symptoms.[21–23]

Despite their grand value for research and therapeutic potential, the limitations of the use of mini-guts in the translational medicine include the lack of peristaltic contractions in the in vitro model and the absence of contact to mesenchymal stem cells, to blood or to microorganisms, which are present in a living gut.[24] These aspects delimitate their use in researches that involve investigations of infectious states, for example. Since such limitations can also be transposed to other organs or systems, another set of tridimensional models were developed, with the promise of an even more realistic translation of a live situation: the organs-on-a-chip. For their construction, biometric microsystems are formed inside a small chip, where different cell types are allocated in different chambers and exposed to "external" environment (e.g. blood, airstream or intestinal microbiota simulations) through semipermeable membranes.[25] It is also possible to exert pressure on the cell cultures to mimic, for example, peristaltic or breathing movements. On account of the short time of their availability on the research field, there are to date still a few number of researches using guts-on-a-chip. Even so, the first investigations present promising results, showing that the use of human intestinal cells to build the devices can reproduce intestinal diseases in a very similar way to what is observed in patients, proving that this methodology can be a valuable tool in the constant search for new therapies.[26–28]

In spite of being a relatively new approach in preclinical experimentation, 3D cultures in the gastrointestinal field have significantly evolved since the first publication of a small intestine organoid[17]; a list containing the main findings with tridimensional cultures in the gastrointestinal field can be seen in Table 1. In less than 10 years, mouse gut-organoids were described, further giving rise to the establishment of human gut-organoids and further to guts-on-a-chip, allowing the contact of the cell cultures to external microenvironments, microorganisms, and peristaltic-like pressure. The more recent approaches already describe the generation of multi-organ-chips (MOC), including whole systems or diverse organs interconnected.[29,30] The expectation is that, in a near future, it will be possible to mimic whole organs in vitro, with the generation of humans-on-a-chip, enabling a wide array of new possibilities of specifically directed research.

One additional aspect of tridimensional models is their potential to be used for patient-specific investigations, in cases where the symptoms vary grandly from individual to individual. One example is cystic fibrosis, where not all patients benefit from the same pattern treatments available. The generation of patient-specific 3D models, mostly organoids in this case, allow the investigation of multiple different treatments or drugs at the same time, culminating in the discovery of the best option for the specific case. Other similar cases could definitely benefit from this approach.

Thus, tridimensional culture models, such as mini-guts and guts-on-a-chip, represent promising methods with great applicability potential in studies of physiopathological aspects

Table 1 – Important landmarks in tridimensional (3D) cell culture in gastroenterology.

Tridimensional model	Institution/country	References
Mouse gastrointestinal organoid	Hubrecht Institute and University Medical Center Utrecht, The Netherlands	[17]
Human gastrointestinal organoid	Hubrecht Institute, KNAW and University Medical Centre Utrecht, the Netherlands	[18]
	Barcelona Institute of Science and Technology, Spain	[19]
Human gut-on-a-chip	Harvard University, Boston, USA	[26,27]
Small intestine-liver on-a-chip	University of Tokyo, Japan	[29]
Multi-organ-chip (MOC)	Technische Universität, Berlin, Germany	[30]
Human-on-a-chip	*To be achieved*	

as well as for testing new therapeutic approaches in chronic degenerative intestinal diseases. From good bench results to successful clinical applications.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Nicoll CS, Russell SM, Mozart. Alexander the Great, and the animal rights/liberation philosophy. FASEB J. 1991;5:2888–92.
2. Mizoguchi A. Animal models of inflammatory bowel disease. Prog Mol Biol Transl Sci. 2012;105:263–320.
3. Kolios G. Animal models of inflammatory bowel disease: how useful are they really? Curr Opin Gastroenterol. 2016;32:251–7.
4. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A. 2005;102:11070–5.
5. Gonçalves J, Araújo F, Cutolo M, Fonseca JE. Biosimilar monoclonal antibodies: preclinical and clinical development aspects. Clin Exp Rheumatol. 2016;34:698–705.
6. Simian M, Bissell MJ. Organoids: a historical perspective of thinking in three dimensions. J Cell Biol. 2017;216:31–40.
7. Marx U, Walles H, Hoffmann S, Lindner G, Horland R, Sonntag F, et al. 'Human-in-a-chip' developments: a translational cutting-edge alternative to systemic safety assessment and efficiency evaluation of substances in laboratory animals and man? Altern Lab Anim. 2012;40:235–57.
8. Abaci HE, Shuler ML. Human-on-a-chip design strategies and principles for physiologically based pharmacokinetics/pharmacodynamics modeling. Integr Biol (Camb). 2015;7:383–91.
9. Muthuswamy SK. Bringing together the organoid field: from early beginnings to the road ahead. Development. 2016;144:963–7.
10. Bernstein P, Dong M, Corbeil D, Gelinsky M, Günther KP, Fickert S. Pellet culture elicits superior chondrogenic redifferentiation than alginate-based systems. Biotechnol Prog. 2009;25:1146–52.
11. Hirschhaeuser F, Menne H, Dittfeld C, West J, Mueller-Klieser W, Kunz-Schughart LA. Multicellular tumor spheroids: an underestimated tool is catching up again. J Biotechnol. 2010;148:3–15.
12. Tseng H, Gage JA, Raphael RM, Moore RH, Killian TC, Grande-Allen KJ, et al. Assembly of a three-dimensional multitype bronchiole coculture model using magnetic levitation. Tissue Eng Part C Methods. 2013;19:665–75.
13. Souza GR, Molina JR, Raphael RM, Ozawa MG, Stark DJ, Levin CS, et al. Three-dimensional tissue culture based on magnetic cell levitation. Nat Nanotechnol. 2010;5:291–6.
14. Nirmalanandhan VS, Duren A, Hendricks P, Vielhauer G, Sittampalam GS. Activity of anticancer agents in a three-dimensional cell culture model. Assay Drug Dev Technol. 2010;8:581–90.
15. Clevers H. Modeling development and disease with organoids. Cell. 2016;165:1586–97.
16. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell. 1975;6:331–43.
17. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009;459:262–5.
18. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brinks S, et al. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. Gastroenterology. 2011;141:1762–72.
19. Jung P, Sommer C, Barriga FM, Buczachi SJ, Hernando-Momblona X, Sevillano M, et al. Isolation of human colon stem cells using surface expression of PTK7. Stem Cell Rep. 2015;5:979–87.
20. Fordham RP, Yui S, Hannan NR, Soendergaard C, Madgwick A, Schweiger PJ, et al. Transplantation of expanded fetal intestinal progenitors contributes to colon regeneration after injury. Cell Stem Cell. 2013;13:734–44.
21. Fukuda M, Mizutani T, Mochizuki W, Matsumoto T, Nozaki K, Sakamaki Y, et al. Small intestinal stem cell identity is maintained with functional Paneth cells in heterotopically grafted epithelium onto the colon. Genes Dev. 2014;28:1752–7.
22. Yui S, Nakamura T, Sato T, Nemoto Y, Mizutani T, Zheng X, et al. Functional engraftment of colon epithelium expanded in vitro from a single adult Lgr5+ stem cell. Nat Med. 2012;18:618–23.
23. Spurrier RG, Grikscheit TC. Tissue engineering the small intestine. Clin Gastroenterol Hepatol. 2013;11:354–8.
24. Liu F, Huang J, Ning B, Liu Z, Chen S, Zhao W. Drug discovery via human-derived stem cell organoids. Front Pharmacol. 2016;7:334.
25. Huh DD. A human breathing lung-on-a-chip. Ann Am Thorac Soc. 2015;12:S42–4.
26. Kim HJ, Huh D, Hamilton G, Ingber DE. Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow. Lab Chip. 2012;12:2165–74.
27. Kim HJ, Li H, Collins JJ, Ingber DE. Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. Proc Natl Acad Sci U S A. 2016;113:E7–15.
28. Lee J, Choi JH, Kim HJ. Human gut-on-a-chip technology: will this revolutionize our understanding of IBD and future treatments? Expert Rev Gastroenterol Hepatol. 2016;10:883–5.
29. Materne EM, Maschmeyer I, Lorenz AK, Horland R, Schimek KM, Busek M, et al. The multi-organ chip—a microfluidic platform for long-term multi-tissue coculture. J Vis Exp. 2015;28:e52526.
30. Kimura H, Ikeda T, Nakayama H, Sakai Y, Fujii T. An on-chip small intestine-liver model for pharmacokinetic studies. J Lab Autom. 2015;20:265–73.