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Opinion

Organoids and 3D In Vitro Models as a Platform for Precision Medicine (PM): An Update

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Abstract: Globally, a number of diseases impact us and while treatment options exist, it is often found that similar treatments have variable effects on different patients with the same disease. Particularly in the case of conditions that are closely associated with genetics (like cancer), the intensity and results of a treatment vary between patients. Even for diseases like arthritis it is not uncommon for only a fraction of patients to achieve remission with the same therapeutic approach. With millions suffering from diseases like cancer and arthritis, precision medicine (PM) has been at the forefront of biomedical and pharmaceutical research since 2015. PM focusses on understanding the genetic and environmental factors affecting the patients and has several platforms. One of the platforms is the use of three-dimensional (3D) in vitro models, especially those derived from the patient themselves. These models, like organ-on-chip (OOC), organoid and spheroid models, 3D biomaterial scaffolds and others, have several advantages over traditional two-dimensional (2D) cell culture approaches. In this opinion paper, the author briefly discusses the different platforms used for PM. Then, the advantages that 3D in vitro models have over traditional 2D models and in vivo models are considered and an overview of their applications is provided. Finally, the author outlines the challenges and future directions and shares their opinion about using 3D in vitro models as a tool for PM towards enhanced patient outcomes.

Keywords: organoids; 3D cell culture; precision medicine; in vitro modelling; disease modelling

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1. Introduction

Precision medicine (PM) by definition refers to approaching a disease or a condition by suitably tailoring it to the people suffering from it [1]. While the previously used term 'personalised medicine' was often mistaken for treatment approaches used only for a single individual, PM conveys that the information from the genetics and environmental factors affecting patients could better help us understand the problem and thus provide tailormade therapy approaches [2]. In 2015, US President Barrack Obama announced the PM Initiative, aiming to create a paradigm shift in modern medicine [3]. This initiative was focused on producing targeted approaches towards diseases and their treatments including information about genetic make-up, environmental and lifestyle factors rather than the traditional generalised research protocols and treatment approaches that use visible symptoms [2].

The general approach towards diseases usually involves the concept of a 'one-size-fits-all' method, wherein all the patients suffering from a particular condition are given the same therapy. While this has been extremely helpful until now and we still continue to benefit from it, there has been an increase in the disparity with the number of people who benefit from this therapeutic approach. This means that while some of the patients given a therapy respond well to it, there are non-responders to the same treatment approach—making it increasingly important to include data on their genetics, lifestyle and environment [4]. The reason for these differences in the observed results are varied and may include differences in age, gender, genetic makeup, lifestyles, ethnicities, addictions and comorbidities. This

has commonly been observed in conditions like rheumatoid arthritis (RA) [5,6], Parkinson's disease [7] and cancer [8].

Thus, there is an increased need to transform the therapeutic approaches used for patients with cancer and their care, providing them with the right treatment at the right time. Even though this is still in development, PM may be broken down into the steps of deep patient phenotyping, processing the deep phenotyped data, utilising diagnostic and prediction models and finally predicting treatment responses best suited for patients [9].

However, with all the steps required to achieve PM, this is a challenging path and multiple platforms or tools are needed to harness its potential. The key players involved in this strategy may be generalised under the following categories: (a) genomics, genetics and sequencing [10–15]; (b) artificial intelligence (AI), big data and machine learning (ML) [13,16–20]; (c) several OMICs like proteomics and epigenomics [21–25]; (d) targeted drug delivery strategies [26–30]; and finally, (e) personalised and three-dimensional (3D) in vitro models like organ-on-a-chip (OOC), spheroids, scaffold-based models and organoids [31–35]. These platforms have been outlined below in Figure 1.

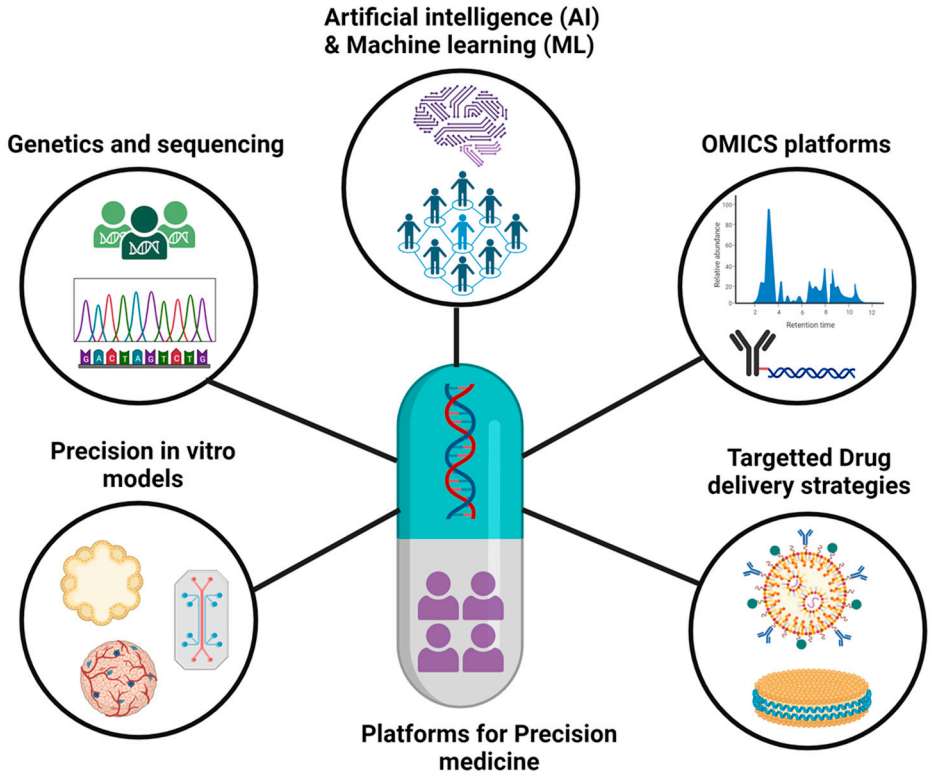

Figure 1. Platforms for precision medicine.

3D in vitro models like organoids, OOCs, cellular scaffolds systems and spheroids are miniature adaptable models and provide enhanced conditions to better mimic the physiological conditions. These can be formulated using different cell types to replicate complex tissue systems for biological and disease modelling [36], which in turn can provide useful information about a target organ in both healthy and diseased conditions [33]. The origin of organoids dates back to 1906–1907 when sponges were identified to have self-renewing abilities [37], around which time Harrison developed the hanging drop cell culture technique [38]. This was followed by success in tissue digestion, the isolation of cells and culturing them in 3D in the 1950s–1970s [39,40]. In 1975, Rheinwald and Green found that co-culturing keratinocytes and fibroblasts could lead to the formation of a self-organised epithelium, resembling stratified skin [41]. This was the first evidence of the long-term culture of untransformed cells from a human source. Later on, in 1987, Mia Bassel cultured cells in 3D using a hydrogel and demonstrated functional differentiation

of alveoli-like structures [42]. It was Hans Clevers' team that reported the formation of 3D organised structures from seeding a single intestinal stem cell in an extracellular matrix (ECM)-like microenvironment [43]. These were known to be the first organoids with published evidence [44]. The last few decades have been instrumental in further contributing to our horizon of knowledge in this field and how patient-derived organoids (PDOs) are essential for PM.

This opinion article will focus on 3D in vitro models, outlining how these are more beneficial compared to traditional 2-dimensional (2D) in vitro models and in vivo models, and discuss their emergence over the last decade as an essential tool for PM. This opinion article then considers patient-derived organoids (PDOs) to underline the advantages and disadvantages of these new models. Finally, it addresses the current challenges and predicts the foreseeable future of these models, providing opinions regarding next-generation techniques. The author aims to encourage the scientific and medical communities at large, including biomedical researchers, pharmaceutical industries and healthcare professionals, to utilise more physiologically relevant methods for a deeper understanding of healthy and diseased conditions for enhanced patient outcomes.

2. 3D In Vitro Models

The essential requirement for targeting any disease is to dissect its underlying mechanism. Biomedical research has gained much from decades of scientific investigations across the globe using traditional 2D cell culture techniques. While these techniques continue to provide us with information, 3D models are able to provide us with a more realistic view of in vivo conditions. Since their discovery, various 3D in vitro disease models have been formulated to mimic the targeted or diseased tissues. Spheroids, OOCs and organoids are among the most investigated and researched models that are increasingly being used for PM in different types of cancer. With the majority of the evidence from organoids research for PM being focused on cancer, this will be our disease of reference for this opinion article [45–47]. Currently, traditional 2D in vitro models and in vivo models are the most widely used platforms for dissecting disease mechanisms, drug testing, phenotyping, identifying drug targets and genotyping. In spite of these models significantly contributing towards our understanding thus far, they still have substantial limitations.

First and foremost, 2D models lack the complexity and intricacy of the human body in vivo and thus fail to replicate the physiological interactions and sophistication. The intercellular interactions, the effects of growth factors and proteins within a complex and dynamic 3D tissue system in vivo, are all reduced by the simple plastic culture dishes single cell type and extremely limited cellular interactions and growth factors in 2D [48]. Second, when in culture, cells are known to undergo a phenomenon known as 'in vitro aging' with every passage, leading the cells towards cellular senescence. This further diminishes the capacity of a 2D cell culture model to provide physiologically relevant information. The biggest advantage that 3D in vitro models like organoids provide is the ability to offer enhanced physiological relevance of in vivo conditions in an in vitro environment. The use of a combination of different cell types along with a continuous supply of media or ECM ensures that the data collected mimic the in vivo conditions. Studies have also found that culturing cells in 3D has a lower cytotoxic impact and a higher viability in comparison to culturing them in 2D [49].

Next, due to the inability to replicate the in vivo conditions, the data and information gathered from these models also do not necessarily provide physiologically relevant details for potential therapeutic targets. Pathways, mechanisms, potential drug targets and therapies revealed from 2D models may or may not materialise in further stages of drug development. Similarly for in vivo models, the system functioning and organisation of mice, rats, sheep, dogs and horses vary significantly form the human body. Thus, despite the popularity of using in vivo systems, a lot of new molecules do not pass the infamous 'valley of death' or phase III of clinical trials [50,51]. Finally, an in vivo research set-up is expensive, time consuming and requires additional ethical involvement as it often implies

euthanasia of the animals at pre-determined time points to enable observation and collection of data. This has led to an increase in the '3R principle' of 'replacement', 'reduction' and 'refinement' to perform animal studies ethically. The answer to this challenge is the use of patient-derived organoids (PDOs) including xenograft models that have increasingly being used for better comprehending diseases like cancer in research and drug development [52]. PDOs provide a system that replicates the in vivo condition of patients, enhancing basic research, prediction abilities and drug screening applications for drug development [53–55]. Table 1 provides examples from studies that investigated PDOs in cancer as a tool for PM in the last 5 years.

Table 1. Examples of patient derived organoids (PDOs) in cancer for precision medicine (PM) in last 5 years.

Target Organ	Main Findings	Reference
Neuroendocrine prostate cancer	PDOs were used to understand the role of the epigenetic modifier EZH2 in disease progression and for high-throughput drug screening.	[56]
Gastrointestinal cancer	Data forecasted that PDOs could provide 100% sensitivity, 93% specificity, 88% positive predictive value in comparison to tissues in vivo.	[57]
Rectal cancer	Chemo-radiation responses in patients were highly matched to PDO responses with 84.43% accuracy, 78.1% sensitivity and 91.97% specificity.	[58]
Follicular lymphoma	PDOs provided a robust platform for advancing PM for treatment evaluation by mimicking the tumour microenvironment signature.	[59]
Breast cancer	Investigation with PDOs resulted in treatment with complete response and progression-free survival, which was more than three times that of previous therapies.	[60]
Lung cancer	Using PDOs reduced the time from organoid establishment to drug testing and may be useful for predicting patient-specific drug responses.	[61]
Endometrial cancer	PDOs maintained specific phenotypes in long-term organoid cultures with potential applications in drug screening applications.	[62]
Brain tumour	PDOs replicated therapeutic effects consistent with patient response to medications with high potential implications in precision medicine.	[63]

Last but not the least, drug discovery and development (DDD) as processes suffer due to lack of specificity in data from 2D cultures. DDD is one of the major pillars of pharmaceutical, biomedical and thus the overall healthcare sectors, providing a platform for new drug molecules to becomes available to patients [64]. However, the process is lengthy, expensive and has been found to have low success rates post animal studies in clinical trials [65]. This has indicated the need for newer techniques for efficacious drug screening processes and toxicological screening [66]. The problem often faced in 2D cultures with respect to DDD is that cells in 2D are more sensitive to drugs due to changes in the cell morphology in 2D in comparison to the cells in vivo. This often leads to heightened responses and statistically significant differences in 2D cultures, that are often not a true representation of the same cells within in vivo conditions. Additionally, these cells in 2D cultures have altered cell receptor exposure compared to the cells in vivo [49,65], thus manipulating the evidence when compared to a 3D system and/or in vivo conditions. This is one of the major reasons why drugs fail in later phases, usually phase III of clinical trials [53,64]. Thus, organoid and 3D in vitro models have several applications and these have been outlined below in Figure 2.

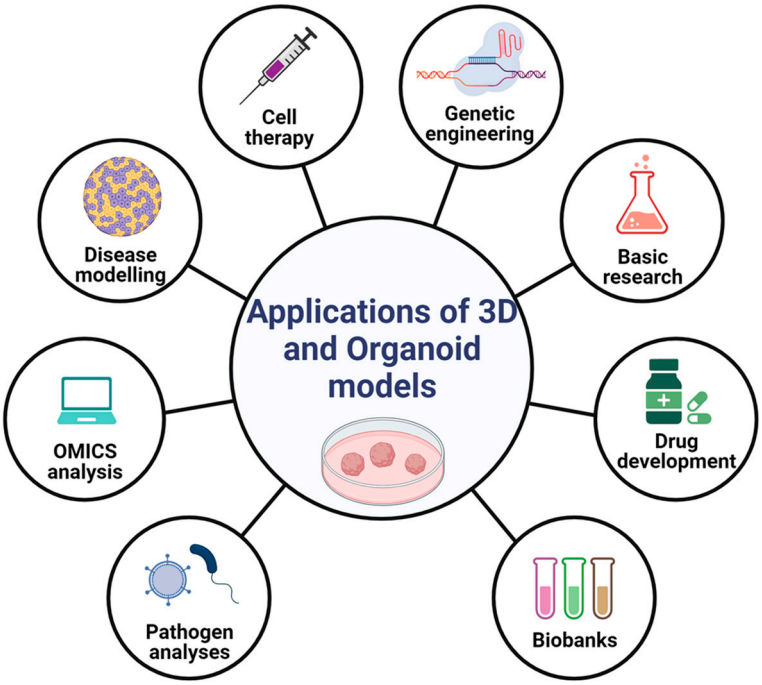

Figure 2. Applications of organoids and 3D models in PM.

Along with these advantages, organoids come with considerations for finances and expenses as their formation, maintenance and clinical applications demand well-equipped and managed laboratories [67]. It is worth noting that the advantages that organoids bring to a research project provide deep insights to the research question [68]. Specifically, for investigations focusing on animal models, the overall cost of maintaining animals for the length of the investigation is often found to be comparable to that of organoids-based research investigations [69,70]. Having said that, it will always be beneficial for individual investigations to explore the cost–benefit analysis of their projects. For example, projects aimed at short-term investigations may not always benefit from investing in 3D models. However, larger projects with three–five years of investment will most likely have much more information and knowledge to gain from 3D models than from traditional or long-established in vivo animal models [71,72].

3. Challenges, Conclusions and Future Directions

With respect to the technical advancements that 3D in vitro models provide, Jensen and Teng rightly pointed out the differences between 2D and 3D cell cultures by comparing several parameters. These included cell shape, cell viability, cell proliferation, drug sensitivity, cell differentiation and cost effectiveness, among others. On comparing these parameters, they found 3D cell culture systems to be more efficient and followed a more comparable rate of cell growth, proliferation, differentiation and expression to the actual physiological conditions [49]. While the possibilities of 3D in vitro models, PDO and PM seem endless, there are certain challenges that still need solutions. To begin with, there is no standardisation of 3D in vitro models like organoid manufacturing. Protocols have been suggested by different groups [73,74]; however, formally harmonising these protocols for their production will provide robust methods for consistency in PDOs. With respect to PM; data acquisition, analysis, its storage, use and research activities also need to be aligned uniformly or in such a way that the techniques are adaptable for different target organs and applications [75]. Additionally, PM will require highly integrated patient data maintenance so that they may be utilised even at a point of care use. This will naturally lead to demands for a robust infrastructure of information technology within institutes, hospitals and other bodies adopting PM.

More importantly, the ethical aspects of using PDOs including patient consent, patient participation, genetic privacy and the potential misuse of data still pose challenges in the field. Regulations protecting both the application of PDO for scientists as well as the interests of patients will be needed to ensure the appropriate use of PDOs [76]. Organoids used for research as well as those for clinical care present challenges that will need to be considered in the future. Organoids for research face the dilemma of choosing the extent to which patients must be informed about the potential future research, especially considering the fact that patients wanted to know the results and be involved in the research use of organoids from their tissues for cystic fibrosis research in the Netherlands [77,78]. Additionally, challenges exist around the guidelines available for the commercialization of organoids for research and these were discussed in detail by Jongh et al. in 2022 [79].

From a public health perspective, the initial requirements for PM may not always be aligned to the overall needs of public health as groups benefitting most from PM would include 'individuals with specific diseases' and the 'high risk general population' [80]. However, considering the vulnerability, burden of diseases and poor quality of life of these two groups (for example, cancer patients), the use of PM will bring them much needed relief. Even for more common diseases like sickle cell anaemia and rheumatoid arthritis where the disease may affect certain phenotypes, or where the same treatment produces variable results in patients, PM will provide a novel approach towards enhancing patient care [81,82]. To realise the full potential of PM, it is essential that appropriate policy guidelines are developed to support all these different aspects of PM [83]. These would include data acquisition and analysis, data sharing, data integration with patient health, its regulation, public information and economic value. A large amount of evidence will be needed to support claims that 3D in vitro models enhance patient outcomes; keeping in mind that the extent of evidence will largely vary depending upon the population size impacted by a particular disease/condition.

The future will include more platforms that are adaptable for different types of cancers and the platforms will make utilising PDOs more feasible as previously demonstrated by Larsen et al. in 2021 with their pan-cancer organoid platform [73]. Additionally, combining PDOs with other next-generation techniques like nanotechnology and genetic sequencing will further provide insights into pathways and mechanisms that may be targeted for future DDD [84,85]. While discussing these are beyond the scope of this opinion article, they have been discussed in more details elsewhere [86,87]. Specifically for PM, a combination of machine learning and artificial intelligence with organoid technology will further strengthen the approach for enhanced patient care [88,89].

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