Revolutionising oral organoids with artificial intelligence

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Key Words:
artificial intelligence; bioprinting; dental stem cells; machine learning; oral disease; oral organoids

From the Contents

ABSTRACT

The convergence of organoid technology and artificial intelligence (AI) is poised to revolutionise oral healthcare. Organoids - three-dimensional structures derived from human tissues - offer invaluable insights into the complex biology of diseases, allowing researchers to effectively study disease mechanisms and test therapeutic interventions in environments that closely mimic in vivo conditions. In this review, we first present the historical development of organoids and delve into the current types of oral organoids, focusing on their use in disease models, regeneration and microbiome intervention. We then compare single-source and multi-lineage oral organoids and assess the latest progress in bioprinted, vascularised and neural-integrated organoids. In the next part of the review, we highlight significant advancements in AI, emphasising how AI algorithms may potentially promote organoid development for early disease detection and diagnosis, personalised treatment, disease prediction and drug screening. However, our main finding is the identification of remaining challenges, such as data integration and the critical need for rigorous validation of AI algorithms to ensure their clinical reliability. Our main viewpoint is that current AI-enabled oral organoids are still limited in applications but, as we look to the future, we offer insights into the potential transformation of AI-integrated oral organoids in oral disease diagnosis, oral microbial interactions and drug discoveries. By synthesising these components, this review aims to provide a comprehensive perspective on the current state and future implications of AI-enabled oral organoids, emphasising their role in advancing oral healthcare and improving patient outcomes.

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Introduction

Organoids represent a groundbreaking advancement in biological research, offering simplified three-dimensional (3D) models of human organs grown from stem cells. These miniature structures replicate key aspects of tissue architecture and function, yielding invaluable insights into organ development, disease mechanisms and potential therapeutic interventions.[1, 2] The evolution of organoids has transitioned from basic cell culture to sophisticated models that mimic the complexity of human tissues, driven by advancements in stem cell biology, tissue engineering and regenerative medicine. Recent studies have demonstrated the potential of organoids to maintain critical physiological and pathological characteristics of their parental tissues to model various diseases and facilitate drug discovery.[3, 4]

Oral diseases present a unique set of complexities due to the diverse cell types involved. Conditions like periodontitis, oral cancer and dental caries involve multifaceted pathological processes that are challenging to study with traditional two-dimensional cell cultures or animal models. Given the high prevalence and significant impact of these diseases on overall health, there is an urgent need for advanced models to elucidate underlying mechanisms and develop effective treatments.[5] Oral organoids have emerged as

particularly significant models, simulating the architecture and functionality of oral tissues such as the gingiva, periodontal ligament and dental pulp.[6] These models pave the way for personalised medicine and novel therapeutic approaches.[7,8]

The integration of artificial intelligence (AI) into oral organoid research marks a significant advancement, enhancing our ability to analyse complex biological systems and improve treatment strategies for oral diseases. AI technologies, particularly machine learning (ML) algorithms, have been employed to analyse high-dimensional data generated from organoid cultures, facilitating the identification of cellular heterogeneity and drug responses.[9] For instance, the application of deep learning (DL) techniques have enabled researchers to classify organoid differentiation states and predict treatment outcomes based on transcriptomic data, streamlining drug discovery processes.[10] AI also plays a vital role in optimising organoid bioprinting, ensuring the precision and reproducibility. Researchers can create more reliable *in vitro* models that closely mimic the physiological conditions of the oral cavity by employing AI to monitor organoid development quality.[11]

Moreover, AI's potential extends to personalised medicine, where ML algorithms can analyse patient-specific data to tailor treatments based on individual responses.[12] This personalised approach enhances therapeutic efficacy and minimises adverse effects, improving patient outcomes in managing oral diseases. As the field evolves, the synergy between AI and organoid technology is expected to yield novel insights into the pathophysiology of oral conditions and drive innovative therapeutic strategies.

Hence, in this narrative review, we examine how oral organoids are revolutionising our understanding of oral health and disease while examining how AI can amplify these advancements. Integrating AI with organoid technology will enable researchers to overcome traditional limitations, enhance experimental precision and accelerate clinical translation, ultimately pushing the frontiers of oral health research (**Figure 1**).

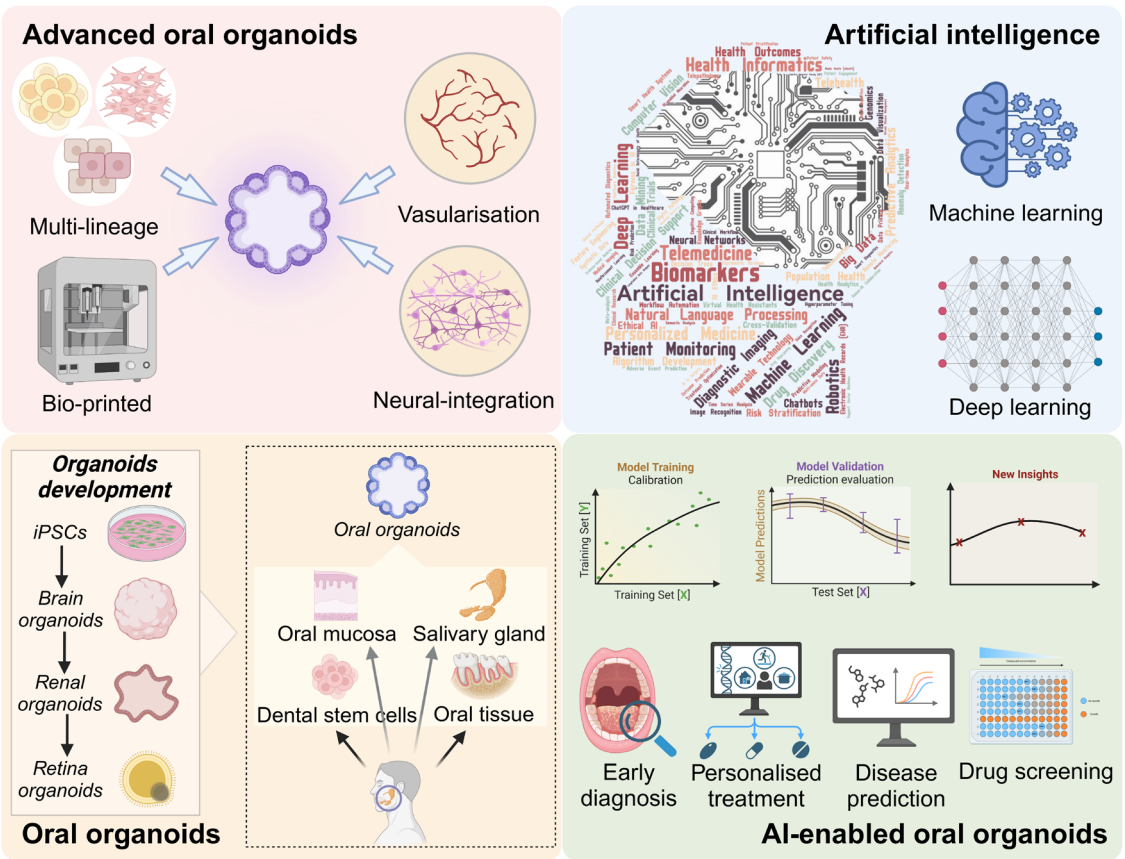

Figure 1. Schematic illustration of the development of oral organoids and perspectives of AI-enabled oral organoids. Created with BioRender.com. AI: artificial intelligence.

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Overview of Oral Organoids

History of organoids

Research over the last half century has shown cells cultured in two-dimensional environments, such as tissue culture polystyrene, do not represent *in vivo* biology.[13, 14] This discrepancy contributes to the high failure rate of new drugs as they translate from benchtop to clinical trials, coining the term "valley of death" to describe this critical bottleneck in drug development.[15] With rising ethical concerns around animal testing, there's an urgent need for alternative models such as 3D culture platforms.[16] Organoids, derived from induced pluripotent stem cells (iPSCs), embryonic stem cells (ESCs), or adult stem cells (ASCs), recreate the structure and function of organs. A critical aspect of organoids is the ability of the construct to self-organise or spatially organise and form clusters. Therefore, here we use a general definition for organoids taken from Sakalem et al.[17] as "refer[ring] to 3D cultures that present multiple cell lineages in co-culture [or more] that are able to spatially organise and form clusters".

Cells are typically supported by a complex mixture of factors in the culture media to create organoids.[18] IPSC and ESC organoids normally involve differentiation protocols with multiple different culture media to mimic developmental cues.[19] Organoids are typically formed by suspending or supporting cell in myriad scaffold materials (like Matrigel®)[20] or scaffold-free techniques including droplets hanging from plates[21] and air-liquid-interface models.[22]

IPSC/ESC-derived organoids generally exhibit more diverse and organ-like functions and can expand more readily than those from ASCs.[23] Organoids derived from ASCs tend to exhibit spheroid shapes while iPSC/ESC tend to show more complex geometries associated with brain, lung and kidney.[24] While iPSCs are technically more complex, they can differentiate into nearly any lineage and better model organ development compared to ASCs, such as mesenchymal stem cells, which are widely studied for their immune-regulating properties.[25, 26]

Oral organoids

Organoids have emerged as a significant tool in dental, oral and craniofacial medicine, addressing critical healthcare challenges in these fields. Organoids facilitate the investigation of complex biological interactions, particularly between ectoderm and mesoderm during tooth development.[27] Here we spotlight key advances in oral organoids for oral mucosa, salivary glands, teeth and other oral tissues.

Oral mucosa organoids

The oral mucosa acts as a barrier against the microbial and physical challenges in the oral environment. Collected surgical oral resection tissue can be cultured with a basement membrane extract and Rho-associated kinase inhibitor to create human oral mucosal organoids, which express basal cell markers, proliferation markers and keratin and overall resemble natural mucosa allowing expansion for up to 6 months.[28] Similarly, lingual mucosa organoids have been developed to exhibit stratified squamous epithelial layers by culturing with epidermal growth factor, the Wnt signalling-activator R-Spondin1 and the transforming growth factor β inhibitor noggin (**Figure 2A**).[29]

Co-cultures are often employed to investigate paracrine signalling and organoid interactions. For instance, oral squamous cell carcinoma organoids co-cultured with fibroblasts demonstrated enhanced organoid formation due to lactate and other secreted factors.[30] Organoid culture conditions vary significantly across different disease states, as evidenced by comparisons between normal oesophageal tissue, gastroesophageal junction, oesophageal squamous cell carcinoma and Barrett's oesophagus.[31]

Salivary gland organoids

Salivary glands are crucial for daily functions like speaking and eating and their dysfunction - especially after radiation therapy - can significantly affect oral health. These glands feature branching duct systems composed mainly of acinar, ductal and myoepithelial cells, with their structure resulting from epithelial-mesenchymal crosstalk.[32] Salivary gland organoids can be established using fragments or ESCs. For instance, Tanaka et al.[33] demonstrated the potential of ESCs to create organoids that showed mature salivary gland function when transplanted into a defective mouse parotid gland model (**Figure 2B**).

Research has revealed that salivary gland organoids grown in hyaluronic acid hydrogels express key functional markers like tight junction proteins and α-amylase[34] and are responsive to neurotransmitters when implanted in resected parotid glands of immunocompromised rats.[35] Rat tail collagen has served as a scaffold for self-renewing organoids derived from submandibular gland cells, which differentiate into acinar and ductal cells.[36]

Branching morphogenesis, essential for salivary gland development, is relevant to other organs as well. Coculturing embryonic submandibular gland epithelial cells with bone marrow-derived mesenchymal stem cells in Matrigel® has produced self-assembling organoids with branching morphology influenced by cell ratios.[37] Factors like laminin 111 and fibroblast growth factor 2 promoted the differentiation of epithelial progenitor cells into terminal buds.[38] These advancements contribute to our understanding of salivary gland branching morphogenesis and potential regenerative strategies.

Tooth and dental stem cell organoids

Engineering of teeth has been a long-held goal given myriad dental diseases and tooth loss. A key challenge in constructing tooth organoids is recreating the spatiotemporal crosstalk between dental epithelium and mesenchyme.[6] An early study combined dissociated dental epithelial and dental mesenchymal cells from tooth germs at the cap stage in a collagen gel, successfully generating tooth-like structure upon implantation.[39] This approach has been adapted for canine[40] and porcine[41] models.

Most tooth-derived stem cells are obtained from human third molars, complicating the creation of anterior tooth organoids. This has prompted the use of pluripotent stem

cells alongside dental mesenchymal stem cells as feeder cells to form tooth organoids that develop tooth-like structures post-implantation.[42] An emerging stem cell for epithelial cells is the cell rests of Malassez,[43] which can differentiate into ameloblasts crucial for dental hard tissue when cultured with dental pulp stem cells (DPSCs).[44]

Other innovative approaches for developing tooth organoids involve culturing adult dental epithelial stem cells in Matrigel®, relying on various signalling pathways to produce highly elongated hydroxyapatite (**Figure 2C**).[45] Additionally, Bektas et al.[46] created methacrylated gelatine microparticles to culture DPSCs and dental epithelial cells, resulting in differentiated dental cells. Calabrese et al.[47] combined dental pulp stem/progenitor cells and periodontal ligament stem/progenitor cells to mimic spatial organisation and mineral patterns of tooth roots, including rudimentary periodontal ligament tissue. These advancements represent promising steps toward effective tooth regeneration.

Other oral tissue organoids

Dental, oral and craniofacial structures are highly diverse, leading to the development of various organoids for their study. For instance, taste bud organoids can be cultured by combing circumvallate epithelium and foliate papillae cells within Matrigel®, which helps localise stem cells and taste receptors along with their innervation (**Figure 2D**).[48] This concept has been extended to engineering artificial tongues using a decellularised extracellular matrix.[49]

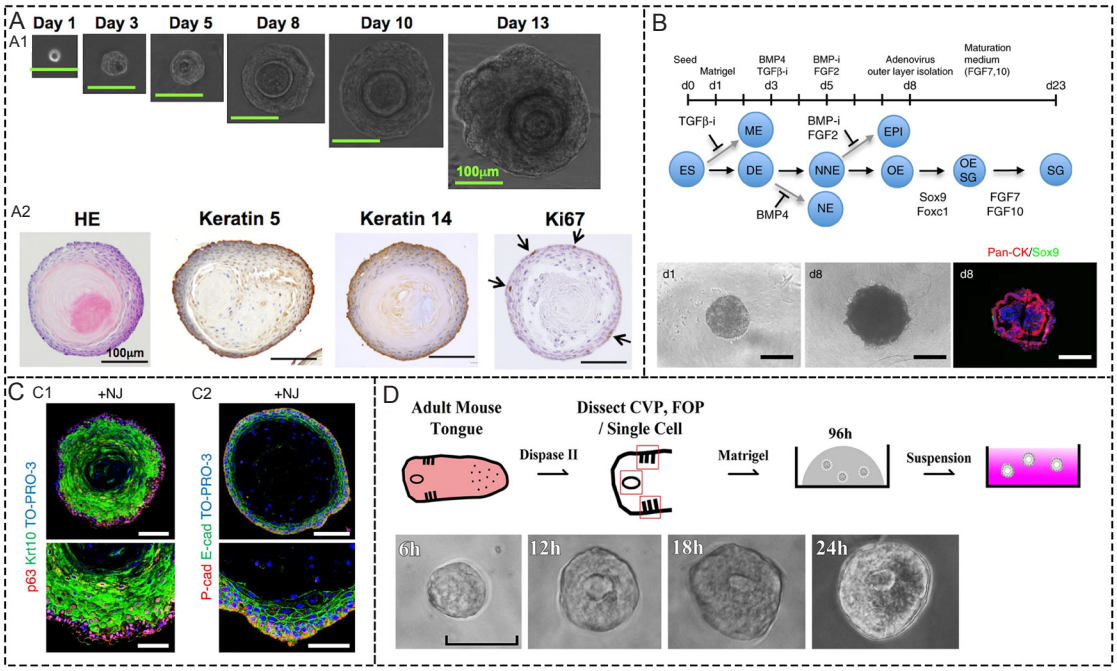

Figure 2. Various oral organoid applications. (A) Lingual mucosa organoid growth and histological analysis of the organoids. Staining for Ki67 showed that some cells actively proliferated in the outer periphery (arrows). Scale bars: 100 μm. Reprinted from Hisha et al.[29] (B) Generation of a salivary gland organoid and immunofluorescence analysis of pan-cytokeratin (Pan-CK, red) and Sox9 (green). Scale bars: 300 μm. Reprinted from Tanaka et al.[33] (C) Confocal images of a 3D culture system for murine dental epithelial organoids. Scale bars: 100 μm (top), 50 μm (bottom). Reprinted from Kim et al.[45] (D) Schematic of suspension-culture method for taste bud organoids and the time-lapse imaging of suspension-cultured organoids under a bright-field microscope. Red squares indicate CVP and FOP. Scale bar: 50 μm. Reprinted from Adpaikar et al.[48] Copyright 2024, Springer Nature. BMP4: bone morphogenetic protein 4; BMP-i: bone morphogenetic protein inhibitor; CVP: adult mice circumvallate papilla; DE: definitive ectoderm; E-cad: E-cadherin; EPI: epidermis; ES: embryonic stem cells; FGF: fibroblast growth factor; FOP: Foliate papillae; Foxc1: forkhead box C1; HE: haematoxylin eosin staining; Ki67: Kiel 67; Krt10: Keratin 10; ME: mesendoderm; NE: neural ectoderm; NJ: Noggin and Jagged1; NNE: non-neural ectoderm; OE: oral ectoderm; OE-SG: salivary gland placode; p63: tumour protein 63; P-cad: P-cadherin; Sox9: SRY-box transcription factor 9; TGFβ-i: transforming growth factor β inhibitor; TO-PRO-3: Thiazole red.

Cartilage exists throughout the head and neck, with hyaline cartilage found in the nose and fibrocartilage in the temporomandibular joint.[50] Crispim and Ito[51] developed hyaline cartilage organoids from chondrocytes in a notochordal cell-derived matrix. These organoids expressed prototypical hyaline cartilage features like type II collagen, type VI collagen, glycosaminoglycans, with SRY-box transcription factor 9-positive cells. Future research may expand on these models by incorporating concepts from other cartilage organoids, such as those related to osteoarthritis and larger joints in the human body.[52]

Applications of oral organoids

Disease model

Oral organoids have become a crucial tool for developing disease models, especially oral cancers and salivary gland disorders. Organoids derived from human salivary glands have been shown to maintain key functional characteristics, making them suitable for modelling salivary gland diseases and testing potential therapeutic interventions (**Figure 3A**).[53, 54] Salivary gland organoids were also utilised to create a swelling model, induced by cholinergic stimulation, to simulate the physiological conditions of saliva secretion, revealing the underlying mechanism of salivary gland disorders and innovative treatments.[53, 54] Patient-derived oral carcinogenesis organoids can accurately reflect tumour characteristics and responses to therapies,[55, 56] making it possible to explore the genetic alterations and tumour microenvironment for mastering cancer biology and developing targeted treatments (**Figure 3B**).[30]

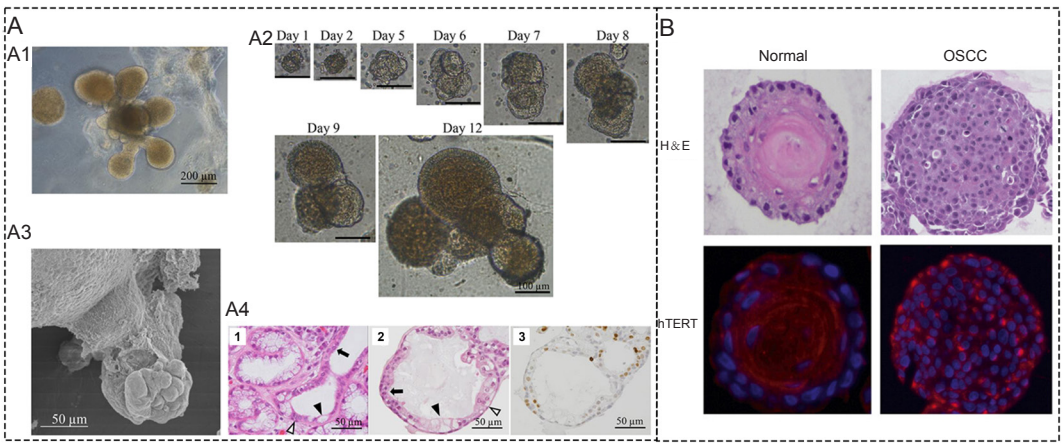

Figure 3. Applications of oral organoids as disease models. (A) Morphological and functional analyses of the human salivary-gland-derived organoids. The organoid has two layers of cells, an inner lining of epithelial (arrow in A4-2) and mucous cells (arrowhead in A4-2) and an outer lining of cells (open arrowhead in A4-2). These inner and outer layers are reminiscent of the luminal inner epithelial (arrow in A4-1) and mucous (arrowhead in A4-1) cells and outer myoepithelial cells (open arrowhead in A4-1), respectively, in the region of the intercalated duct connecting to the secretory end piece of the normal salivary gland. Scale bar: 200 μm (A1), 100 μm (A2), 50 μm (A3, A4). Reprinted from Yoshimoto et al.[53] (B) H&E analysis and hTERT expression in normal oral and OSCC organoids. Reprinted from Yoon et al.[55] hTERT: human telomerase reverse transcriptase; H&E: haematoxylin eosin staining; OSCC: oral squamous cell carcinoma.

The application of organoids extends beyond cancer modelling. Gao et al.[6] discussed how oral organoids can be used to study various diseases, including maxillofacial tumours and tooth dysplasia, thereby contributing to a better understanding of oral health and disease mechanisms. Furthermore, the progressive clustered regularly interspaced palindromic repeats clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (CRISPR/Cas9) for gene editing has enabled oral organoids to model specific genetic alterations associated with oral diseases, enhancing the relevance of these models for translational research.[56]

Regeneration

Advances in bioprinting technology have enabled the creation of salivary gland organoids for drug discovery and regenerative therapies, minimising the reliance on animal models.[57, 58] Studies have found that bioengineered salivary glands, developed using embryonic organ-inductive potential stem cells, can regenerate fully functional salivary glands *in vitro*, highlighting the potential for clinical applications in treating conditions like xerostomia.[57, 59, 60] Oral organoids derived from dental stem cells present promising opportunities for tooth regeneration. These stem cells can differentiate into various cell types necessary for the regeneration of dental tissues, including dentine and periodontal ligaments.[61, 62] Creating organoids from these cells opens up possibilities for investigating odontogenic mechanisms and developing biomimetic artificial teeth, potentially transforming dental restorative practices (**Figure 4**).[60, 63, 64]

Oral organoids are also valuable for studying the mechanisms of tissue regeneration and repair. The application of intermittent compressive forces has been proven to enhance cell cycling and reduce apoptosis in embryoid bodies derived from iPSCs, suggesting that mechanical stimuli can positively influence the regenerative capacity of these cells.[65] Furthermore, oral organoids help in addressing diseases that affect oral health.

Microbiome interaction

Oral organoids' 3D structures assist in investigating the oral microbiome and host-tissue interactions in a controlled condition. Traditional methods often miss the dynamic nature of the oral cavity. Dysbiosis, or an imbalance in microbial communities, can lead to oral diseases, including periodontitis and dental caries.[66, 67] Oral organoids can simulate the oral environment and examine how specific microbial species interact with epithelial cells, potentially leading to inflammation and tissue damage. This approach allows for a more nuanced understanding of the mechanisms underlying oral diseases and the role of microbial communities in their progression.

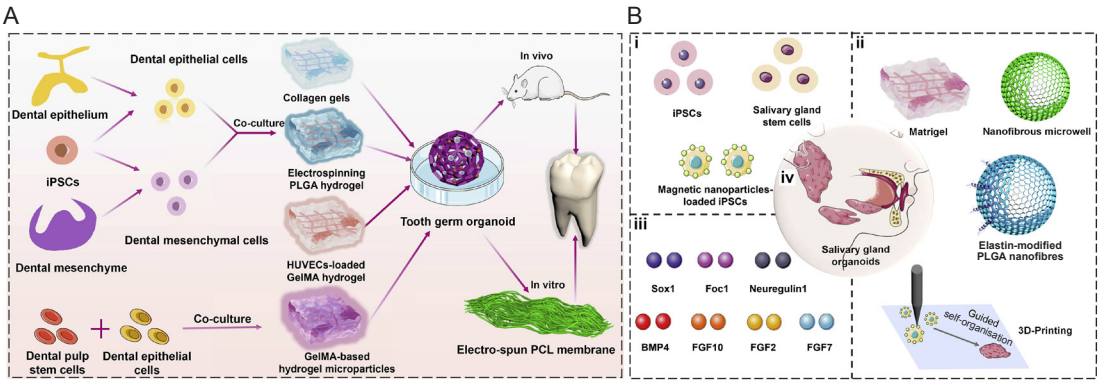

Figure 4. Oral organoid applications for tooth and salivary gland formation. (A) Scheme of engineered tooth germ organoids fabrication for tooth regeneration. Reprinted from Wang and Sun.[60] (B) Scheme of generation of engineered salivary gland organoids. Reprinted from Wang and Sun.[60] 3D: three-dimensional; BMP4: bone morphogenetic protein 4; FGF: fibroblast growth factor; Foc1: *Fusarium oxysporum* f. sp. cubense race 1; GelMA: gelatine methacrylate; HUVECs: human umbilical vein endothelial cells; iPSCs: induced pluripotent stem cells; PCL: polycaprolactone; PLGA: poly(lactic-co-glycolic acid); Sox1: SRY-box transcription factor 1.

Moreover, oral organoids can be employed to investigate how microbes transmit between the oral cavity and other body sites, like the gastrointestinal tract. Salivary gland organoids are being employed to investigate the regeneration mechanisms in the context of inflammatory bowel disease, where salivary gland impairment occurs due to systemic inflammation.[68] This research could lead to novel therapeutic strategies for managing oral symptoms associated with such diseases. By using organoids derived from oral and intestinal tissues, researchers can study how oral microbes influence gut microbiome composition and function, which is relevant for systemic diseases linked to oral health, such as cardiovascular disease and diabetes.[69, 70]

Another exciting area of research involves modelling host-pathogen interactions using oral organoids. Forbester et al.[71] have demonstrated their utility in studying how pathogenic bacteria interact with epithelial cells, assisting in developing targeted therapies. Organoids are valuable for evaluating various treatments on microbial communities are provide insights into restoring a healthy microbiome balance.[72]

Advanced oral organoids

Single-source vs. multi-lineage oral organoids

Single-source oral organoids, derived from a single progenitor cell type, replicate the architecture and function of specific oral tissues, enabling controlled experiments to study cellular behaviours. Hemeryck et al.[73] created an organoid model from human dental follicle tissue, showing a tooth epithelial stemness phenotype similar to the epithelial cell rests of Malassez, confirmed through single-cell transcriptomics. Song et al.[74] demonstrated that transforming growth factor β1 promotes differentiation in organoids derived from human gingival mesenchymal stem cells without compromising viability. However, the limited cellular diversity of single-source organoids restricts their ability to model complex biological processes.[75] In contrast, multi-lineage oral organoids, developed from various progenitor cell types, offer a more accurate representation of oral tissues. This closely mimics the physiological environment and facilitates the study of interactions between different cell types.[76] Recent advancements in growth factors and small molecule cocktails that simulate organ stem cell niches enhance their capacity for differentiation and self-renewal into complex structures.[6]

Furthermore, advanced imaging and sequencing technologies enable real-time monitoring and gene expression analysis, enriching our understanding of organoid biology.[3] Multi-lineage oral organoids are particularly promising for drug testing and personalised medicine, allowing for the assessment of individual responses to therapies using patient-derived cells. This approach is especially relevant for oral diseases where patient-specific factors significantly influence treatment outcomes, making multi-lineage organoids a powerful platform to explore cellular dynamics and processes like tissue regeneration, inflammation and cancer progression.[77, 78]

Bioprinted oral organoids

3D bioprinting technology is revolutionising oral organoid manufacturing by overcoming limitations of traditional 3D culture methods, such as size constraints and lack of reproducibility, as well as insufficient vascular and immune cell interactions.[79] By layering bio-inks, bioprinting allows precise control over organoid composition and structure, facilitating the creation of larger, more complex tissue-like models. This is particularly beneficial for studying oral cancers, as chimeric organoids combining normal and cancerous cells can be developed to explore tumour microenvironments and cellular interactions.[56, 80, 81] Enhanced reproducibility and structural fidelity enable organoids to be engineered with specific traits essential for accurate disease modelling, which is especially important in oral cancer research for recreating tumour heterogeneity.[56, 81, 82]

Bio-inks derived from natural materials have further improved organoid viability and functionality, creating a more physiologically relevant environment.[83] Integrating microfluidic systems with bioprinted organoids enables real-time analysis of dynamic cellular behaviour and drug response,[84] simulating physiological conditions like nutrient flow.[85] Magnetic 3D bioprinting has also been used to create salivary gland organoids, replicating functional epithelial compartments responsive to neurotransmitters (**Figure 5A**).[86, 87] These neuroepithelial organoids hold promise for drug discovery and cytotoxicity screening, particularly for conditions like dry mouth syndrome.

Vascularised oral organoids

Vascularisation is essential for organoid survival and functionality, providing nutrients and oxygen while removing waste. Xu et al.[64] created dental pulp organoids with endothelial and DPSCs, highlighting the role of vascularisation in organoid development, especially in studying dentin and pulp interactions in teeth.[62] IPSCs can differentiate into endothelial cells crucial for vascular structures. Recently, 3D human iPSC-derived blood vessel organoids resembling native vessels were created.[88] Vascularised liver organoids derived from iPSCs further showcase the potential of stem cells in forming vascularisation (**Figure 5B**).[89] Additionally, some specific growth factors, such as Wnt family member 2B, have been reported to significantly enhance vascular development in organoids by increasing endothelial cell number.[90] Hence, with advancements in stem cells and specific growth factors, vascularised oral organoids with complex structures and adequate functions will be designed for promising applications. Recent efforts to enhance vascularisation have been drawn from various organ systems. Holkom et al.[91] designed oral organoids to examine how the tumour microenvironment regulates angiogenesis in oral squamous cell carcinoma, identifying nicotinamide N-methyltransferase as a potential target for antiangiogenic therapy (**Figure 5C**). Kidney organoids cultured on kidney-decellularised extracellular matrices exhibited strong vascularisation, suggesting similar methods could benefit oral organoids.[92]

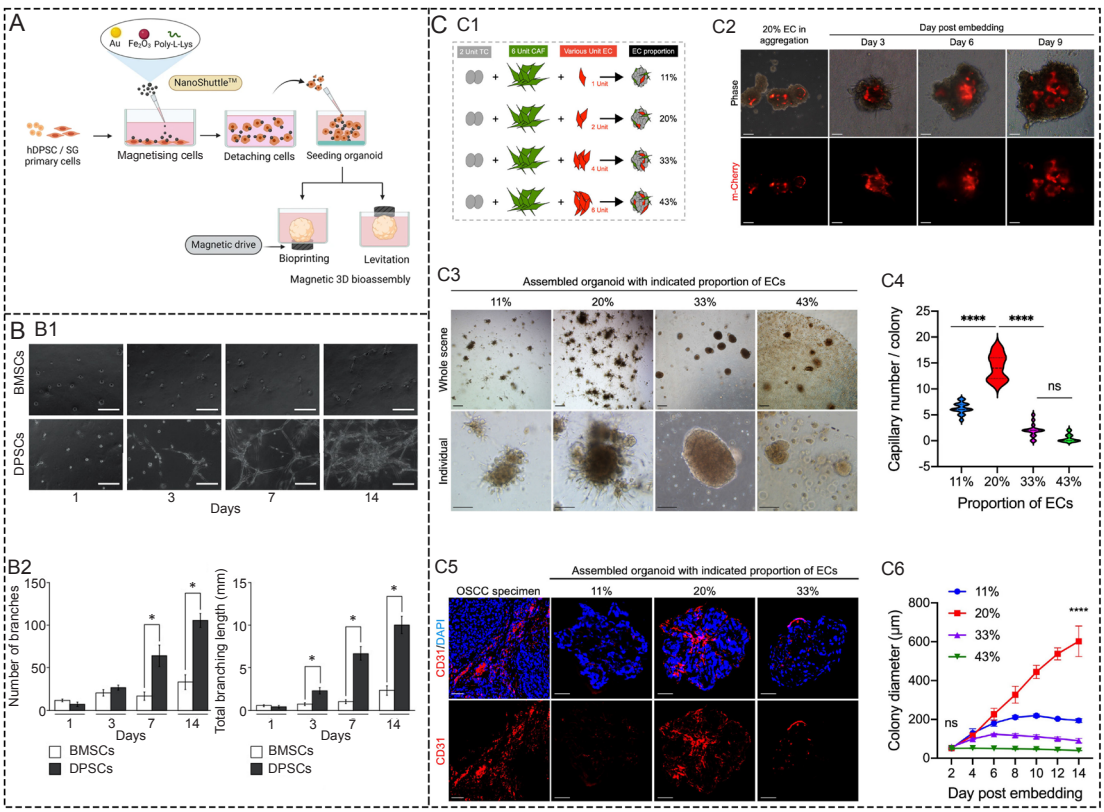

Figure 5. Applications of advanced oral organoids. (A) SG organoid biofabrication workflow utilising two different magnetic 3D bioassembly platforms. Reprinted from Klangprapan et al.[87] (B) Comparison of the sprouting ability of BMSCs and DPSCs, showcasing the potential of stem cells in forming vascularised organoids. *P < 0.05. Scale bars: 200 μm. Reprinted from Li et al.[89] (C) Generation of assembled organoid comprising ECs, fibroblasts and cancer cells. ****P < 0.0001. Scale bars: 100 μm. Reprinted from Holkom et al.[91] Copyright 2024, with permission from Wiley. 3D: three-dimensional; Au: gold; BMSCs: bone marrow-derived mesenchymal stem cells; CAF: cancer-associated fibroblast; CD31: cluster of differentiation 31; DAPI: blue-fluorescent DNA stain; DPSCs: dental pulp stem cells; EC: endothelial cells; FAO: fibroblast-attached organoid; Fe_2O_3: ferric oxide; hDPSC: human dental pulp stem cells; ns: not significant; OSCC: oral squamous cell carcinoma; Poly-L-Lys: polylysine; SG: salivary gland; TC: tumour cell.

Neural-integrated oral organoids

The development of neural-integrated oral organoids is a cutting-edge approach for creating more physiologically relevant models for studying oral health and disease by combining neural and oral epithelial tissues. 3D bioprinting technology enables the precise spatial arrangement of various cell types, including neural and epithelial cells, within oral organoids. Li et al.[93] have demonstrated the potential of bioprinting to create brain-like co-culture constructs, which could be adapted for oral organoid fabrication. Such oral organoids could provide insights into the interactions between neural and epithelial cells in the oral cavity, which are crucial for understanding conditions like pain, inflammation and cancer.

Integrating neural stem cells into oral organoids enhances their functionality by mimicking *in vivo* neural-epithelial interactions. Research has emphasised the importance of incorporating both vascular and neural populations to improve physiological relevance and enable longitudinal analyses of functional development.[94] Additionally, the development of an integrated transcriptomic cell atlas of human neural organoids has provided insights into cellular composition and differentiation pathways.[95] This methodology can be applied to oral organoids to explore how neural integration affects gene expression and cellular interactions, ultimately improving organoid functionality.

Overview of Artificial Intelligence

With its ability to mimic human intelligence and perform tasks that typically require human cognition, AI is reshaping the landscape of healthcare, finance, education and more. In healthcare, AI is being utilised to predict disease outbreaks, assist in surgical procedures and optimise treatment protocols, paving the way for a future where AI technology plays a crucial role in improving human health and well-being.[96] Here we generally introduce the evolution of AI through several significant stages, the concept of integrating AI into organoids and provide specific examples of their use in various applications.

Evolution of artificial intelligence

During the 1950s and 1960s, AI research focused on symbolic reasoning and rule-based systems, which laid the groundwork for early AI applications. Pioneers like Alan Turing and John McCarthy were instrumental in shaping foundational ML and AI concepts. Alan Turing introduced the Turing Test in 1950 that set a benchmark for evaluating machine intelligence, while John McCarthy officially created the term "artificial intelligence" at the 1956 Dartmouth Conference.[97] Following that, people were optimistic about AI research, as the established machines could solve complex problems, making computers intelligent.[98]

In the 1980s, AI research shifted towards symbolic AI and the development of "expert systems", which aimed to emulate human decision-making in specific domains like medicine and finance.[99, 100] Expert systems relied on a knowledge base and an inference engine that processed and combined these symbols to make decisions. Despite their initial success and the potential to handle complex tasks, expert systems were limited by their inability to learn and adapt over time, relying on static representations of knowledge and rules.[98]

AI evolution met a pivotal turning point as ML emerged in the 1990s. ML introduced algorithms that could learn from data and improve performance without explicit programming. This led to supervised learning models[101] and unsupervised learning models,[102] expanding AI applications in areas such as image recognition,[103] natural language processing[104] and recommendation systems.[105] Inspired by biological neural networks, these advancements allowed machines to learn from data rather than relying solely on pre-defined rules.

Today, AI has evolved into advanced systems incorporating reinforcement learning, generative models and autonomous systems. These technologies enable the creation of intelligent agents that can tackle complex real-world challenges. Integrating ML with emerging technologies highlights AI's transformative influence across sectors, such as healthcare, finance and transportation, significantly shaping modern society.[106]

How is artificial intelligence assisting organoids development?

Initially, the development of organoids relied on traditional biological methods, focusing on isolating and culturing stem cells to create 3D structures.[6] Organoid design and applications are exploring new avenues as AI is booming. As an essential component of AI, ML makes computers effectively process and analyse vast amounts of data (**Figure 6**).[107, 108] ML algorithms have been employed to analyse single-cell transcriptomics data[109] and identify organoid phenotypes by automating the analysis of biological images.[110] Indeed, in oral medicine in general, machine learning approaches for clinical radiographic image analysis is booming with some countries granting approval for clinical use.[111] In organoid research, ML helps optimise growth conditions by analysing data from experiments to identify the best combinations of growth factors and extracellular matrix components.[112] This data-driven approach improves the reproducibility and functionality of organoids.

DL is a subset of ML that significantly advances data analysis through multi-layer neural networks to enhance feature extraction and transformation.[113] Unlike traditional ML methods, DL processes raw data directly via neural networks, allowing DL to efficiently train models end-to-end and perform exceptionally well with large datasets.[114] Techniques like convolutional neural networks excel in image classification, including organoid detection.[115-117] The development of OrgaQuant, a deep convolutional neural network, automates the detection of human intestinal organoids in bright-field images, significantly increasing research efficiency.[118] Image analysis may be particularly powerful in oral organoids for systems looking at the interactions between cell populations during tooth formation, like ectoderm and mesoderm cells during tooth development.[27]

Additionally, AI integration into bioprinting technologies optimises 3D printing parameters, facilitating the creation of complex and functional organoids that better mimic native tissue architecture.[119] This has wide potential application for printing of the multi-tissue periodontal complex.

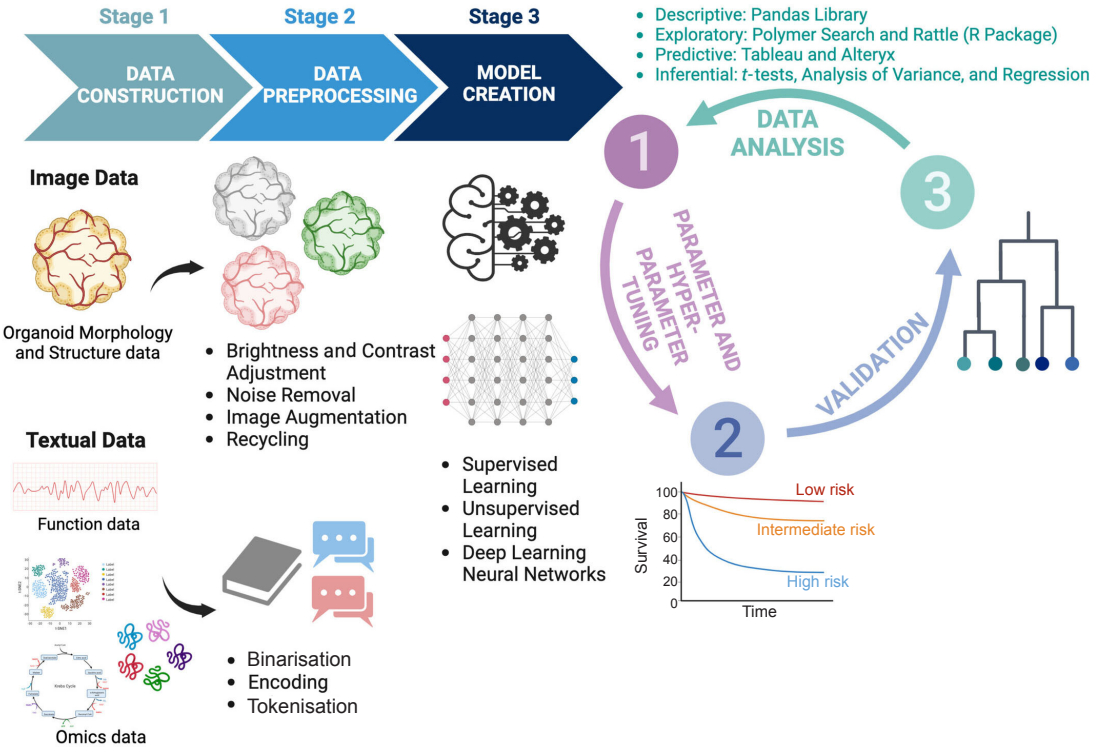

Figure 6. Integration of AI organoid system in three steps: data construction, data preprocessing and model creation. Reprinted from Maramraju et al.[108] AI: artificial intelligence.

Artificial intelligence-enabled organoids

Early detection and diagnosis

AI-enabled organoids are proving to be valuable tools for early detection and diagnosis, offering a unique platform for studying tumour biology and drug responses. By using organoids derived from patient biopsies, researchers can closely examine the biological characteristics of tumours and identify novel biomarkers that inform clinical decision-making.[120] This approach is especially important in cancers like gastrointestinal stromal tumours, where patient-derived organoids have been instrumental in evaluating drug sensitivity and predicting treatment outcomes.[120, 121] Moreover, the integration of AI into the analysis of organoid data significantly enhances the accuracy of these assessments. AI algorithms can detect subtle changes that may signal disease progression, thereby improving our understanding of tumour dynamics and enabling more tailored treatment strategies.[4]

In addition, AI-enabled organoids facilitate more precise and efficient analysis of complex biological systems, deepening our understanding of disease mechanisms and refining diagnostic capabilities. As highlighted by Maramraju et al.[108] AI-driven analyses yield results that surpass traditional human assessment, a critical factor for the accuracy needed in preclinical trials and diagnostics. This sentiment is echoed by Lampart et al.[122] who developed analytical tools that enable high-throughput and high-content screenings, further demonstrating how ML enriches organoid research. The ability to sift through extensive datasets generated by organoid studies is critical for detecting subtle phenotypic changes that may signal disease progression or therapeutic responses. Advanced imaging techniques exemplify AI's transformative role in this field. Gritti et al.[123] have introduced ML-based Organoids Analysis (MOrgAna) software for quantitatively analysing organoids, enabling researchers to conduct detailed morphological assessments (**Figure 7A**). This capability allows for the observation and analysis of dynamic changes in organoid structures over time, offering critical insights into developmental processes and disease states.

Personalised treatment and health monitoring

AI-enabled organoids are valuable in the realm of personalised medicine, offering the ability to create patient-derived organoids that closely replicate the characteristics of individual tumours or tissues. This innovation allows for a precise assessment of how specific therapeutic agents will affect individual patients. Studies have demonstrated that pancreatic cancer organoids can be used for drug screening directly on patient cells, facilitating treatment planning tailored to the unique genetic makeup of the tumour.[4, 124] A potential use for AI and salivary glands is identification of early markers of saliva gland dysfunction from cultured organoids. The incorporation of microfluidic systems in organoid culture further enhances this process by enabling real-time monitoring of drug responses, which is critical for developing personalised treatment plans. Natarajan et al.[125] have showcased a microfluidic co-culture system that allows for live monitoring of T cell interactions with liver organoids, providing insights into immune responses and therapeutic strategies against viral infections. Takahashi[126] emphasised the role of organoids in drug discovery and personalised medicine,

noting their ability to resemble various organs and respond to treatments in ways that reflect patient-specific responses. By integrating AI into the analysis of organoid data, researchers can enhance the efficiency of drug screening processes, leading to the identification of effective therapies tailored to individual patients. Park et al.[127] investigated the use of AI in predicting the differentiation of kidney organoids derived from human iPSCs. By utilising AI algorithms to assess the maturity of these organoids, researchers can select the most suitable models for clinical applications, thereby enhancing the reliability of drug testing and improving treatment outcomes for kidney-related diseases.

In terms of health monitoring, AI-enabled organoids serve as a dynamic platform for assessing disease progression and treatment efficacy. By integrating AI algorithms, researchers can analyse complex data generated from organoid experiments, gaining a comprehensive understanding of how individual organoids respond to treatments over time. The organoid brightfield identification-based therapy screening (OrBITS) platform developed by Deben et al.[128] enables label-free, time-lapse monitoring of patient-derived organoids, significantly enhancing the ability to track organoid health and therapy responses (**Figure 7B**). This capability is essential for making timely adjustments to treatment strategies based on real-time data. Furthermore, applying AI to analyse organoid growth kinetics addresses the intra-tumoural heterogeneity commonly observed in cancers. By utilising ML techniques to assess individual organoid responses rather than relying on bulk cultures, researchers can uncover the variability in treatment responses among different tumour cells.[129]

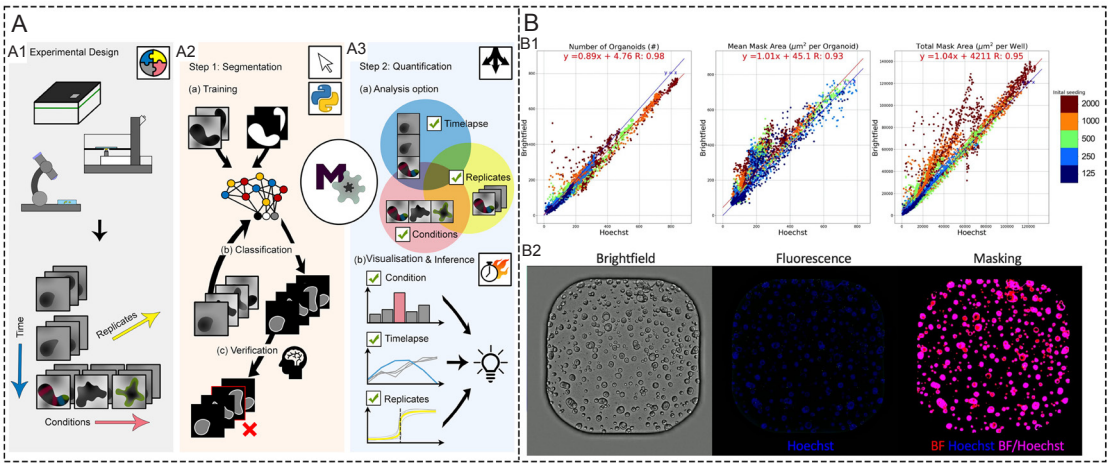

Figure 7. AI-enabled organoids for early diagnosis and health monitoring. (A) MOrgAna workflow schematic: experimental design, segmentation and quantification. Reprinted from Gritti et al.[123] (B) Brightfield *versus* Hoechst analysis of organoids. Reprinted from Deben et al.[128] AI: artificial intelligence; BF: brightfield; MOrgAna: machine-learning based organoids analysis; OrBITS: organoid brightfield identification-based therapy screening.

Disease prediction

AI-enabled organoids are revolutionising human disease modelling by closely mimicking tissue physiological and pathological characteristics. Cai et al.[130] have explored the potential of brain organoids, combined with AI, to create predictive models that simulate disease progression and response to therapies. In the realm of retinal diseases, Kegeles et al.[131] have demonstrated that convolutional neural networks could accurately predict retinal differentiation in retinal organoids (**Figure 8A**). This predictive capability is vital for early diagnosis and intervention, enabling the identification of individuals at risk of developing vision-threatening conditions. The ability to forecast disease progression based on organoid behaviour enhances the potential for timely therapeutic strategies.

AI-enabled organoids are also valuable in studying neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. Esmail and Danter[132] have developed iPSC-derived brain organoids to model the genetics of Alzheimer's progression, yielding insights into the disease's pathophysiology. Similarly, Monzel et al.[133] employed ML techniques to predict neurotoxicity in human midbrain organoids, demonstrating the potential for AI to assess the impact of environmental toxins on neuronal health. By utilising AI to analyse organoid responses to various stimuli, researchers can predict disease progression and identify potential therapeutic interventions. In oral medicine, this will likely be most immediately useful for oral squamous cell carcinoma cancer organoids, which are more advanced and ready for AI-integration than other oral organoid systems.

Drug screening

As discussed above, organoids derived from patient-specific cells replicate an individual's unique genetic and phenotypic characteristics, providing a platform for drug testing that closely mimics the patient's tissues. The work of Phan et

al.[134] demonstrated that high-throughput screening using patient-derived tumour organoids can identify actionable drug sensitivities, creating a preclinical platform for precision medicine.

AI-enabled organoids further enhance the establishment of high-throughput drug screening platforms that can efficiently evaluate the efficacy of various compounds. Takahashi et al. conducted large-scale drug cytotoxicity screenings using human intestinal organoids, demonstrating the feasibility of screening extensive libraries of pharmacologically active compounds.[135] By utilising dispersed intestinal epithelial cells from organoids, they achieved a homogeneous cell population, which is crucial for minimising variability in drug response assessments. This method exemplifies how organoid technology can be adapted for high-throughput applications, making it a valuable tool in drug discovery.

Integrating ML algorithms into organoid drug screening enhances data analysis and interpretation. Branciforti et al.[129] have highlighted the use of DL-based pipelines to analyse drug screening results from cancer organoids, combining imaging techniques with gene expression and protein interaction data. This comprehensive analysis leads to a more nuanced understanding of drug efficacy and mechanisms of action, ultimately improving the predictive power of drug screening assays. For example, the oral squamous cell carcinoma organoid system developed by Zhang et al.[136] for drug screening could be enhanced with AI. Furthermore, the automated microfluidic platforms developed by Schuster et al.[137] enable dynamic and combinatorial drug screening of tumour organoids, allowing for the simultaneous testing of multiple drug combinations (**Figure 8B**). This approach not only accelerates the drug discovery process but also provides insights into the interactions between different therapeutic agents, which is essential for developing effective combination therapies.

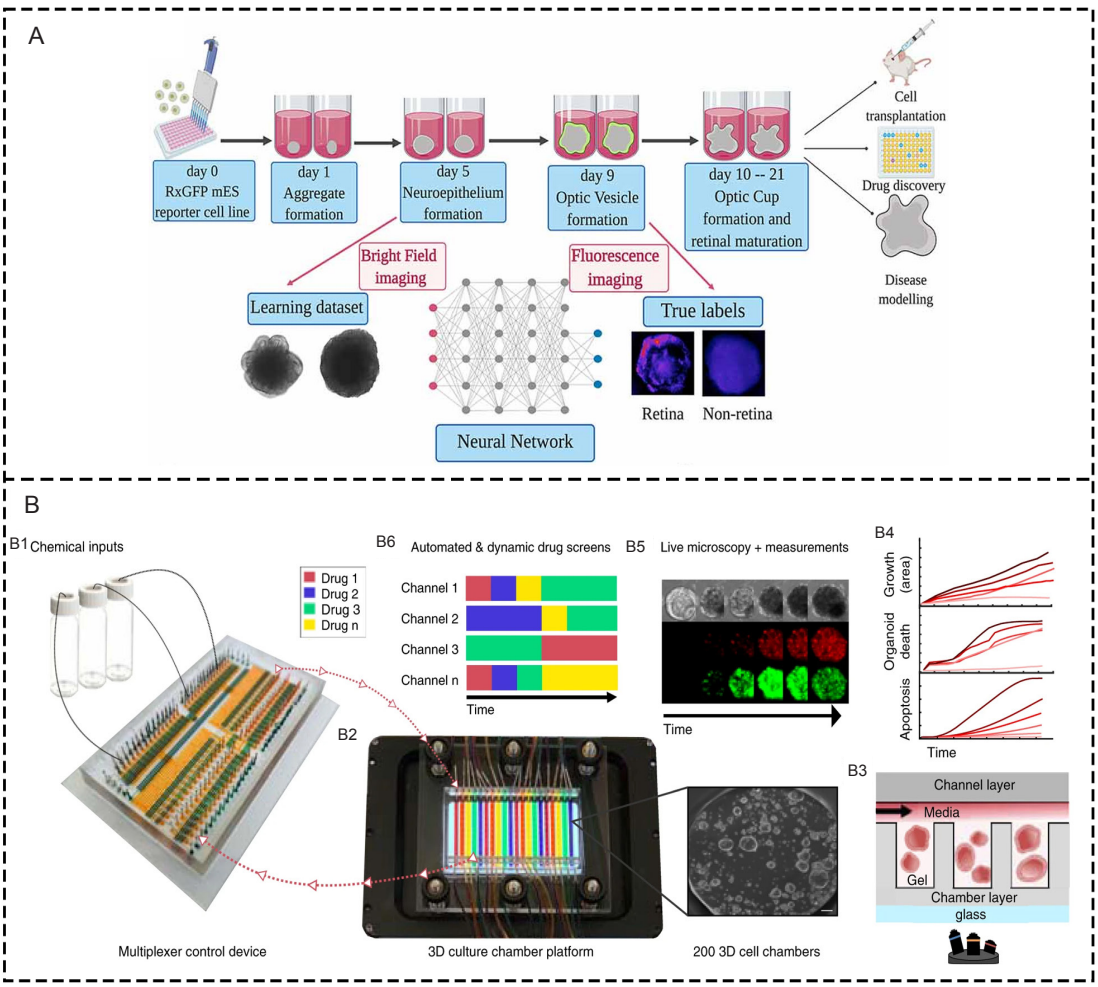

Figure 8. AI-enabled organoids for disease prediction and drug screening. (A) Schematic flow of retinal differentiation experiments using a neural network to predict retinal differentiation. Reprinted from Kegeles et al.[131] (B) Automated microfluidic 3D cellular and organoid culture platform for dynamical drug perturbations. Reprinted from Schuster et al.[137] 3D: three-dimensional; AI: artificial intelligence; mES: mouse embryonic stem cell; RxGFP: mES reporter cell line.

Challenges and Perspectives

Challenges in developing oral organoids

Oral organoids, miniature models of oral tissues, have greatly expanded knowledge of oral biology and disease, yet several challenges remain in their development and applications. Tissues like the gingiva, periodontal ligament and dental pulp exhibit complex cellular interactions that are difficult to replicate *in vitro*. Achieving functional organoids requires precise control over the microenvironment and signalling pathways to accurately mirror the native tissue complexity.[138] Stringent quality control and standardisation are vital to ensure reproducibility and reliability in research outcomes. Ahn et al.[139] highlighted that replicating the *in vivo* environment poses significant challenges, necessitating integration of oral organoids into regulatory frameworks that prioritise quality and safety.

Without established manufacturing standards, variability in oral organoid quality can lead to inconsistent findings, complicating the translation of results into clinical applications.[140] Differences in the quality and type of stem cells, whether derived from different individuals or tissues, further complicate the development of consistent oral organoids. For instance, oral organoids derived from DPSCs and periodontal ligament stem cells may show differing differentiation potentials based on their origin.[56] Addressing these challenges is crucial for advancing oral organoid technology in research and clinical settings.

Moreover, ensuring equitable access to organoid technology is vital for its broader adoption in clinical practice, especially in resource-limited settings where specialised equipment and trained personnel may be lacking.[141] The high costs associated with organoid production and analysis may be prohibitive for some patients, hindering their access to personalised treatments.

Challenges in artificial intelligence-driven oral organoids

Integrating AI into the development of oral organoids offers promising opportunities, yet critical considerations need to be addressed to ensure ethical and reliable application of AI. The process involves analysing sensitive health data from patient-derived tissues, raising concerns about data privacy and algorithmic bias that could reinforce existing inequalities.[142, 143] Mahmood et al.[144] underscored the necessity of context-specific quality assurance measures, which include acceptance testing prior to clinical use and continuous quality control monitoring. By addressing biases in the training data and ensuring that the data used for AI algorithms is representative and of high quality, researchers can improve the reliability of predictions made by AI systems in organoid studies. Furthermore, as AI technologies progress, regulatory frameworks need to adapt to ensure the safety and efficacy of AI-driven organoid applications. Ranjbar et al.[145] emphasised the importance of establishing new management systems and quality assurance mechanisms in healthcare organisations to accommodate the unique challenges posed by AI systems.

Rigorous validation of AI-driven models against experimental results is crucial due to the complexity of biological systems, which may limit predictive accuracy.[146] Variability in organoid culture conditions, such as differences in media composition and environmental factors, can lead to inconsistent organoid characteristics, which may affect the outcomes of experiments.[108] The extensive data generated from organoid studies often surpasses the capabilities of traditional analysis methods, increasing the risk of data overload and model overfitting.[147, 148] Thus, developing AI models capable of accurately capturing complex biological behaviours remains a significant challenge.

Ensuring the quality and consistency of oral organoids is critical for reliable research outcomes. While AI can assist in monitoring and analysing organoid characteristics, it also necessitates new quality control protocols, as traditional methods may be inadequate. Without robust quality assurance, the reproducibility of AI-driven results may be compromised, hindering further clinical translation. In addition, generating AI-enabled oral organoids requires seamless interoperability among various platforms and technologies to effectively analyse and provide actionable insights.

Perspectives of artificial intelligence-enabled oral organoids

While AI-driven oral organoids are still relatively rare, advancements in other types of organoids inspire their designs and applications. Integrating AI with oral organoids can significantly enhance diagnostic and treatment capabilities, analyse the interactions between oral microbiota and host cells and facilitate novel oral drug development.

For diagnostics, AI algorithms can analyse high-resolution imaging data from organoids, identifying disease markers and treatment responses for quicker and more accurate diagnoses. The landscape of AI models for predicting oral diseases has evolved, leveraging ML and DL techniques to enhance diagnostic accuracy and improve patient management. In treatment planning, AI optimises the testing of therapeutic drugs on organoids, allowing for patient-specific therapy adjustments.[149, 150] By simulating disease dynamics and analysing drug efficacy, AI-driven models can identify resistance mechanisms and early intervention biomarkers.[151, 152]

Moreover, ML techniques can mine multi-omics data to uncover patterns related to disease states and microbial interactions. AI can analyse the interplay between oral microbiota and host cells in organoid systems, identifying microbial signatures associated with disease and facilitating the development of personalised preventive strategies.[153, 154] Understanding the roles of specific bacterial communities in periodontitis can inform the design of probiotics or other therapeutic interventions aimed at restoring a healthy oral microbiome. AI-driven organoids can model these interactions, providing insights into how microbial communities influence tissue health and disease progression. By analysing data from organoid studies, AI can identify patterns of microbial colonisation and their effects on host responses, potentially leading to the discovery of novel therapeutic targets. This research is vital for developing strategies to manipulate the

oral microbiome in favour of health, thereby preventing or mitigating diseases such as periodontitis.

AI-enabled organoids are revolutionising drug discovery processes in oral healthcare. By utilising organoids that closely mimic human oral tissues, researchers can conduct high-throughput screening of potential therapeutic compounds.[155] AI algorithms can analyse the responses of these organoids to various drugs, identifying effective candidates for further development.[156] This approach not only accelerates the drug discovery timeline but also reduces the reliance on animal models, aligning with ethical considerations in research.[157] Moreover, AI can assist in predicting treatment outcomes based on organoid responses, thereby facilitating the development of more effective and targeted therapies for oral diseases.

Furthermore, AI can help to deal with the challenges in developing oral organoids mentioned above. AI has the potential to improve accessibility to oral organoids by streamlining production processes, ultimately reducing costs and resource demands. By leveraging AI algorithms, oral organoid culture conditions can be analysed and optimised to enhance yield and efficiency, making organoid technology more viable in diverse clinical settings.[108] AI can significantly contribute to quality control by utilising ML to monitor and evaluate oral organoid development in real-time, ensuring more consistent and reliable outcomes.[118] This early detection of deviations allows for timely interventions to maintain quality standards. Scalability is a common challenge in oral organoid production[158] and AI can address this issue by automating aspects of the organoid culture process, such as monitoring environmental conditions and adjusting parameters in real-time to optimise growth.[112] Additionally, AI-driven predictive models can assist in prioritising organoid development based on their potential for successful drug responses, optimising resource allocation and enhancing efficiency.[108]

Although the discussion of oral organoids and AI is ever-growing, due to the limited applications of AI-enabled oral organoids, we hereby encapsulate the progress made in the development of oral organoids and AI, offering our perspectives on the potential for AI to transform oral organoids in the future. Overall, the development of AI-driven oral organoid technology suggests a transformative shift in oral healthcare, emphasising personalised treatment, early disease detection and continuous patient monitoring. These organoids can streamline therapeutic interventions and improve outcomes by harnessing AI for predictive analytics and drug development.

Author contributions

JY and ZY conceptualised the review; JY and NGF drafted the manuscript; ZY revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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Conflicts of interest statement

The authors declare no conflicts of interest.

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