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Organoid intelligence: Integration of organoid technology and artificial intelligence in the new era of in vitro models

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ABSTRACT

Organoid Intelligence ushers in a new era by seamlessly integrating cutting-edge organoid technology with the power of artificial intelligence. Organoids, three-dimensional miniature organ-like structures cultivated from stem cells, offer an unparalleled opportunity to simulate complex human organ systems in vitro. Through the convergence of organoid technology and AI, researchers gain the means to accelerate discoveries and insights across various disciplines. Artificial intelligence algorithms enable the comprehensive analysis of intricate organoid behaviors, intricate cellular interactions, and dynamic responses to stimuli. This synergy empowers the development of predictive models, precise disease simulations, and personalized medicine approaches, revolutionizing our understanding of human development, disease mechanisms, and therapeutic interventions. Organoid Intelligence holds the promise of reshaping how we perceive in vitro modeling, propelling us toward a future where these advanced systems play a pivotal role in biomedical research and drug development.

1. Introduction

In the past few decades, a dynamic collaboration has unfolded between the fields of stem cell biology and bioengineering. This synergy has led to the creation of diverse culture systems capable of orchestrating the assembly of stem cells into distinct organoid types. These include remarkable achievements such as kidney organoids [1,2], intestine organoids [3,4], stomach organoids [5,6], optic cup organoids [7,8], and more recently cardiac organoids [9,10]. These innovative strides have not only deepened our insights into the complex realm of embryonic development but have also propelled the frontiers of regenerative medicine [11] [–] [14]. Organoids are three-dimensional (3D) structures developed from stem cells or specific types of progenitor cells. In contrast to the traditional 2D tissue cultures, organoids possess an inherent complexity, replicating the intricate cellular compositions and 3D architectures that characterize natural organs [15]. For instance, human-induced pluripotent stem cells (hiPSCs)-derived cardiac organoids with designated geometric features were generated using spatial-patterned Poly (ethylene glycol) (PEG) hydrogel substrates to investigate the early development of cardiovascular tissues [16]. In another study, organoids derived from the intestinal stem cells of a cystic fibrosis patient harboring a rare genetic mutation were used to test existing drugs, demonstrating the potential benefits of organoid technology in drug screening and the treatment of inherited diseases [17]. By mimicking the genetic makeup and physiological characteristics of individual patients, organoids offer a platform to tailor medical interventions to unique cases, thus heralding a paradigm shift in how we approach disease treatment and drug development.

In recent years, the emerging high-throughput organoid culture systems have enabled the mass production of different types of organoids [18] [–] [21]. Microfluidic technology, in particular, has emerged as a key player in this landscape. By encapsulating suspension-cultured self-assembling stem cell organoids within hydrogels, this technology has managed to curtail heterogeneity and enhance quality control on a

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large scale [22]. However, this exciting leap in scalability also introduces its own set of challenges, particularly in terms of sample characterization and comprehensive data analysis, which have become pivotal considerations in managing the deluge of information generated by high-throughput platforms. Traditional analytical tools have demonstrated promise in generating robust results [23] [–] [25], yet they currently face the formidable task of handling the vast amount of data stemming from these samples. Balancing the benefits of high-throughput screening with the need for accurate, in-depth data analysis presents a unique challenge that the field must address as it embraces the era of organoid intelligence.

Artificial Intelligence (AI) has emerged as a transformative force in biomedical research. AI algorithms have demonstrated exceptional proficiency in processing vast volumes of genomic, proteomic, and clinical data, identifying patterns and correlations that might elude human observation. Machine learning algorithms are mathematical-based models using existing input to reveal the underlying patterns in the new data by optimizing the global minima of different cost functions [26]. These algorithms have found expansive application in biomedical research, including in the dimension reduction of high-dimensional data to visualizable lower embeddings [27]. For example, Dr. McDonnell harnessed mass spectrometry imaging with t-distributed stochastic neighbor embedding (t-SNE) algorithm to distinguish breast cancer patients and gastric tumor patients into subpopulations, revealing the intratumor heterogeneity [28]. In another study, Dr. Frieboes trained various supervised machine models on a dataset containing the surveillance, epidemiology, and end result of cancer patients, achieving an accuracy rate of over 90 % in predicting lung cancer patient survival rates [29]. Benefited from the advances in computing power, deep learning is increasingly revealing its latency for enabling a more nuanced understanding of the structural and developmental intricacies of organoids, particularly in the realm of image processing, where a growing number of classifiers are being trained on CNN algorithms to analyze organoid histology section image [30,31]. The integration of algorithms that combine image segmentation [32], feature extraction [33], and classification [34] functions has resulted in a remarkable elevation in the analysis of high-content microscopy-based datasets [35].

The convergence of stem cell biology, bioengineering, and AI has ushered in a new era of organoid research (Fig. 1). In this article, we provide an overview of the recent advances in organoid research, only focusing on the emerging applications of AI algorithms in this research area. Through stem cell technology, precision engineering, and data-driven insights, organoid intelligence is redefining our understanding of developmental biology, disease mechanisms, and therapeutic strategies.

2. Application of machine learning models

Machine learning algorithms started to emerge in the 1950s, coinciding with the term "artificial intelligence or AI." This pivotal moment marked a significant milestone in the progression of machine learning concepts [36]. These algorithms can be broadly classified into two fundamental domains: supervised machine learning and unsupervised machine learning [37]. For supervised machine learning, algorithms train models using labeled data, where inputs are paired with corresponding target labels or outcomes [38]. During the late 1950s, one of the early supervised learning models emerged with a single-layer neural network tailored for classification tasks [39]. As computational capabilities advanced, a diverse array of supervised machine learning models, including Decision Trees [40], the k-Nearest Neighbor algorithm (k-NN) [41], Support Vector Machines (SVMs) [42], and Naive Bayes [43], were developed and refined. This refinement led to enhanced predictive accuracy for classification tasks.

In contrast, unsupervised machine learning algorithms are trained on unlabeled data and focus on revealing patterns, structures, or relationships within inputs without predefined categories [44]. The evolution of unsupervised machine learning algorithms is closely intertwined with the broader landscape of AI and machine learning. An early example, the k-Means algorithm, proposed by Stuart Lloyd in 1957 [45], stands as one of the earliest clustering algorithms. Another notable contributor,

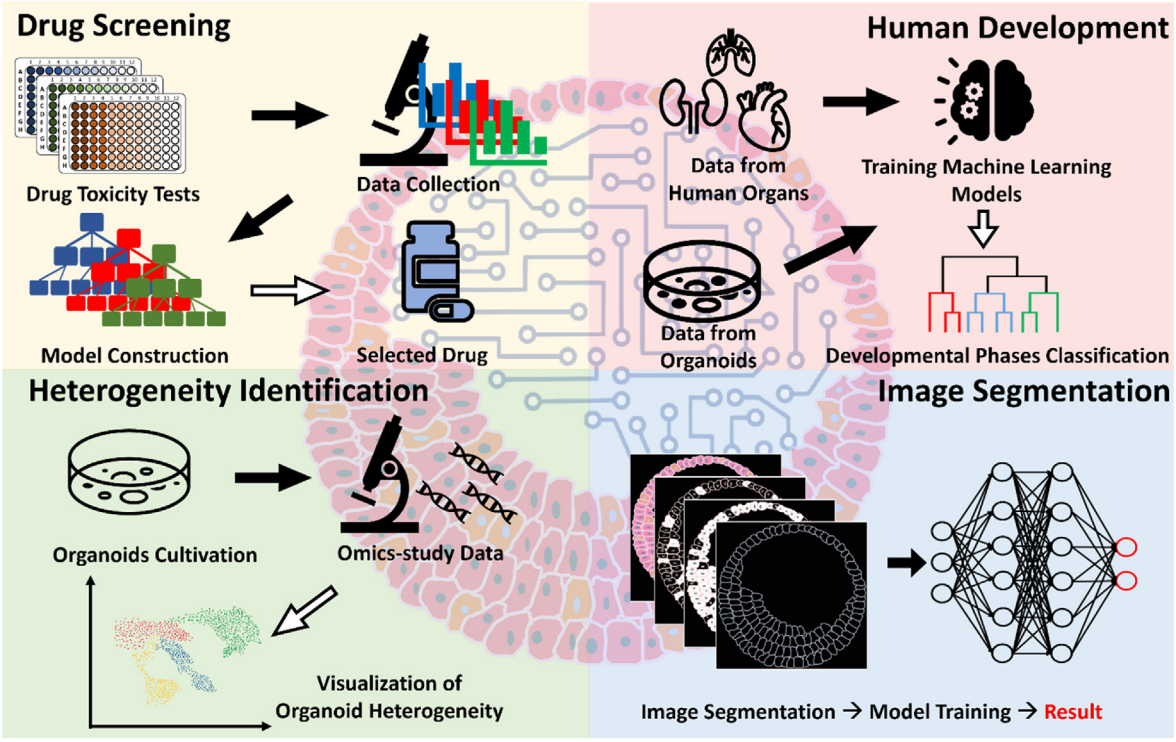

Fig. 1. Organoid Intelligence. The integration of organoid technology and artificial intelligence for studying organoid properties, modeling human development, and assisting drug screening workflow.

Principal Component Analysis (PCA), introduced in 1901 and widely embraced in the 2000s, has proven invaluable in biological studies for tasks such as data visualization, noise reduction, and feature extraction [46]. Furthermore, the non-linear dimensionality reduction techniques, such as t-Distributed Stochastic Neighbor Embedding (t-SNE) and Uniform Manifold Approximation and Projection (UMAP), showcased remarkable potential in visualizing high-dimensional data by transforming it into low-dimensional embeddings [47].

2.1. Machine learning in organoid model of human development

Organoids are new forms of 3D cell culture models that faithfully mimic the complexity and organization of human tissues or organs. These advanced models recapitulate key features of various organs, such as the brain and gastrointestinal tract, making them invaluable tools for studying human development, disease mechanisms, and personalized medicine [48,49]. Through deciphering the intricate patterns within the complex organoid datasets, machine learning algorithms have played a crucial role in validating the similarities between in-vivo organs and in-vitro organoid models. For example, through comparing high-throughput RNA-seq data from organoids to the organs by machine learning algorithms, precise identification of cell types and inference of gene regulatory networks in organoids are achieved [50].

In a study focusing on brain development, researchers used UMAP to cluster RNA-seq data from organoids at various time points, thereby revealing the cell subtype composition during development. Subsequently, these datasets were integrated into a model trained using EEG features from preterm infants, yielding prediction results with heightened consistency in Week 25 organoids. These findings suggest a parallel developmental trajectory of cortical organoids with fetal human brains, indicating similarities in their changing network electrophysiological properties over time [51]. Notably, the development of BOMA, a brain and organoid manifold alignment algorithm, has enabled comparative analysis of gene expression between brains and organoids. Utilizing this unsupervised machine learning algorithm, researchers have revealed spatiotemporal and species-wise gene expression patterns hidden in bulk tissue sequencing data and single-cell RNA sequencing (scRNA-seq) data, thus confirming the legitimacy of brain organoids as in-vitro counterparts for studying human neural system development [52]. Harnessing the spatiotemporal transcriptome atlas of the human brain, researchers trained a supervised classifier named "CoNTExT" to identify developmental maturity with 96.9 % accuracy in brain regional identification [53–55] The CoNTExT classifier confirmed the neuroanatomical identity of Week 5 and Week 14 organoids as cortex, thus demonstrating the preservation of molecular changes between organoids and the human fetal cortex during development [56].

The application of machine learning has transcended the confines of transcriptome datasets and ventured into diverse domains. For instance, SVM algorithms were applied to classify salivary gland organoids treated with EGF and FGF2 based on Raman spectral data, signifying the potential to facilitate comprehension of cellular changes during organoid formation [57]. In a cardiac organoid study, the random forest-based computational approach has been proved to be a robust method for annotating scRNA-seq data obtained from hiPSC-derived heart organoids, which helped effectively remove non-heart cells between fibroblasts and cardiomyocytes. The model has also demonstrated its potential in distinguishing cardiac tissues generated from 3D organoids, conventional 2D protocol, as well as organoids generated from gene mutation hiPSC cell lines. These distinctions are evident in the differential cross-classification pattern in cell type annotations between ventricular and atrial cardiomyocytes [58].

2.2. Machine learning in precision drug screening

The capability to replicate intricate organ structures and phenotypes has positioned organoids as invaluable tools for expediting drug development. This is evident through their exceptional efficiency in assessing the toxicities of drug candidates and emulating disease heterogeneity [59,60]. Furthermore, the integration of machine learning algorithms has ushered in a new phase of advancement, enhancing this process by predicting drug responses and identifying therapeutic targets. Employing t-SNE as a dimensionality reduction technique, researchers have visualized the heterogeneity of colorectal tumor organoids in response to oxaliplatin treatment within a two-dimensional representation. Subsequently, DBSCAN (Density-based spatial clustering of applications with noise) was employed on the low-dimensional outcome to categorize the embedding into distinct clusters. The comparison of clustering outcomes prior to and post-treatment has engendered the delineation of distinct subtypes within cellular populations, encompassing the drug-insensitive, drug-sensitive, and drug-ultrasensitive groups, showcasing considerable potential in cancer chemotherapeutic applications [61]. In a study aimed at building a neurotoxin-induced Parkinson's disease (PD) organoid model, hiPSC-induced human midbrain organoids (hMOs) were treated with 6-hydroxydopamine (6-OHDA) to damage the dopaminergic system. Using high-content confocal image-based data of this PD model, researchers trained a classification random forest model, which achieved an impressive 86 % accuracy in predicting control and treatment organoids, highlighting the potential of supervised machine learning algorithms for neurotoxicity prediction [62].

In line with the goal of advancing precision medicine, a network-incorporated machine learning model was developed to pinpoint drug biomarkers. This model stratified colorectal cancer patients into drug responders and non-responders. The classification outcome was then evaluated in drug-response prediction, revealing that drug responder patients exhibited significantly elongated overall survival following 5-Fluorouracil treatment. Subsequently, this model underwent validation using datasets from patients afflicted by bladder cancer and undergoing cisplatin treatment, thereby exemplifying the model's capacity for generalization across diverse clinical scenarios [63]. Another study utilizes a random forest prediction model fed with survival fraction data from rectal cancer patient-derived tumor organoids subjected to pre-neoadjuvant chemoradiotherapy. Remarkably, the model achieves over 89 % accuracy in predicting tumor regression grade. Furthermore, the study establishes a significant positive correlation between patient and organoid irradiation responses, underscoring the value of organoid intelligence in understanding treatment outcomes and suggesting improved strategies for cancer therapy [64]. These studies emanate a promising signal in personalized therapy, enabling researchers to assess the efficacy of chemotherapy drugs more precisely, thereby minimizing unnecessary side effects.

3. Application of deep learning models

Advances in computing power and the accessibility of extensive training datasets have paved the way for researchers to push the boundaries of neural networks, giving rise to more sophisticated architectures composed of multiple processing layers. These advancements have enabled neural networks to decipher intricate and intricate patterns and structures inherent in high-dimensional data. Noteworthy among these architectures are convolutional neural networks (CNNs), which have demonstrated remarkable potential in the realm of biomedical engineering [65,66]. CNNs generally work by processing data through multiple layers to extract information from local connections, leveraging shared weights, and pooling these connections across the many layers of a deep neural network. CNNs exhibit a particularly strong aptitude for extracting salient features from the multidimensional data intrinsic to imagery, as is often seen in microscopy images. Convolutional networks have had great success in detection, segmentation, and detection of features within images which have translated successfully to biomedical imaging analysis.

3.1. Deep learning for organoid tracking

One of the most difficult and time intensive aspects of organoid research is the analysis of the images and videos generated by microscopy. A wealth of information is available within the video and image captures of organoids relating to changes within organoids morphology, size, number and functioning that relay critical information about organoid development and response to changes within their environment. However, the sheer volume of data presents a bottleneck in the interpretation of experimental results, thus leading to a pivotal need to implement deep learning technology in this field [67]. Considerable effort has been dedicated to developing investigative tools that harness the power of deep learning for organoid image analysis and tracking. The ability to automatically quantify changes in organoids is immensely advantageous, as manual screening of organoids is not only laborious but also prone to human errors. High-throughput image acquisition is a common approach for capturing images of a wide organoid area. However, this method comes with its own set of challenges, stemming from factors such as organoid density, depth, movement, and focus-related issues.

To address these obstacles, a specialized high-throughput organoid image dataset was designed specifically for organoid detection and tracking [68]. By employing a CNN architecture known as ResNet [69], the dynamic evolution of organoid cultures was successfully tracked to profile the growth and morphological changes throughout the organoid developmental process. The impressive speed and accuracy in organoid tracking throughout the entire culture period marks a significant progress that not only advances organoid research but also showcases the potential of deep learning in this domain. Several open-source packages have emerged to facilitate the training of deep learning architectures for organoid analysis. Among these, the python-based tool MOrgAna utilizes a combined approach of deep learning and classical machine learning to analyze brain organoids from brightfield microscopic images. To discern organoid pixels from debris or background noise in the images, a multilayer perceptron neural network is extended into a deeper architecture. This expansion of logistic regression-based machine learning approach with deep learning network enhances the accuracy beyond traditional quantification tools [70].

OrganoID utilizes a CNN network derived from U-NET for pixel-by-pixel organoid detection from brightfield microscopy images. This enables automatic counting and tracking of the size of cancerous organoids. While initially trained on pancreatic cancer organoids, this algorithm has been validated on diverse images, encompassing pancreatic, lung, colon, and adenoid cystic carcinoma organoids. The achieved accuracy rates are noteworthy, with individual count accuracy at 95 % and individual organoid size accuracy at 97 % [71]. Similarly, autoencoder CNNs, like Deep Image Prior (DIP) [72], has been applied to enhance microscopy resolution. This innovative super-resolution framework employs an encoder-decoder architecture to improve the resolution of time-lapse videos involving tumor and immune cells as the focal objects. Extensive validation, covering both synthetic and real videos, has been conducted. In comparison to other techniques, this model demonstrates its value through an unsupervised architecture that effectively complements edge location and edge detection methods, thereby contributing to the advancement of high-resolution microscopy.

3.2. Deep learning for organoid characterization

Identification of abstract and nuanced alterations in organoid morphology has posed significant challenges. However, deep learning approaches have proven successful in quantifying and capturing these subtle morphological changes, which often elude empirical and consistent observation through traditional analysis methods. D-CryptO, a tool for analysis colorectal organoids, was developed by leveraging a comparison of six popular deep learning architectures on brightfield microscopy to detect the opacity and budding of these organoids. The study revealed that D-CryptO achieved remarkable outcomes by not only accurately predicting the overall opacity of individual organoids but also identifying instances of organoid budding with an impressively high level of accuracy [73]. 3D retinal organoids, derived from either mouse or human pluripotent cells, have exhibited a distinct resemblance to the native tissue. Despite their translational potential, the current approach to select functional retinal organoids relies heavily on subjective assessment of organoid morphology and features extracted from brightfield imaging. Addressing this challenge, a CNN-based system was designed to predict retinal differentiation based on early-stage retinal organoid brightfield images [74]. After cross-validation and hyperparameter tuning, the CNN was established using the ResNet50v2 architecture. Notably, the CNN algorithm outperformed experts in predicting organoid fate, even prior to the initiation of reporters. This advancement effectively obviates the need for intricate imaging techniques or the use of fluorescent probes. Moreover, the proposed approach is highly versatile and can be seamlessly incorporated into laboratories for streamlined implementation.

Deep learning autoencoder-decoders have emerged as promising tools for high-throughput drug screening, leveraging the latent vectors of autoencoders to capture the underlying phenotypic structures of organoids. These latent vectors effectively represent the organoids' phenotypic characteristics in a reduced dimension compared to the comprehensive microscopic capture. Metzger et al. pioneered a drug screening platform that successfully distinguished neural tube organoids between wild-type and diseased ones carrying the genetic mutations responsible for Huntington's disease (HD). This discrimination was achieved with remarkable accuracy by analyzing changes within the condensed phenotypic space of the latent vectors. The model was further extended to quantitatively assess the therapeutic efficacy of drugs designed to treat HD. This extension involved measuring the extent to which therapeutic agents brought the HD organoids closer to a phenotypic space resembling that of the wild-type organoids. Additionally, the platform enabled the exploration of potential adverse drug effects. By examining how much the addition of therapeutic agents altered the phenotypic space of the organoids from their original latent state, both for the diseased and wild-type organoids, insights into potential adverse effects could be obtained [66].

4. Conclusions and future perspectives

Organoid technology holds immense promise in a wide range from animal-free drug testing that can investigate traditionally difficult areas from rare genetic diseases and developmental toxicology to personalized precision medicine for testing therapeutic strategies for patients [15]. As researchers create more complex and intricate organoid models to further replicate the structure and functionality of their *in-vivo* counterparts, our analytical tools must advance in sophistication to match. AI has proven to be fast, efficient, and quantitatively capable of extracting and analyzing the high-dimensional data generated from high-throughput organoid models with a high degree of accuracy. Future organoid models will continue to utilize powerful AI algorithms to fully investigate and model the complex and dynamic nature of organoids, facilitating the development of life-saving and groundbreaking discoveries. The future for organoids and AI may even become further entwined with the development of organoid intelligence [75] which seeks to utilize brain organoids for biological computing to implement a hybrid system for the future of artificial intelligence and organoid technology.

Explainable AI (XAI) refers to the capability of AI systems to provide understandable and interpretable explanations for their decisions or predictions [76]. In the context of advancing research on organoid analysis, XAI can play a crucial role in enhancing our understanding of complex biological processes and improving the accuracy and reliability of analysis techniques. Integrating XAI and organoid technology will provide insights into how AI models arrive at a specific conclusion about organoid behaviors or properties. Researchers can gain a clearer view of

which features or patterns that AI is focusing on, leading to increased trust and confidence in the results. By revealing the relationships between various organoid features, XAI can help researchers identify novel correlations (i.e., markers), leading to the discovery of new organoid characteristics that might be associated with certain biological conditions or diseases [77]. Researchers can understand which features or combinations of features are most influential in making predictions, potentially uncovering subtle biomarkers that would have been overlooked otherwise. In addition, AI models can learn irrelevant information from the data and then give high accuracy on biased predictions. This often occurs in biological data where many correlated factors presented to differentiate data might be irrelevant to the target application. Explaining the model outcomes based on XAI will reveal such bias and help develop unbiased AI models.

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Data availability

Data sharing is not applicable to this article as no new data was created or analyzed in this study.

Credit Author Statement

Conceptualization – Zhen Ma & Huaxiao Yang; Original draft – Huaiyu Shi, Andrew Kowalczewski, Danny Vu; Review & editing –; Huaiyu Shi, Zhen Ma, Huaxiao Yang, Asif Salekin; Project administration – Zhen Ma & Huaxiao Yang; Funding acquisition – Zhen Ma & Huaxiao Yang. Huaiyu Shi and Andrew Kowalczewski equally contribute to this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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