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REVIEW

The feasible application of microfluidic tissue/organ-on-a-chip as an impersonator of oral tissues and organs: a direction for future research

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Abstract

Currently, cell culture models play a key role in determining cell behavior under various conditions. However, the accurate simulation of cellular behavior that imitates the body's conditions remains a challenge. Therefore, to overcome this obstacle, three-dimensional cell culture models have been developed. Microfluidic tissues/organs-on-chips (TOOCs) are new devices that have provided the opportunity to culture cells in a medium that is almost similar to the physiological conditions of the body. TOOCs can be designed in simple or complex models, which are mostly fabricated by soft lithography. These novel structures have been developed to mimic the conditions of various tissues and organs; however, microfluidic models for oral and dental tissues have not yet been widely used. The application of TOOCs for oral tissues/organs can provide the opportunity to study cell interactions with biomaterials used in dentistry. Furthermore, TOOCs can provide the opportunity to study the cellular interactions and developmental stages of oral tissues/organs more accurately. This review of the current advances in the field of TOOC development for oral tissues provides a comprehensive understanding of this burgeoning concept, shows the progress and applications of these novel models in the imitation of oral tissues/organs thus far, and reveals the limitations that TOOCs confront. Moreover, it suggests further perspectives for future applications.

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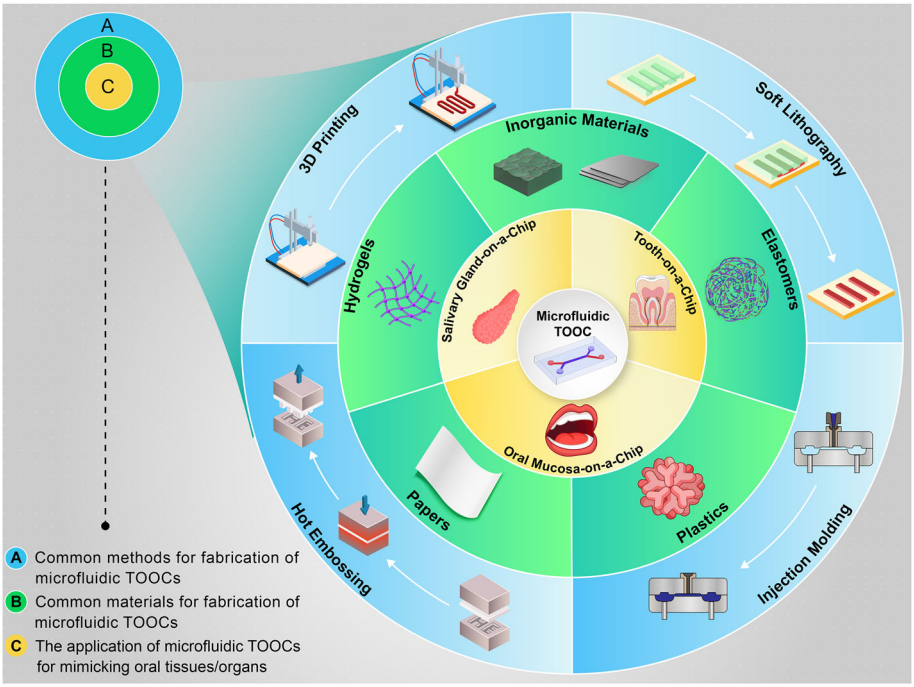
Graphic abstract



Keywords Microfluidics · Tissue-on-a-chip · Organ-on-a-chip · Tooth-on-a-chip · Oral mucosa-on-a-chip · Salivary gland-on-a-chip

Introduction

Various tissues (bone, cartilage, muscle, skin, and so on) are composed of different cell types connected to the extracellular matrix (ECM) and cellular junctions and distinctively in a three-dimensional (3D) framework [1–4]. Cells manifest organ-specific variations (such as dynamic mechanical stress, flow shear stress (FSS), and concentration gradient), which regulate their growth, function, and survival [1, 2, 5]. Understanding cell–cell and cell–ECM interactions, tissue interfaces and organ-specific dynamic variations is crucial to enhance future progress in particular fields such as oral biology and drug development [1, 6–8].

Conventional two-dimensional (2D) cell cultures are reductionist models that are designed by researchers to address many biological questions [6], although they are not effective for anticipating many cellular functions and monitoring in vivo physical and chemical microenvironments precisely [9, 10]. These drawbacks have drawn additional attention toward complex 2D systems that incorporate multiple cell types or cell patterning, resulting in serious deliberation on 3D systems, which properly mimic the spatial and chemical intricacies of living tissues [10, 11]. Moreover, 3D cell cultures are usually composed of either natural or synthetic hydrogels, which stimulate the polarization and interaction of cells with neighboring cells [12–14]. These 3D systems can take several forms, which range from random interspersing of cells in ECM to clusters of cells in organoids[1] [11, 12]. Compared with 2D systems, the employment of a 3D system is very practical for analyzing molecular tissue functions, obtaining signaling pathways, and responding to drugs in some disease states [11]. However, concerning the limitations of 3D systems, 3D organoids have various forms and sizes, and it is difficult to keep the exact location of their cells for analysis [11]. In addition, functional analysis, such as transcellular transport, secretion, and absorption, and biochemical and genetic analysis of cultivated cells are difficult in 3D systems [9, 11]. Many cell culture systems lack multiscale design, tissue–tissue interface, and exposure to mechanical cues (tension, compression, and FSS), which are crucial for the development and functioning of an organ in health/disease conditions [9, 11].

Large and small animal models are currently regarded as gold standards in biomedical research [15–18]. As is customary now, new medications or biomaterials should be tested through animal models prior to human testing for

[1] The 3D multicellular tissue constructs that are formed in vitro and mimic their corresponding in vivo organs to study different aspects of those organs in the laboratory.

safety approval [15, 19]. Regrettably, using these models is often expensive, time-consuming, cumbersome, and ethically controversial, and these models often do not mimic human physiological or pathological conditions [6, 15, 19]. These drawbacks can possibly be overcome by the employment of microfluidic tissue/organ-on-a-chip (TOOC) [13, 20, 21]. Hence, the notion of this review is to focus on microfluidic TOOCs and reflect their potential superior capacity to recapitulate physiological functions of human oral tissues and organs and signify it as a possible bridge for the gaps between the present biological systems.

What are microfluidic TOOCs?

TOOCs are microfluidic devices for cultivating living cells in constantly perfused and microsized chambers to mimic the physiological function of tissues and organs. Its main aim is to construct minimal functional units that can potentially replicate essential tissue and organ functions for the intended application instead of constructing an entire organ [11, 22]. There can be two principal forms of these systems, including simple devices (single, perfused microfluidic chamber of one kind of cultivated cell) or complex devices (two or more microchannels connected by a porous membrane, containing various cultivated cells to replicate tissue interfaces) [11, 22]. Furthermore, organ-specific analysis can be performed using chips containing cells from various organs that are connected by fluid [23]. These connections are either direct from one interstitial tissue compartment to another or indirect through a second microchannel containing vascular endothelium to investigate functional interactions of different tissues/organs or drug distribution in vitro [11, 23]. The term "chip" in TOOC is derived from a modified form of photolithography used in producing computer microchips [24]. This fabrication method has been selected since it can control surface characteristics (shapes and sizes) on a similar scale (nm to μm) as cells in their natural tissue environment sense and react [11, 24]. Likewise, the majority of microfluidic culture systems are built by soft lithography (replica molding processes) [25, 26] through the polymerization of a liquid polymer on an etched silicon substrate to create a rubber stamp [26, 27]. Soft lithography was first introduced to design a microscale ECM island to determine the form, position, and function of cultivated cells on silicon chips and later conventional cultivation substrates [11]. Polydimethylsiloxane (PDMS) is the most commonly used material in fabricating microfluidic TOOCs due to its several advantages, such as high biocompatibility, gas permeability, optical clarity, and flexibility [22, 25, 28]. The optical clarity of PDMS culture systems permits real-time, high-resolution optical imaging of cellular responses to environmental factors [11, 29]. Similar systems were later manufactured from various materials (inorganic materials, elastomers, plastics, papers, hydrogels, etc.) using different fabrication techniques (hot embossing, injection molding, 3D printing, etc.) [11, 30]. Common fabrication materials and methods used to create TOOCs will be discussed in the following two sections.

Common materials for fabrication of microfluidic TOOCs

To date, only a few specific materials can be used for the structure of TOOCs. The materials of TOOCs should be suitable for both laboratory research and commercialization. Therefore, manufacturers should consider all aspects, including the ease of prototyping, the performance of the device, the cost of production, and the ease of use [31]. Moreover, it has been discovered that acceptable mechanical properties and optical transparency are critical for producing these chips [32]. These features lead to different preferences in materials for device fabrication, and each has its own advantages and disadvantages (Table 1). Ultimately, the materials can be modified or combined to fabricate the desired devices for specific targets [31]. Below, the most common materials for the fabrication of microfluidic TOOCs are discussed.

Inorganic materials

As the first generation, silicon and glass are the main inorganic materials for the fabrication of microfluidic TOOCs. Although many other chip materials have been introduced, silicon and glass are still commonly used due to their transparency, resistance to organic solvents, ease of metal deposition, high thermoconductivity, and stable electroosmotic mobility [33].

Silicon, a common material in microfluidic devices, is used as a substrate and in the process of microfabrication (sacrificial layers) [34]. It is used in different forms, such as single-crystal silicon (SCS), polycrystalline silicon, silicon dioxide (SiO_2), and silicon nitride [35]. Its favorable mechanical properties make it suitable for different microfabrication processes, such as etching (wet or plasma etching), laser processing, and various bonding methods [36]. However, silicon is opaque, so glass-based devices can be more helpful in the design and fabrication of microfluidic devices.

In contrast to opaque silicon, glass is ideal for real-time imaging and transformative studies due to its transparency and ability to reduce the absorbance and adsorption of hydrophobic biomolecules [37]. There are three types of glass used in microfluidic chips: soda lime, quartz, and borosilicate, which are a mixture of SiO_2 with other oxides, such as CaO and MgO [38]. It is worth mentioning that when glass chips have enclosed channels, they will no longer be suitable for long-term cell culture since glass is not gas permeable, and its employment might lead to channel plugging.

Table 1 Typical materials for fabrication of microfluidic tissues/organs-on-chips (TOOCs)

Category	Common materials	Main features	Limitations	Reference
Inorganic materials	Silicon	Favorable mechanical properties Inexpensive	Opaque Not gas permeable	[31]
	Glass	Surface stability Optically transparent Electrically insulating	Not gas permeable High cost of fabrication Time-consuming fabrication	[93]
Elastomers	Polydimethylsiloxane (PDMS)	Ease and low cost of fabrication High elasticity High gas permeability Biocompatible Rapid prototyping Optically transparent	Hydrophobicity Strong adsorption of biomolecules Not compatible with organic solvents	[31, 77, 78]
Plastics	Plastics	Optically transparent Low absorption Rigid Suitable for mass production	Poor gas permeability Unsuitable for prototyping	[53, 54]
Papers	Papers	Highly porous Matrix of cellulose Potable and low cost	Limited detection methods Difficult to integrate microcomponents	[31, 137, 138]
Hydrogels	Natural Collagen	Biocompatible Enzymatically degradable Similar in structural and mechanical properties to ECM Good cell adhesion	Weak mechanical properties	[139–141]

Table 1 (continued)

Category	Common materials		Main features	Limitations	Reference
		Gelatin	Biocompatible Biodegradable Similar in composition to collagen Good cell adhesion Tunable properties by the addition of functional groups	Weak mechanical properties Rapid degradation	[142–144]
		Alginate	Biocompatible Biodegradable Easy functionalization Immediate gelation at mild condition	Weak mechanical properties Poor cell adhesion Uncontrollable degradation	[145–148]
	Synthetic	Polyethylene glycol (PEG)	Biocompatible Tunable and precise mechanical and degradation properties Relatively low protein adsorption	Less cell adhesive Limited biodegradation	[149–151]
		Polyvinyl alcohol (PVA)	Chemical stability Nontoxicity Good biocompatibility Biological aging resistance High water-absorbing capacity Easy processing	Biologically inert Weak ability to adsorb proteins Inability to adhere to cells	[152]
		Gelatin methacryloyl (GelMA)	Excellent biocompatibility Biodegradability Moldability Low viscosity Rapid photo-crosslinkability	Poor mechanical strength Fast biodegradability	[63]

Glass is typically processed with standard photolithography and etching, which is tedious and expensive. To overcome these limitations, 3D glass-based TOOCs are made using the femtosecond laser ablation technique [39, 40]. Moreover, liquid glass has also been developed and used to make low-cost prototyping of glass-based microfluidics. Nevertheless, due to the aforementioned limitations of silicon- or glass-based TOOCs, significant care must be taken in the fabrication of these microfluidic devices [41].

Elastomers

Elastomers are polymers with elasticity and a higher yield strain compared to other materials. PDMS is the most popular elastomer in microfluidics and was introduced several years after silicon- or glass-based microfluidic chips [42, 43]. Because polymers have a wide range of properties, they provide a great deal of flexibility in regard to selecting a suitable material [44, 45]. A PDMS chip can be reversibly sealed to another piece of PDMS, glass, or other substrates by simply making contact [46]. An optimized blend of PDMS and methacrylate has also been used for 3D stereolithography with mechanical properties similar to conventional thermally cured PDMS. It is worth mentioning that the 3D-printable PDMS resin makes the fabrication of PDMS-based TOOC platforms easier [47].

Ease and low cost of microfabrication, low surface tension, gas permeability, high biocompatibility, and optical transparency are interesting features of PDMS. Because of these features, PDMS is broadly used in the fabrication of various TOOCs, cell culture scaffolding, and screening and biochemical assays [46]. Despite its popularity and benefits mentioned above, PDMS has some drawbacks and has limited applications in specific fields [48]. These characteristics include incompatibility with organic solvents, hydrophobicity, and strong adsorption of biomolecules. In this regard, a variety of methods have been introduced to improve the surface properties of PDMS-based microfluidic chips using plasma treatment, ultraviolet (UV) treatment, and coating [49]. Coatings include some metals or metal oxides (e.g., titanium oxide and gold) or solgel coating and surface silanization techniques (i.e., creation of Si–O–Si bonds by an amine, carboxyl, thiol, etc.) which can reduce the surface energy of PDMS but still cannot fully overcome its limitations [49–52]. Therefore, the application of PDMS-based microfluidic devices is restricted to aqueous solutions.

Plastics

Polymethyl methacrylate (PMMA), polycarbonate (PC), polystyrene (PS), cyclic olefin polymer (COP), and cyclic olefin copolymer (COC) are typical plastic materials for microfluidics fabrication. Among them, PMMA has been used as a substrate material for organ-on-a-chip (OOC) devices due to its rigidity and transparency [53]. PC membranes are porous, so they are used for tissue–tissue interface modeling [54]. PS is optimal for cell growth and adhesion due to its biocompatibility [55]. COP and COC have high quality in fluorescence imaging since they are optically transmissive in both the visible and UV ranges, allowing for high-quality fluorescence imaging. Recently, polylactic acid (PLA) has been proposed as a suitable alternative to other plastics for TOOC applications due to its low absorption and low autofluorescence [56].

Recently, thermoplastics[2] have been increasingly used to produce microfluidic devices due to the drawbacks of PDMS- or glass-based microfluidic chips regarding surface treatment instability and the absorption of molecules. Thermoplastics, in addition to the convenient features of other plastics, are more resistant to pressure and temperature fluctuations since they have linear and branched molecules [57]. However, there are several restrictions to the usage of thermoplastic materials. Nontransparent thermoplastic polymers (e.g., polyether ether ketone (PEEK) and polypropylene (PP)) interfere with microscopic or imaging observations. Some of these materials have strong autofluorescence properties and are not suitable for detection purposes and prototyping. Additionally, these materials have poor gas permeability, which has a negative impact on the long-term cell culture of microfluidic TOOCs [31].

Papers

Most microfluidic devices were prepared with sealed channels before paper-based devices were produced. The advantages of paper-based microfluidics, including light weight, easy use, and low cost, make these materials applicable for the fabrication of TOOCs. In addition, the cellulose matrix of paper and its porous structure make it good for cell growth in a 3D format. However, the detection methods relatively limit these devices because of the presence of the fabric matrix in the channel area and its difficulty in integrating its microcomponents [58]. It is worth mentioning that paper-based microfluidics with dynamic adjustment of physiological conditions can be utilized as high-output test platforms by shaping on multilayered paper. In addition, by specifically joining a luminescent detecting film, the spatiotemporal oxygen utilization rates or pH slopes can be checked in real time through quantitative image analysis.

Having several similarities with paper, nitrocellulose membranes, threads, and clothes have also been used as scaffolds for cell culture in microfluidic TOOCs since they have

[2] A thermoplastic is a plastic polymer material that becomes pliable or moldable at a certain elevated temperature and solidifies upon cooling.

stronger, higher controllable rates for fluid mixing and lower environmental impact [59, 60].

Hydrogels

Hydrogels, mimicking the extracellular matrix, have been widely applied to embed cells for various applications owing to their high biocompatibility and tunable properties, including elasticity, porosity, permeability, stiffness, and degradability [58, 61]. Hydrogels are 3D networks of hydrophilic polymer chains that span an aqueous medium of over 99% water. Generally, there are two types of hydrogels, including natural hydrogels (collagen, gelatin, alginate, etc.) and synthetic ones (polyethylene glycol (PEG), polyvinyl alcohol (PVA), gelatin methacryloyl (GelMA), etc.) [30, 58, 62, 63]. Although natural hydrogels are biocompatible, biodegradable, and low cytotoxic, they suffer from some shortcomings, such as relatively low mechanical properties and restricted long-term stability. Therefore, they are often combined with synthetic hydrogels [64].

Hydrogels are highly porous with controllable pore sizes and have a combination of aqueous nature and permeability. Therefore, they are perfect for encapsulating cells for 3D cultures [30]. Moreover, microchannels can be built into them to diffuse bioparticles and solutions through them, which is similar to the bifurcating vasculature's function [65, 66]. However, the diffusion of nutrition and oxygen through the bulk gel is not yet adequate, and necrosis typically starts to occur at a depth of several hundred micrometers [67]. Additionally, despite their aforementioned advantages, hydrogels have a major flaw, which is low stiffness, so they are not widely used in microfluidic devices, especially TOOCs [68].

Currently used materials for fabrication of oral microfluidic TOOCs

Previous investigations adopted PDMS to fabricate different tooth-on-a-chip, oral mucosa-on-a-chip, and salivary gland-on-a-chip [69–76]. Positive features of PDMS, such as ease and low cost of fabrication, high gas permeability, biocompatibility, and optical transparency, have made this material suitable for the fabrication of oral microfluidic TOOCs [31, 71, 77, 78]. Moreover, PMMA was used for the fabrication of the mold rather than the chip itself in some studies [69, 76], but recently, in a study by Hu et al. [79], PMMA was the material of choice for the fabrication of a microfluidic tooth-on-a-chip due to its rigidity and transparency [53]. However, the application of TOOCs in the oral and craniofacial fields has been limited to date. Furthermore, there are still many other oral and craniofacial tissues/organs that could be promising subjects for future TOOC investigations; therefore, utilization of materials other than PDMS and PMMA could be possible.

Common methods for fabrication of microfluidic TOOCs

The design of microfluidic devices entails delicate and precise processes. As mentioned earlier, various biomaterials are introduced in the fabrication of microfluidic TOOCs. Since each material has a specific characteristic and behavior, the fabrication methods should be optimized based on the biomaterials. Moreover, the cost-effectiveness of microfluidic TOOCs is another issue to consider for fabrication. Since these devices cannot be cleaned easily, they are often used once and then disposed [80]. This implies the importance of the economic feasibility of the fabrication of these devices. Generally, there are two approaches for designing microfluidic TOOCs: top-down and bottom-up [30]. In a top-down approach, the microstructures are first fabricated, and the cells are seeded inside the microenvironment [30]. In a bottom-up approach, the cells are seeded in a microenvironment to develop the desired microstructure [30, 81]. Among the techniques used for microfluidic TOOCs, soft lithography, hot embossing, injection molding, and 3D printing are among the most common methods [30], which are described below.

Soft lithography

Soft lithography, also known as "replica molding," is one of the most commonly used methods to fabricate microfluidic TOOCs [82, 83]. This method was developed from the photolithography technique to circumvent the obstacles of this method, such as poor surface control, high cost, and incompatibility with curved substrates [84]. The advantages offered by this technique are lower cost, rapid prototyping, and high-resolution replica fabrication [83]. Soft lithography uses elastomeric polymers and surface patterning by PDMS stamps [27, 83, 85, 86]. This technique consists of four major steps, including pattern design, mask and master fabrication, PDMS stamp production, and production of micro- or nanostructures with a stamp through printing, molding, and embossing [27]. The fabrication of microfluidic TOOC devices through this method can be performed out of the clean room in a routine chemical laboratory, especially when the master has processed the rest of the procedures, which can be continued outside of the clean room by using the printing or molding technique [27]. Figures 1a and 1b briefly illustrate the fabrication protocol of microfluidic chips by PDMS using soft lithography.

Hot embossing

This method is mostly known for its flexibility and convenience [30, 87]. In the hot embossing technique, the thermoplastic polymer is pressed against the master mold

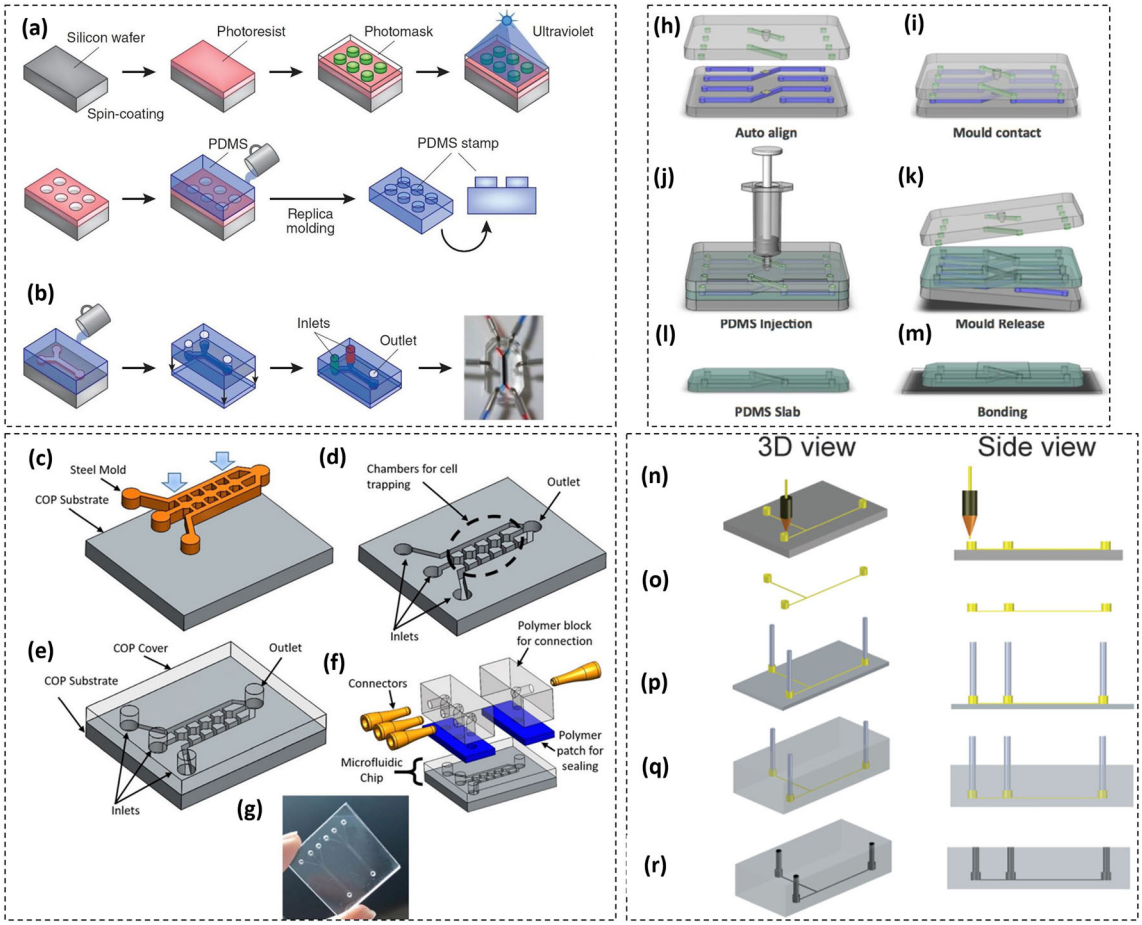


Fig. 1 **a**, **b** Microfluidic chip manufacturing using the soft lithography method. **a** Illustration of the replica molding process. First, a photosensitive layer (photoresist) is placed on the silicon chip. Then, the photomask layer is placed on the photoresist layer and exposed to ultraviolet light. After the dissolution of the photoresist layer, the microscale pattern of the photomask layer remains on the photoresist layer, which makes it ready to fabricate polydimethylsiloxane (PDMS) stamps by pouring liquid prepolymerized PDMS on the photoresist layer. **b** The fabrication of a single-layer microfluidic device by manufacturing a PDMS stamp. This process is performed by one outlet, two inlets, and one major channel. Reproduced from [11], Copyright 2014, with permission from Nature Publishing Group. **c**–**g** Illustration of the tissue/organ-on-a-chip (TOOC) fabrication process by hot embossing. **c** The steel mold is applied to the desired substrate (cyclic olefin polymer, COP). **d** Formation of trenches in the COP substrate after hot embossing. **e** Thermocompression of the COP substrate and the COP cover containing the outlet and inlets. **f** Outlet–inlet port insertion into the reactor. **g** Representation of the final microfluidic device. Reproduced from [134], Copyright 2017, with permission from Springer Science+Business Media New York. **h**–**m** Illustration of the TOOC fabrication process by injection molding. **h**, **i** Complementary molds are integrated. **j** The degassed PDMS is injected into the integrated structure and fills the voids. **k**, **l** The mold is removed, and the final PDMS is obtained. **m** The channels are sealed with glass slides. Reproduced from [135], Copyright 2016, with permission from The Royal Society of Chemistry. **n**–**r** Fabrication of microfluidic devices based on the three-dimensional (3D) printing technique. **n** Fabrication of polyvinyl alcohol (PVA) mold using the 3D printing technique. **o** Mold removal from the printing bed. **p** Placement of PVA mold on cured polymer with inserted tubes of polytetrafluoroethylene (PTFE) in inlet and outlet sockets. **q** The process of casting with uncured polymer. **r** Removing mold with water. Reproduced from [136], Copyright 2018, with permission from WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

under a certain amount of contact pressure and heat to produce the replica [30, 83, 88]. This technique offers the advantage of higher delicate designs, which results in the fabrication of a high aspect ratio and micropin lamella in the microstructure polymer [30]. Moreover, one of the other advantages of this process is its low cost [30]. Aside from the advantages of this method, the practitioner must carefully adjust the temperature and other parameters, such as pressure [30, 89, 90]; furthermore, the application of this technique is only limited to thermoplastic materials and polymers [82, 83]. This method is schematically represented in Figs. 1c–1g.

Injection molding

This method is also similar to the hot embossing technique; however, instead of pouring the thermoplastic polymer, it

is injected into a cavity with a heated master mold [82, 83, 88]. While injecting the polymer into the heated master mold cavity, the pressure should be maintained, whereas the temperature decreases below the glass transition level of the polymer [83]. Despite the low cost and lower complexity of this method, the hot embossing technique is thought to be more effective in producing more precise replicas due to the lack of thermal changes and subsequent dimensional variations of the master mold [83]. Both injection molding and hot embossing techniques are limited to thermoplastic materials, such as PDMS, and master models are usually produced by photolithography [82, 83]. Injection molding by PDMS polymer is illustrated in Figs. 1h–1m.

3D printing

Three-dimensional (3D) printing is a relatively novel technology in developing microfluidic TOOCs [78]. This technology enables the practitioner to fabricate complex microfluidic systems with a wide diversity of materials [91–94]. Some of the well-known techniques in 3D printing are multijet molding, inkjet molding, focused-deposition molding, and suspended liquid subtractive lithography [78, 94]. The main advantages of this technique are rapid prototyping and direct fabrication of the microfluidic models by obviating the necessity to develop the master model by other methods, such as photolithography [95]. Although this technique offers various advantages in developing microfluidic devices, this technology still requires further progress in small dimensions (the smallest 3D printed channel is 350 microns) [30, 96]. Additionally, the low z-resolution of the printing system, restricted types of transparent materials for application, required finished smooth surfaces, and challenging precise production of hollow sections are other shortcomings that should be addressed in future studies [83, 97]. Three approaches to 3D printing are represented in Figs. 1n–1r.

Currently used methods for fabrication of oral microfluidic TOOCs

To date, the majority of the previous investigations on oral microfluidic TOOCs employed soft lithography with PDMS to fabricate different tooth-on-a-chip and oral mucosa-on-a-chip [69–74]. Additionally, in a recent study [75], a salivary gland-on-a-chip was designed using gas expansion molding with PDMS. Moreover, in a study in 2022 [79], a tooth-on-a-chip device was fabricated using micromilling with PMMA. Although hot embossing and injection molding have not been used in the fabrication of any oral microfluidic TOOC to date, they are suitable fabrication methods that can be employed with thermoplastic materials [82, 83]. Moreover, future progress in enhancing the precision of 3D printers might enable researchers to fabricate microscale TOOCs that better impersonate oral and craniofacial tissues/organs.

Features and advantages of microfluidic TOOCs

There are many beneficial features and advantages for microfluidic TOOCs, which enable them to perform physiological analysis and monitor system parameters more accurately than other systems [9, 98]. First, the flexibility of microfluidic TOOCs can deliver microenvironments through perfusion or 3D structures. In these 3D structures, cells are incorporated into natural or synthetic polymers (collagen, Matrigel, and hydrogel) [23, 29]. These 3D systems are feasible for signal pathways, drug reactions, and tissue functions [9, 13]. Second, the main advantage of TOOCs lies in the precise control of parameters. By containing a microflow that matches real exposure volumes with accurately controlled administration of test substances and nutrients and the removal of waste products, these devices provide a more physiologically relevant environment [99, 100]. On this matter, fluid flow in these microfluidic chips is crucial since viscous forces dominate inertial forces on a smaller scale. Therefore, when the channel diameter is less than 1 mm, the flow is laminar and enables chemical and physical gradients to be produced [9, 24, 101]. The FSS can be adjusted by varying the channel size/flow rate or by using a nonporous membrane that disconnects cells from the flow path or inhibits the passage of cells by microengineered posts[3] [5, 9, 102]. Additionally, fluid mechanical computational models can optimize the microchannel geometry to enhance cell survival and function by increasing nutrition and oxygen delivery [9, 103]. Thus, these chips can simulate the organs' sophisticated mechanical microenvironment in vitro [25]. In contrast to in vitro well plate culture, microfluidic TOOCs, which allow flow in luminal channels, are able to mimic the flow of saliva or gingival crevicular fluid [73]. Third, in addition to the controllable flow, the inner surface topography of the TOOCs and the amount of pressure on the cells can also be controlled. In that manner, modification of the inner surface of TOOCs results in favorable cell location patterning [13], and flexible side chambers and cyclic suction[4] of these chips can be used to establish cyclic mechanical strains[5]. Moreover, enhanced pressure can be used to compress the tissues that usually react to compression [11]. These features

[3] Microengineered pieces that are set upright in a microchip.

[4] A cyclic vacuum occurring with a specific frequency in flexible chamber(s) that affects adjacent cells to imitate the natural strains that cells confront in some organs in the body such as lungs, gut, etc.

[5] Repeated or fluctuating deformities showing the relative displacement among particles in the body of a material.

could add other dimensions that are not available in standard tissue culture formats [13]. Fourth, various cell types can be plated in designated patterns or in direct competition with the same planar substrate in microchannels by methods including plating cells/ECM proteins using laminar stream, applying complex microchannel path designs to contact adhesive substrates, situating microposts between adjacent cells or microprinting ECM in various positions in microchannels [11, 25]. Fifth, the substrates can be shaped into an organoid form using molding techniques. Chip designs contain embedded cells in 3D ECM gels and tissue-engineered multicellular structures [9, 30]. The integration of porous substrates for the separation of two microchannels allows the aforementioned functional analyses, which were difficult in 3D systems. A culture of different cells on opposite sides of the substrate produces tissue–tissue interfaces that imitate almost all organ interactions [9, 11].

TOOC is of benefit in studying the physiology as well as the pathological conditions of tissue/organ models. It is also appropriate for the study of biological processes that are dependent on tissue structure and perfusion [11, 28]. TOOC can be used as a substitute for other cell cultivations and animal models in various diseases or drug studies [28, 104]. Since these chips can control fluid flow, cell survival and differentiation can be increased [28, 105]. This technology shows the potential to advance the development of tissue, organ physiology, disease etiology, dental biomaterial efficacy, and drug development. Their feasible applications can emerge in a new era in oral biology. In summary, Table 2 concisely compares the advantages and disadvantages of microfluidic TOOCs and other in vitro culture models.

The application of microfluidic TOOCs for mimicking oral tissues/organs, the current portfolio

To date, microfluidic TOOCs have been broadly developed and fabricated for different tissues and organs of the body. However, TOOC models for stimulating specific human oral tissues and organs, including teeth, oral mucosae, salivary glands, and their composite assemblies, have not been widely developed [6]. These models will be able to mimic conditions of oral tissues and organs, such as architecture, organization, multi-tissue interphases, physiology, and pathological conditions [6, 106]. Prior to the emergence of microfluidic TOOCs, conventional co-cultures were employed to provide a beneficial way to evaluate and control the interactions of cells and their target cells/tissues/organs in a regulated and isolated environment [107, 108]. However, these co-cultures do not optimally mimic the in vivo situation [107, 108]. Being appropriate for co-culturing, microfluidic systems can preserve the physiological properties of specific cell populations by providing an environment for different culture media [108]. Recent studies have investigated the application of microfluidic TOOCs for mimicking oral tissues/organs. The following sections explain the impact of each platform in advancing the knowledge in oral and craniofacial research and their accomplishments thus far. Table 3 also illustrates the current studies in which the practicality of microfluidic TOOCs for mimicking oral tissues/organs was investigated.

Tooth-on-a-chip

For the first time, in 2014, a microfluidic system involving dental tissues was developed to analyze the crosstalk of tooth germs with trigeminal innervation [107]. In this study, Pagella et al. [107] compared this microfluidic system to a conventional co-culture of embryonic trigeminal ganglia and tooth germ. In a conventional co-culture, trigeminal ganglia and tooth germ were cultured in a medium optimized for trigeminal ganglia survival and growth (Figs. 2a–2e) [107]. The results showed that most of the axons did not approach the tooth germ and did not penetrate it during the culture period (Fig. 2c) [18]. Additionally, the tooth germ could not grow properly, and signs of degeneration were observed after long periods of culture (Figs. 2c and 2e) [107]. On the other hand, microfluidic systems were able to provide optimal co-culture conditions for the growth of trigeminal ganglia and tooth germ (Figs. 2f and 2g) [107]. To recapitulate the in vivo pattern of tooth innervation, the trigeminal ganglia were co-cultured with postnatal (Figs. 2h–2k) or embryonic tooth germ for 10 days. The results of the microfluidic system showed that the trigeminal ganglia spread neurites toward the tooth germ without showing any sign of repulsion (Fig. 2i). Moreover, neurons entered the dental pulp mesenchyme in the postnatal molar, and the tooth germ preserved its structure (Figs. 2i and 2j) [107]. This pioneering study [107] indicated that mouse trigeminal ganglia and teeth can persist in this microfluidic system for an expanded culture time, and teeth maintained attractive and without repulsive effects on trigeminal ganglia, as previously observed in vivo. From these findings, microfluidic co-culture systems have been proven to be an optimal option for studying the relationship between neuronal and dental tissues and exploring the role of innervations in developing teeth [107]. These are valuable tools for future investigations on the behavior of neurons during orofacial development, imitating the in vivo situation [107].

Lam et al. [71] in 2016 designed a tooth-on-a-chip microfluidic system to mimic the performance cultivation and analyses of two oral bacteria (*Streptococci species* and *Fusobacterium nucleatum*) in biofilms under different dissolved gas and sucrose concentrations. Microbes within biofilms are often less prone to antibacterial agents in comparison to planktonic cells; hence, inhibiting biofilm

Table 2 Comparison of advantages and disadvantages of microfluidic tissues/organs-on-chips (TOOCs) and other in-vitro two-dimensional (2D) and three-dimensional (3D) culture models [153]

In-vitro culture models	Advantages	Disadvantages
2D cell culture	Reductionist model with a well-established protocol Easy conduction and quantification	Static condition Inefficient in simulating in vivo physical and chemical microenvironments due to the lack of physical and biochemical signals Media volume with a large scale Considerable variety in nutrient and waste concentration
3D cell culture	Ability to take several forms Responding to drug treatments Consisting of cel–ECM (extracellular matrix) and cell–cell interactions	Static condition Difficult to maintain the exact location of cells on 3D organoids Difficult functional analysis
Microfluidic tissue/organ-on-a-chip (TOOC)	Ability to deliver microenvironment Vital fluid flow due to the domination of viscous forces on microscale High ability in integration with various technologies	Difficult to microfabricate and standardize with high accuracy Adsorption of molecules of interest on the inner linings due to the dominant surface effect

formation has always been a great challenge for related dental therapies [71, 109]. Several biofilm culture systems (e.g., biofilm reactors, "Centers for Disease Control" biofilm reactors [110], biofilm fermenters, and biofilm microarrays) have been produced thus far [71]. Many of these systems are inefficient in carrying out parallel cultures with concurrent regulation of several environmental factors, which would result in limiting the screening throughput [71]. Conversely, this high-throughput microfluidic device (containing 128 culture chambers) could be applied to screen the development of biofilm properties under combinations of specified growth parameters, such as seeding bacteria populations, growth medium compositions, medium flow rates, and dissolved gas levels (Fig. 3) [71]. The main fabrication method for microchannel layers in this device was the multilayer soft lithography of PDMS [71]. The study suggests that this multichamber microfluidic system could assess biofilm morphology, colonization density, and bacterial spatial arrangement in maturing dental biofilms by providing an independent microenvironment in each culturing chamber for monitoring bacterial biofilm development and colonization. Additionally, this platform could provide comprehensive applications for future dental biofilm research [71].

In 2019, Niu et al. [70] designed a microfluidic chip that enabled the growth of both the body and the processes of odontoblasts. Odontoblasts contain two parts: the body and the processes. It is essential for studies that aim to investigate the function of these cells and their role in dentin hypersensitivity to provide a similar condition to the oral cavity for these cells to grow their natural morphology. These cells have been examined in vivo, in animal models, and in various conventional in vitro culturing systems thus far [70]. Animal model studies may confront ethical problems, and their experimental conditions may be poorly controlled [23]. However, conventional in vitro culturing systems provide controllable experimental conditions, and to date, various in vitro culturing systems have been developed. In these systems, despite the formation of the body of the odontoblasts, the processes did not form or appear [70]. On the other hand, an odontoblast microfluidic chip was shown to induce the growth of odontoblast processes [70]. This microfluidic device was fabricated using conventional soft lithography. Figure 4b portrays the steps of developing this microfluidic device. First, the structures were designed via Auto CAD and then transferred to photomasks. The mold was fabricated via two-step photolithography. In this regard, the first layer was spin-coated on a silicon wafer and exposed, resulting in microchannels. Next, the second layer was lithographed, which formed the culture chambers. Afterward, PDMS overflowed on top of the mold, degassed for 2 h, and then cured at 80 °C for 1 h [70]. Microchambers in this microfluidic chip were formed for culturing odontoblasts, and microchannels with geometric constraints were made for growing odontoblast processes (Fig. 4e) [70]. These microchannels impersonated the dentin tubules and with a 2 μm width (consistent with the size of dentin tubules in an in vivo environment) could successfully induce the growth of odontoblast processes [70]. These odontoblast processes showed similar morphology and functions to those in in vivo experiments (Figs. 4f–4h) [70]. The odontoblast microfluidic chip is a very useful tool for investigating the biology and pathology of odontoblast processes and will assist in developing treatment solutions for dentin hypersensitivity and other dental diseases [70].

In 2020, França et al. [69] designed a two-chamber tooth-on-a-chip that was made of molded PDMS separated by a

Table 3 Current studies in which the feasibility of microfluidic tissues/organs-on-chips (TOOCs) for mimicking oral tissues/organs was investigated

Organ model	Authors (publication year)	Fabrication material	Fabrication method	Types of mono-/co-culture	Aim(s)	Main finding(s)
Tooth	Hu et al. [79] (2022)	PMMA	Micromilling	DPSCs	To characterize the cytotoxicity potential of SDF on DPSCs in a tooth-on-a-chip model	DPSCs exposed directly to SDF showed a dose-dependent reduction in cell viability (IC50: 0.001%). Inlet channels (internal control) of the tooth-on-a-chip exposed to PBS and SDF-exposed dentin disks showed >85% DPSC viability. In contrast, the outlet channels of SDF-exposed dentin disks showed a decreased viability of <31% and 0% (1.5 and ≤1.0 mm thick dentin disk, respectively) (P<0.01)
	Rodrigues et al. [76] (2021)	PDMS	Soft lithography	DPSCs	To compare the effects of three different CSCs on the viability and proliferation of DPSCs and investigate whether these events correlate with pH variations and the release of TGFβ on-chip To conduct a live and dead assay to test the antimicrobial capability of ProRoot in real time	ProRoot significantly increased the extraction of TGFβ (P<0.05) within 24 to 72 h and, along with Biodentine, induced higher cell proliferation (P>0.05), while TheraCal decreased cell viability and provoked atypical changes in cell morphology No correlation between TGFβ levels and pH was observed The bacterial cells within biofilms treated by ProRoot were mostly killed, both at the biofilm's outer and inner layers (>90%)

Table 3 (continued)

Organ model	Authors (publication year)	Fabrication material	Fabrication method	Types of mono-/co-culture	Aim(s)	Main finding(s)
	França et al. [69] (2020)	PDMS	Soft lithography	SCAPs	To distinguish the responses of pulp cells to dental materials on-chip and compare them with the off-chip system	HEMA, PA, and SB had cytotoxic effects in the on-chip and off-chip systems in the following order: HEMA>SB>PA (P<0.05) Cells presented consistently higher metabolic activity on-chip than off-chip (P<0.05)
	Niu et al. [70] (2019)	PDMS	Soft lithography	Odontoblasts	To determine the appropriate size of the microchannel to induce the growth of odontoblast processes	Microchannels with a 2-μm width could better mimic dentin tubules and successfully induced the growth of odontoblast processes The obtained odontoblast processes presented a similar morphology to those in vivo and could express the marker protein Aquaporin-4
	Lam et al. [71] (2016)	PDMS	Soft lithography	*Streptococci species* and *Fusobacterium nucleatum*	To design a microfluidic "artificial teeth" device that can control microenvironmental factors for quantitative characteristics of long-term dental bacteria growth and biofilm development	This device provides extended applications for general biofilm research, including monitoring developing biofilm properties under combined growth parameters such as seeding bacteria populations, growth medium compositions, medium flow rates, and dissolved gas levels

Table 3 (continued)

Organ model	Authors (publication year)	Fabrication material	Fabrication method	Types of mono-/co-culture	Aim(s)	Main finding(s)
	Pagella et al. [107] (2014)	NR	NR	Teeth and trigeminal ganglia	To study the efficacy of microfluidic systems for imitating the behavior of neurons during orofacial organs/tissues' development in vivo	Mouse trigeminal ganglia and teeth could survive for long culture periods and teeth maintained the repulsive effect on trigeminal neurites (the projections from the cell body of the neurons of the trigeminal nerve) as had been observed in vivo
Oral Mucosa	Ly et al. [72] (2021)	PDMS	Soft lithography	Epithelial and subepithelial cells including keratinocytes and fibroblasts	To determine if the mucosa-chip could replace traditional platforms to test HEMA by providing multilayered tissue geometry	Mucosa-chips were deemed more sensitive to assess HEMA-altered cell viability compared to well-plate culture, especially at lower doses (1.56 and 6.25 mmol/L)
	Rahimi et al. [73] (2018)	PDMS	Soft lithography	Epithelial and subepithelial cells including keratinocytes and fibroblasts	To develop a co-cultured microfluidic mucosal model on-chip to assess the remodeling of mucosa and the responses of epithelial and subepithelial layers to challenges typically found in the oral environment	Exposure to HEMA lowered mucosal cell viability Exposure to the bacteria lowered transepithelial electrical resistance

Table 3 (continued)

Organ model	Authors (publication year)	Fabrication material	Fabrication method	Types of mono-/co-culture	Aim(s)	Main finding(s)
	Kang et al. [74] (2016)	PDMS	Soft lithography	SHEDs, PDLSCs, and hGFs	To explore the oral cell interactions during differentiation	Osteoblast gene expression levels in SHEDs incubated with hGF media decreased compared to those in SHEDs treated with PDLSC media, suggesting that indirect co-culture of SHEDs with hGFs may inhibit osteogenic cytodifferentiation
Salivary Gland	Song et al. [75] (2021)	PDMS	Gas expansion molding	The acinar cell clusters and intercalated ducts isolated from human and mouse	To combine engineered extracellular matrices with MB array technology to develop functional tissue mimetics for mouse and human salivary glands	The SGms responded to calcium signaling agonists and secrete salivary proteins SGm exhibited phenotypic and functional parameters of salivary glands and provided an enabling technology for high-content/throughput drug testing

PMMA: polymethyl methacrylate; PDMS: polydimethylsiloxane; SDF: silver diamine fluoride; PBS: phosphate-buffered saline; CSC: calcium silicate cements; DPSCs: dental pulp stem cells; TGFβ: transforming growth factor–β; SCAPs: stem cells from apical papilla; HEMA: 2-hydroxyethylmethacrylate; PA: phosphoric acid; SB: Adper Scotchbond; SHEDs: stem cells from human exfoliated deciduous teeth; PDLSCs: periodontal ligament stem cells; hGFs: human gingival fibroblasts; SGm salivary gland mimetic; MB: microbubble; NR: not reported

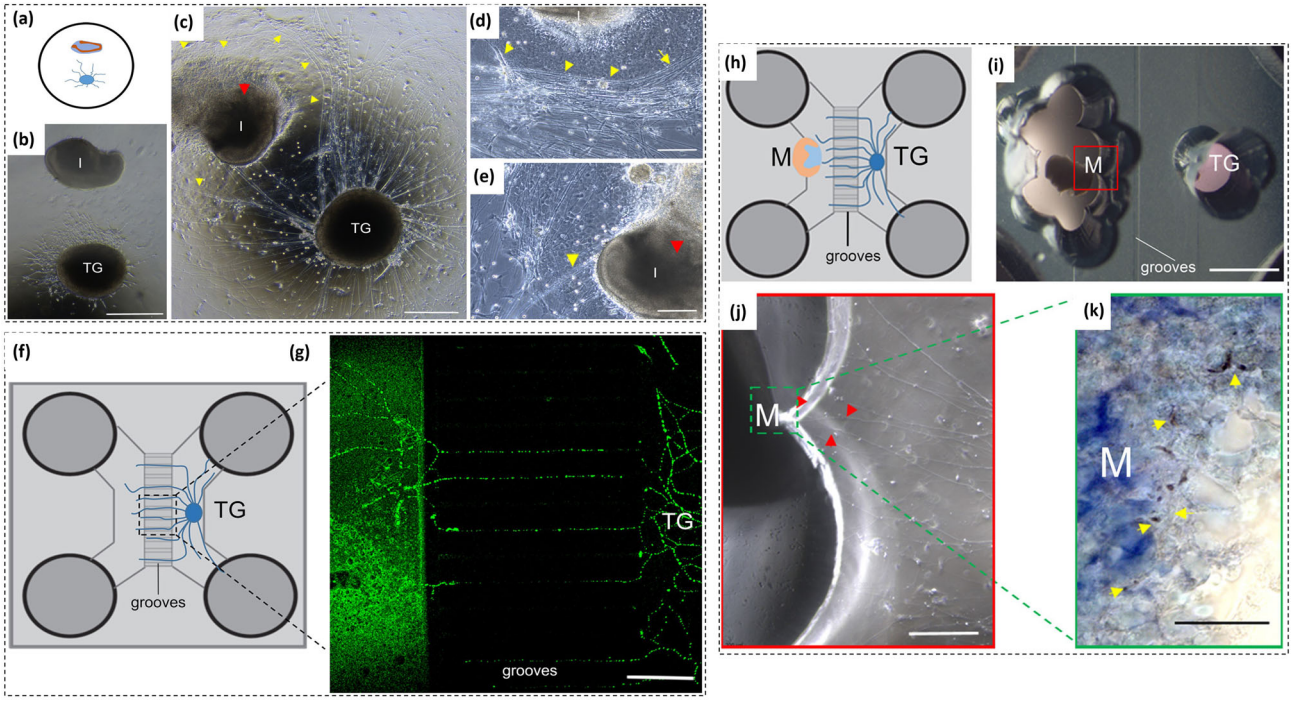


Fig. 2 **a**–**e** Conventional co-cultures of trigeminal ganglia and incisors. **a** Schematic representation of the deposition of embryonic trigeminal ganglion and incisor in co-culture. **b**, **c** Trigeminal ganglion (TG) and incisor (I) after 4 and 10 days in co-culture, respectively. Axons (yellow arrows) are clearly repealed by the incisor. The incisor presents signs of degeneration that are evident (red arrows). **d** Neurites grow through the layer of cells surrounding the incisor germ (yellow arrows). **e** Few neurites grow toward the labial cervical loop (yellow arrows). Signs of tooth degeneration are clear (red arrows). Scale bars: 500 μm for (**b**, **c**) and 200 μm for (**d**, **e**). **f**, **g** General setup and growth of neurites through microgrooves of the microfluidic device. **f** Schematic view of a microfluidic co-culture system device. **g** Both β-tubulin (green) and neurofilament (not shown) immunostaining show extensive neurite outgrowth toward the empty compartment of the device after 4 days of culture. Scale bars: 150 μm. **h**–**k** Co-culture of trigeminal ganglia and postnatal molars (M). **h** Schematic representation showing the disposition of TG and postnatal molars in the microfluidics chamber. The molar is oriented with the pulp facing the ganglion. **i** Molar (on the left) and TG after 10 days of culture. Red box: magnification in panel **j**. **j** Neurites (red arrowheads) grow toward the tooth germ (green line) and penetrate into the tooth germ. **k** Section of the cultured tooth germ. Neurofilament staining shows that neurites have contacted the dental pulp (yellow arrowheads). Scale bars: 2 mm for **i**, 200 μm for **j**, and 20 μm for **k**. Reproduced from [107], Copyright 2014, licensed under the terms of the Creative Commons Attribution License (CC BY)

dentin fragment to mimic the pulp–dentin interface and its interaction with different dental biomaterials. For designing this device, a positive template was laser-cut in a PMMA board using computer-aided design (CAD) software. Thereafter, the templates were molded with PDMS prepolymer and cured at 80 °C overnight using a soft lithography technique [69]. Figures 5a–5h portray the steps of developing a tooth-on-a-chip with a dentin fragment at the center to simulate the tissue interactions. This in vitro model can replicate the dentin–pulp interface, allowing the morphological, metabolic, and functional effects of dental biomaterials on pulp cells to be better understood [69]. To imitate the responses of pulp cells to dental biomaterials such as 2-hydroxyethyl methacrylate (HEMA), phosphoric acid (PA), and a dental adhesive (Adper-Scotchbond; SB) on-chip, the dentin surface was seeded with stem cells from the apical papilla (SCAPs) and observed using live-cell microscopy (Figs. 5i–5q) [69]. Standard dental biomaterials used clinically were compared to standardized off-chip controls by testing their cytotoxicity, cell morphology, and metabolic activity on-chip (Figs. 5q1–5q16) [69]. Using this tooth-on-a-chip platform enables future research to study the dental pulp cell response to biomaterials by replicating the near-physiological conditions of the pulp–dentin interface [69]. To appraise the differences between the on-chip model and standard off-chip models, the authors [69] replicated each experiment on both models. During a 7-day period, it was observed that the cells in the on-chip model decreased in number and showed visible morphological changes after 7 days compared to the first day (Figs. 5q2–5q4, 5q10–5q12), whereas in the off-chip model, cells that were treated with HEMA, PA, and SB were very few in number with faint cytoplasmic staining after 7 days (Figs. 5q14–5q16) [69]. Another considerable difference between the models was concerned with the type of reaction against the biomaterial

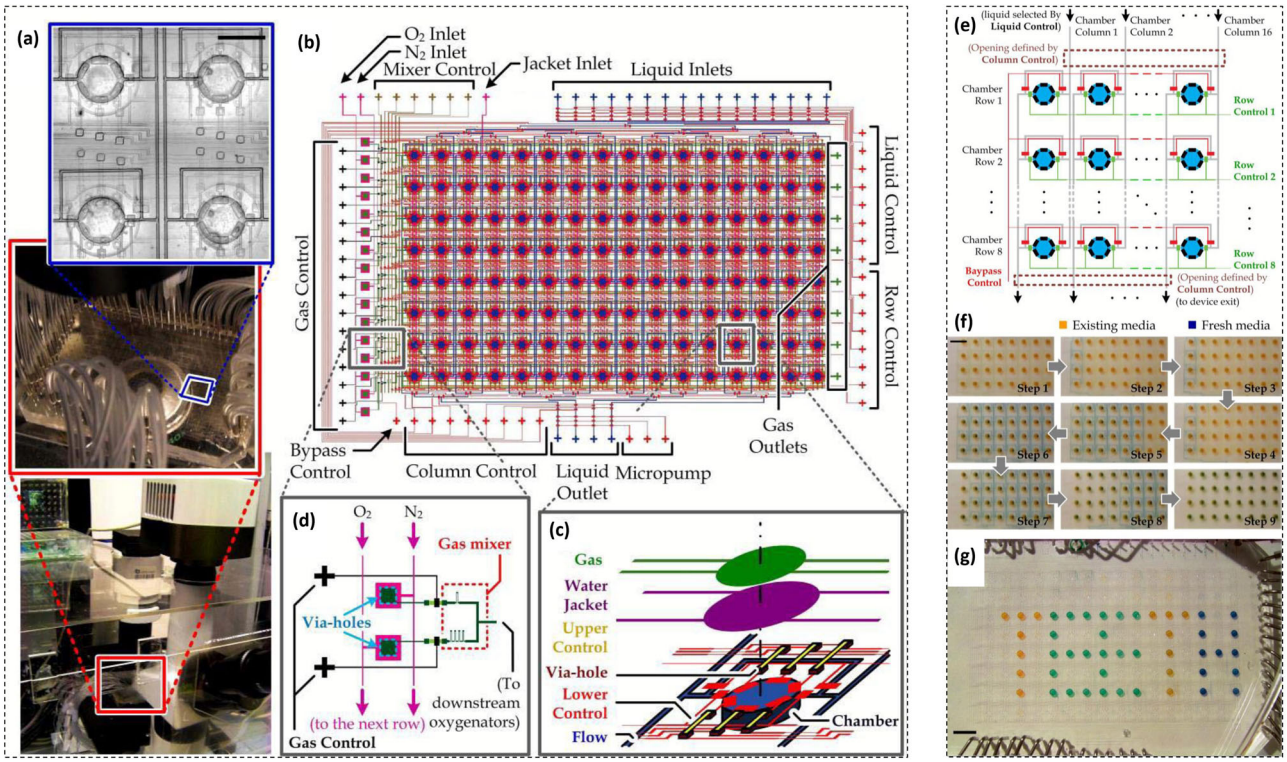


Fig. 3 **a** Fabricated microfluidic "artificial teeth" device placed on the automated microfluidic control platform. Red inset: zoomed-in photograph of the artificial teeth connected with tubing. Blue inset: micrograph of the culture regions. **b** Design layout of the 128-chamber artificial teeth chip. **c** Sketch of multiple structural layers in a culture region: gas channel (green) controlled by microvalves in the overhead gas control layer (not shown), water jacket (purple), upper flow control layer (yellow), via holes (dark red), lower flow control layer (red), flow channels (blue) and a culture chamber (lower blue). **d** The design of the gas micromixer includes two common gas inlet channels (purple) flowing with O_2 and N_2 to the mixer (green) through the gas via holes (cyan). The gas flow can be selectively gated by the "gas control" microvalves (black) located above the reflowed mixer inlet channel sections (light green). **e** Arrangement of culture chambers (blue), flow channels (gray), chamber control (red), row control (green), and micromixers (black). **f** The lower left quarter of the chamber array (rows 5–8 and columns 1–8) in artificial teeth during medium replacement procedures (steps 1–9) demonstrated with orange and blue dyes, representing fresh and old media, respectively. **g** Selected chambers of the device filled with different color dyes showing the word "TEETH." Reproduced from [71], Copyright 2016, with permission from The Royal Society of Chemistry

exposures [69]. The cells in the off-chip models were much more sensitive to HEMA exposure and 15 s of exposure to 35% phosphoric acid. The contributing factor to this observation was thought to be the lack of dentin, as a semipermeable barrier, and the cell matrix contacts with dentin for the dental pulp cells, which could have deviated the cells' response from the standard in vivo-like interactions and made them more vulnerable to external injuries [69]. Moreover, the application of such microdevices to simulate cell behaviors in the presence of dental biomaterials can provide opportunities since these biomaterials have illustrated various mechanisms to affect dental pulp cells, such as mechanical, chemical, and gradual diffusion through dentinal porosities, which elicit indirect reactions [69]. All the mentioned mechanisms of biomaterial interactions cannot be assessed by a single conventional in vitro model; nevertheless, on-chip models can imitate body conditions more realistically by combining the various mechanisms of biomaterial interactions with cells in a single study [69]. Furthermore, this study [69] has corroborated the possibility of performing the same clinical protocols, such as acid etching, and producing a hybrid layer in the in vitro setting by using on-chip models, which can be considered groundbreaking in the field of in vitro studies.

Rodrigues et al. [76] in 2021 developed another tooth-on-a-chip with a similar structure and fabrication method to a previous study [69] to compare the effect of three different types of calcium silicate cement (TheraCal, Biodentine, and ProRoot) on the viability and proliferation of dental pulp stem cells (DPSCs), and test the pH variations and the release of transforming growth factor–β (TGFβ) within the pulp chamber as a substitute for the bioactive dentin matrix molecules [76]. Additionally, a biofilm of *S. mutans* was established on this chip to test the antimicrobial capability of ProRoot on the biomaterial–biofilm–dentin interface in real time through a live and dead assay [76]. The results of cell culture revealed that those cells that were treated with ProRoot and Biodentine

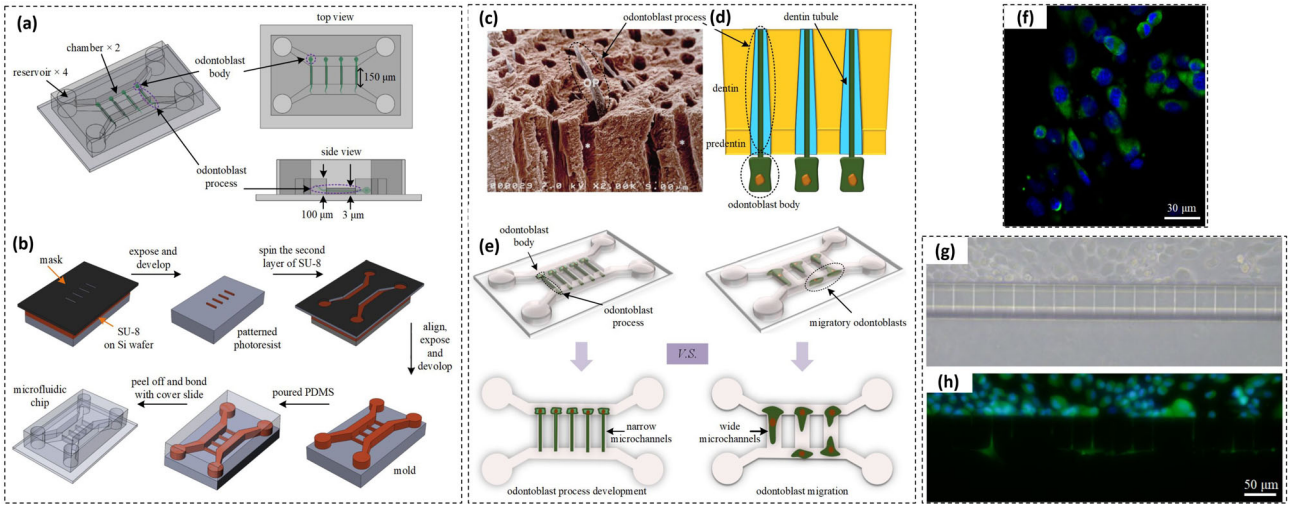


Fig. 4 **a**, **b** Design and fabrication process of the microfluidic chip. **a** Structure of the microfluidic chip. The chip consists of four reservoirs, two chambers, and hundreds of microchannels. **b** The fabrication process of the microfluidic chip. **c–e** Schematic of an odontoblast process and odontoblast microfluidic chip. **c** Scanning electron microscopy (SEM) image of an odontoblast. An odontoblast process is an extension of the odontoblast that stretches into the dentin tubule. **d** Illustration of an odontoblast process and its surrounding microstructure. **e** Schematic of the microfluidic chip for odontoblast process growth. Narrow microchannels with an appropriate size can induce the growth of odontoblast processes. However, wide microchannels will lead to the migration of odontoblasts. **f–h** Characterization of the odontoblast marker protein Aquaporin-4 (AQP4). **f** Immunofluorescence image of odontoblasts after staining with AQP4 (green). The cells were also stained with 4′,6-diamidino-2-phenylindole (DAPI) to visualize the nuclei (blue). The odontoblasts were cultured and stained in a petri dish. **g** Optical microscope image of odontoblasts in the chip. **h** Immunofluorescence image of odontoblasts cultured in the chip. Reproduced from [70], Copyright 2019, with permission from American Chemical Society

were similar in density and shape to the control group after 7 days, while cells in the TheraCal group presented atypical morphology and were fewer in number [76]. Moreover, all three types of calcium silicate cement showed a statistically similar increase in PH after 48 h, 72 h, and 7 days, and all were higher than the control group [76]. Additionally, ProRoot and TheraCal induced the highest and the lowest release of TGFβ from the dentin treated with each of them, respectively, throughout the experiment [76]. Furthermore, the results of the ProRoot effect on the *S. mutans* biofilm showed that most of the bacteria were killed due to exposure to calcium ions released from ProRoot and the high-pH microenvironment created by this material [76]. This microfluidic tooth-on-a-chip system enabled direct characterization of biomaterial interactions with dentin, pulp cells, and live biofilms that were previously unattainable [76].

Recently, Hu et al. [79] in 2022 established a microfluidic tooth-on-a-chip to analyze the cytotoxic potential of silver diamine fluoride (SDF) on DPSCs under flow conditions. This tooth-on-a-chip model was designed using CAD software; then, it was fabricated by thermal bonding of four microstructured PMMA sheets in a vacuum oven [79]. By using computer numerically controlled (CNC) micromilling, chambers and microchannels were microfabricated within the PMMA sheet [79]. In this device, there was a central cylindrical chamber (dentin chamber) to hold the dentin disk. Additionally, beneath the central chamber, there was a perfusable rhomboid-shaped microchannel ("pulp channel") that had circular openings at either end (inlet and outlet ports) to seed cells and perfuse reagents by using tubings connected to a peristaltic pump (Figs. 6a–6c) [79]. On this device, DPSCs were evenly seeded throughout the length of the pulp channel (Fig. 6d), and the dentin disks (prepared in three different thicknesses of 0.5, 1.0, and 1.5 mm) were placed within the microchamber [79]. First, the culture medium was perfused through the pulp channel; then, SDF was directly added on top of the dentin disk. Thereafter, the culture medium was replaced with medium containing calcein acetoxymethyl ester (calcein-AM) and propidium iodide to stain the live and dead cells, respectively [79]. As there was a unidirectional flow in this device, those cells that were seeded in the inlet side of the pulp channel were unexposed to SDF and, therefore, were used as an internal control for comparison of their viability with those cells seeded in the outlet side of the pulp channel (exposed to SDF) (Figs. 6e–6h) [79]. The results showed 100% cell death for dentin disks with 0.5 mm and 1.0 mm thicknesses that were exposed to SDF in the outlet channel compared with >90% cell viability in the inlet channel (Figs. 6i and 6j) [79]. In contrast, the 1.5 mm dentin disks revealed (30.6±8.6)% cell viability in the outlet channels (Fig. 6i); however, the viable cells showed a

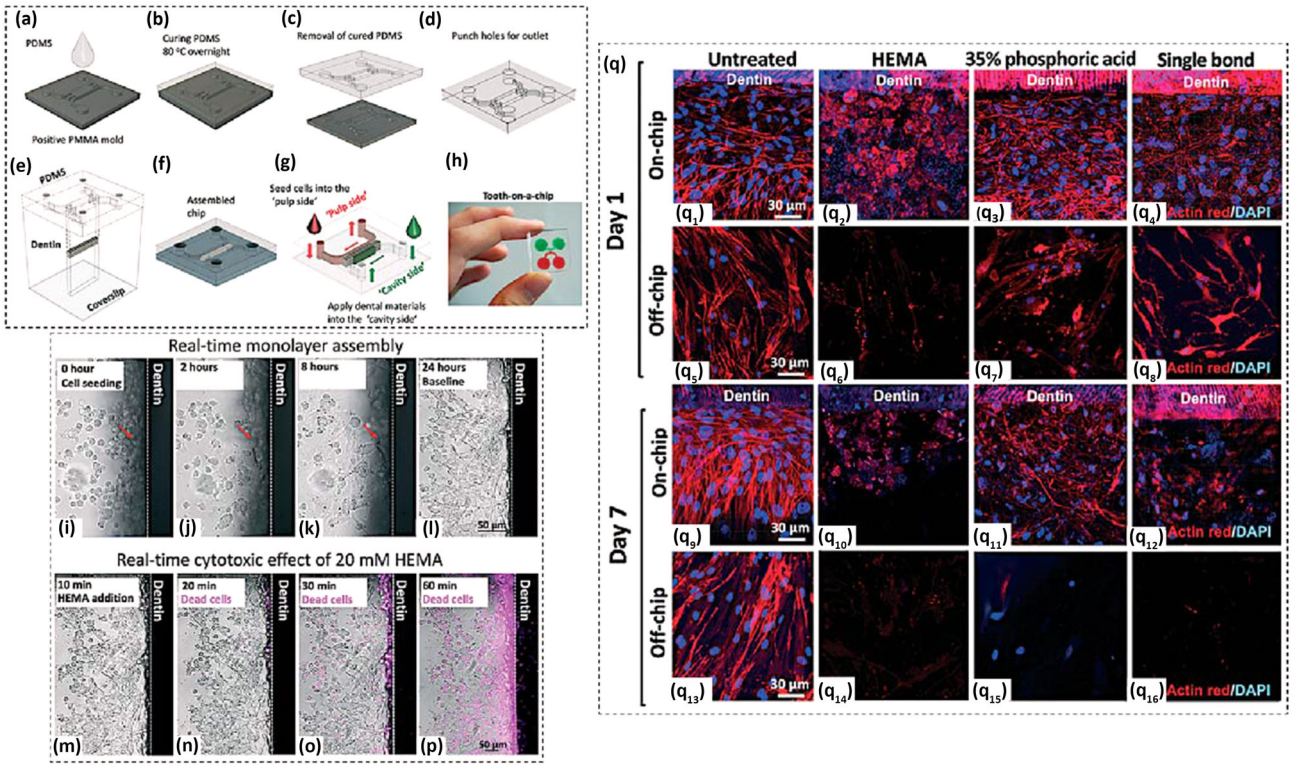


Fig. 5 **a**–**h** Protocol of tooth-on-a-chip production. First, polydimethylsiloxane (PDMS) is applied to the polymethyl methacrylate (PMMA) mold (**a**). After curing PDMS at 80 °C overnight (**b**), the PDMS is separated from the PMMA mold (**c**). To fabricate reservoirs, several holes are made in the PDMS (**d**). Then, the PDMS and the coverslips will be treated with plasma, and a dentin fragment will be placed between the two chambers at the center (**e**). The system is finally assembled (**f**). The two chambers represent the pulp side and the cavity side of the dentin (**g**, **h**). **i**–**p** Live-cell imaging on-chip. A total of 10^5 stem cells from apical papilla (SCAPs) were seeded on-chip (**i**) and spread in 2 h (**j**) to 8 h (**k**). After 24 h, the monolayer was completely formed (**l**). Arrows show the morphological changes of a single cell, which goes from being round and unattached to a spread morphology as it attaches to the dentin wall after 8 h (**i**–**k**, arrow). Cells cultured on-chip after 24 h were incubated with a DNA dye (Helix NP NIR) and imaged, demonstrating initial cell viability near 100% (**m**). Next, 20 mmol/L 2-hydroxyethylmethacrylate (HEMA) was added to the "cavity side" of the chip, and after 10 min of incubation, the cells still showed high viability (**n**). After 30 min, almost 50% of cells had their nuclei stained, which is suggestive of cell death (**o**). After 60 min, nearly all cells were not viable (**p**). $\mathbf{q}_1$–$\mathbf{q}_{16}$ SCAP morphology after biomaterial treatment on-chip and off-chip. On day 1, untreated samples ($\mathbf{q}_1$) had SCAP monolayers that were morphologically stable for at least 7 days, while the HEMA ($\mathbf{q}_2$), phosphoric acid (PA) ($\mathbf{q}_3$), and Adper-Scotchbond (SB) ($\mathbf{q}_4$) groups showed significant cell morphology changes and decreased cell numbers on-chip. Cells cultured off-chip on day 1 showed polygonal morphology with oval nuclei in the control group ($\mathbf{q}_5$), and almost no cells were visible after HEMA treatment ($\mathbf{q}_6$). Severe cytoplasmic changes and apparently fragmented nuclei were observed in the PA ($\mathbf{q}_7$) and SB ($\mathbf{q}_8$) groups. On day 7, untreated cells ($\mathbf{q}_9$) on-chip showed little change from day 1, while samples treated with HEMA ($\mathbf{q}_{10}$), PA ($\mathbf{q}_{11}$), and SB ($\mathbf{q}_{12}$) had visible morphological changes. Cells cultured off-chip that were not treated ($\mathbf{q}_{13}$) showed confluent monolayers, whereas HEMA ($\mathbf{q}_{14}$), PA ($\mathbf{q}_{15}$), and SB ($\mathbf{q}_{16}$) had very few cells with faint cytoplasmic staining. Reproduced from [69], Copyright 2020, with permission from Royal Society of Chemistry

rounded morphology compared with the elongated spindle-shaped morphology of the cells in the inlet channel [79]. The results of this microfluidic tooth-on-a-chip showed that SDF was cytotoxic to the DPSCs at very low concentrations (0.001%) and could penetrate the dentin with a low thickness ($\leq$1.0 mm), which resulted in cell death in vitro [79]. This microfluidic device enabled the culture of cells under dynamic flow conditions and impersonated the pulp–dentin interface as well as the assessment of biomaterial permeation through a dentin barrier under flow conditions [79].

Oral mucosa-on-a-chip

Rahimi et al. [73] in 2018 developed another co-cultured microfluidic system, a mucosal model on-chip, for rapid evaluation of layer-specific responses to typical challenges present in the oral environment. This system can lead to a more thorough understanding of mucosal homeostasis and pathological conditions [73]. The fabrication method used for this device was soft lithography [73]. The mold of this microfluidic device was custom fabricated on a silicon wafer using conventional photolithography with a spin coater and an exposure masking system. Then, PDMS was poured on

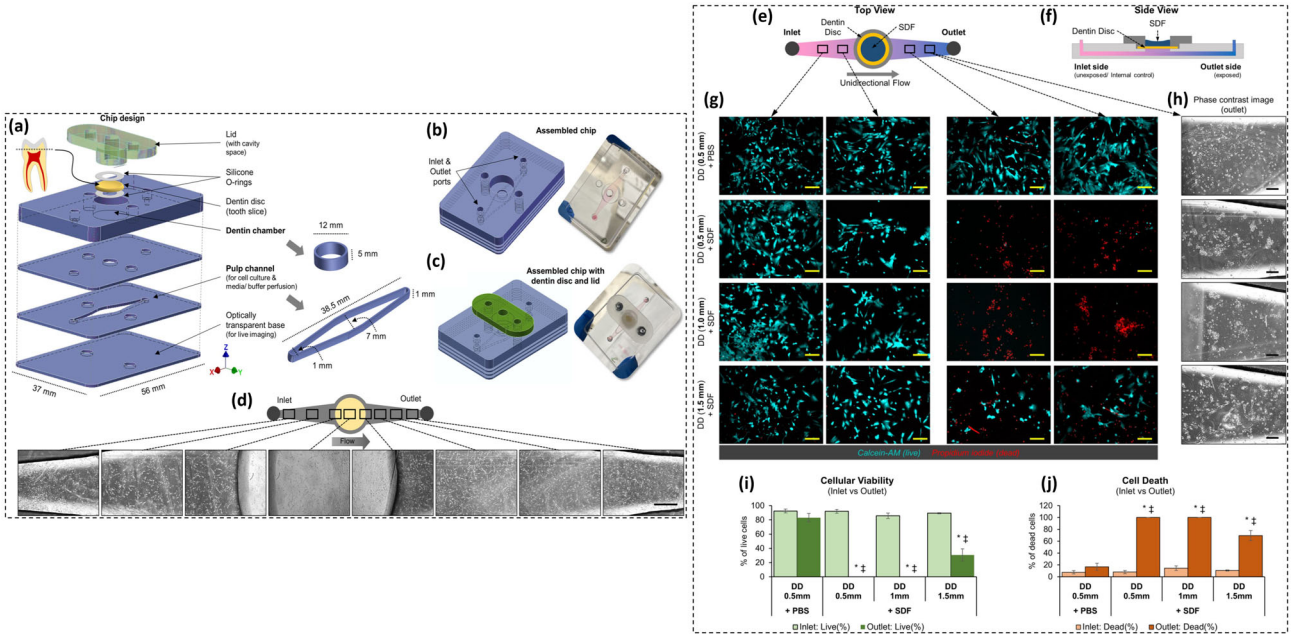


Fig. 6 **a**–**d** Schematic illustration showing the features of the microfluidic tooth-on-a-chip device. The exploded view (**a**) and the assembled device (**b**, **c**) show the different features of the device, including an optically transparent base, pulp channels, dentin chamber, removable lid, and ports for inlet and outlet. **d** The sequence of phase contrast images shows the ability to visualize the cells and the even distribution of dental pulp stem cells (DPSCs) cultured across the pulp channel and under peristaltic flow conditions. **e**–**j** Effect of silver diamine fluoride (SDF) on DPSCs cultured under flow and dentin barrier conditions. Schematic illustration demonstrates the working principle of the tooth-on-a-chip device as seen from the top (**e**) and side (**f**) views. The sequence of fluorescent (**g**) and phase contrast (**h**) images from the inlet and outlet sides of the pulp channel show DPSCs stained with calcein-AM (live, cyan) and propidium iodide (dead, red). The bar graphs show the percentage of live (**i**) and dead (**j**) cells within the inlet and outlet sides of the pulp channel upon exposure to SDF and different dentin disk (DD) thicknesses. Data presented as the mean±standard deviation (SD), n=3, *p<0.05 (inlet vs. outlet), ‡p<0.05 (phosphate-buffered saline (PBS)-exposed vs. SDF-exposed dentin disks). Reproduced from [79], Copyright 2022, with permission from The Academy of Dental Materials

top of the mold in a homemade aluminum foil container, degassed in a vacuum chamber, and cured at 65 °C for 4 h [73]. Assembled in its central channel with interconnecting pores, the three-channel microfluidic chamber comprised a gingival fibroblast-laden collagen hydrogel, followed by a keratinocyte layer attachment to the collagen exposed in the pores (Figs. 7a–7c) [73]. The mucosal construct was exposed to HEMA, a dental monomer, and a prevalent oral bacterium, *S. mutans* (Figs. 7f–7o) [73]. The results of this study [73] showed that exposure to HEMA decreased cell viability, whereas exposure to *S. mutans* reduced transepithelial electrical resistance (Figs. 7f–7h and 7o). The authors of the current study [73] have noted that the possible advantage of the on-chip model was its ability to observe keratinocytes, fibroblasts, and labeled signals in one outlook. Moreover, this system enabled the researchers to monitor the flow of fluid in luminal channels. The potential benefit of this flow in the luminal channels is the ability to mimic the flow of the saliva or the gingival crevicular fluid that can slough keratinocytes and stabilize the epithelial layer morphology [111].

In this regard, to assess the responses of gingival fibroblasts and keratinocytes to varied HEMA concentrations (twofold serial dilutions ranging from 1.56 to 25 mmol/L), Ly et al. [72] in 2021 conducted a study on the same mucosal model on-chip and compared it to the conventional well plate monoculture. The mucosal model on-chip in this study [72] was designed with a similar fabrication method, structure, and cell culture to the previous study [73]. In a conventional well plate monoculture in this study [72], keratinocytes and fibroblasts were cultured separately in 96-well plates and exposed to a twofold serial dilution of HEMA. To evaluate the efficacy and the possible advantages of this mucosa-on-a-chip model compared to the conventional model utilized in this study [72], the authors [72] have stated that one of the shortcomings of traditional models is the lack of ability to simulate multilayer cell interactions, which limits the chance of assessing layer-specific cell responses. In contrast, mucosa-on-a-chip models offer a more accurate multilayered architecture of cells, as well as accessibility to layer-specific cell information [72]. The results of this study [72] have

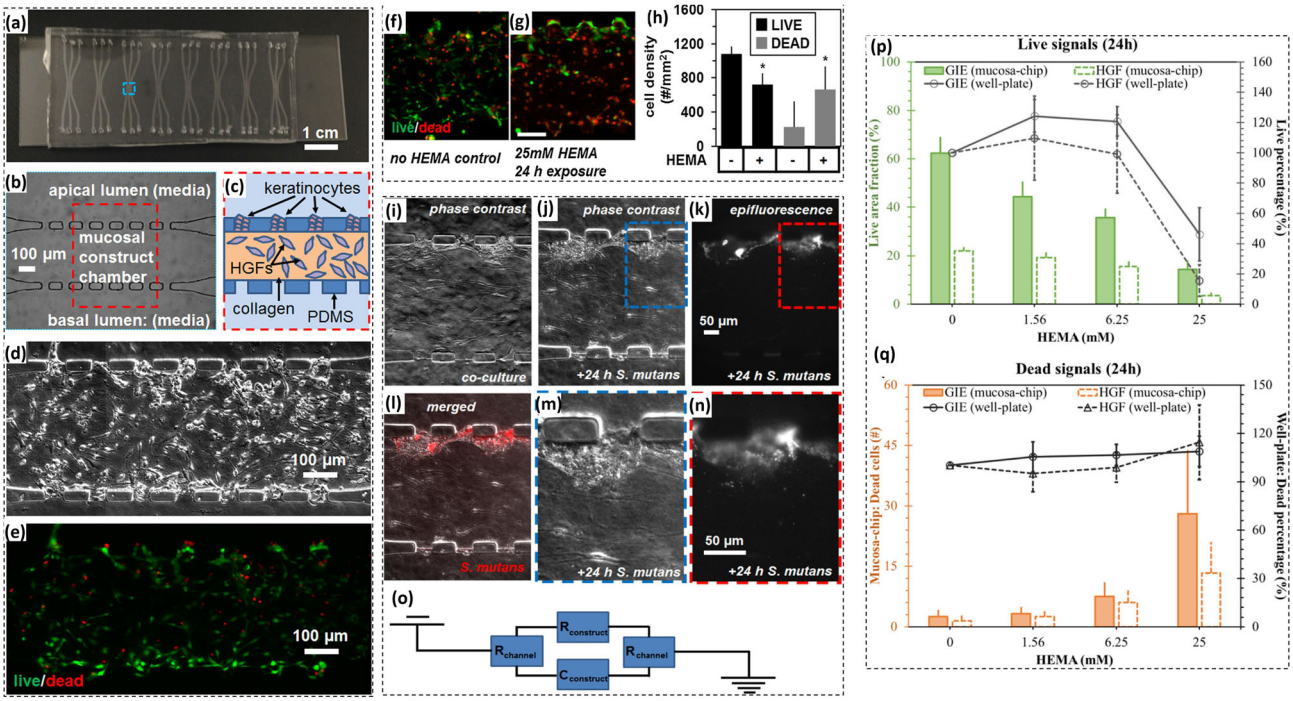


Fig. 7 **a**–**e** Configuration of the microfluidic device and tissue construct. **a** A photograph of the chip containing eight three-channel devices. **b** A phase contrast micrograph from the blue dashed box in **a**, and **c** schematic of the construct culture chamber from the red dashed box in **b**, showing channel and pore microstructure and cell placement. Mosaicked phase contrast (**d**) and epifluorescence (**e**) micrographs of the live-dead stained co-culture, showing the morphology of cells, viable cells in green, and dead cell nuclei in red. **f**–**h** Effect of 2-hydroxyethylmethacrylate (HEMA) on the layer viability of the mucosa-on-a-chip. Merged epifluorescence micrographs of calcein-AM (live, green) and ethidium homodimer (dead, red) signals before (**f**) and after (**g**) 24 h of exposure to 25 mmol/L soluble HEMA. **h** The density of live and dead cells was counted within each condition. **i**–**o** Exposure of the mucosa-on-a-chip to *Streptococcus mutans*. **i**, **j** Phase contrast and **k** epifluorescence micrographs of microfluidic constructs before (**i**) and after (**j**), (**k**) 24 h culture of intravitally stained (DiI) *S. mutans* in the apical channel adjacent to keratinocytes. **l** Overlay of phase contrast (greyscale) and epifluorescence (red signal, DiI fluorescence) micrographs from **j** and **k**, respectively. **m** Zoomed-in region of **j**. **n** Zoomed-in region of **k**. **o** The equivalent circuit of the device is used to measure transepithelial resistance, connected across the tissue construct from the luminal to basal channels, including components contributing to resistance (R) and capacitance (C). **p**, **q** Comparison of **p** live signals and **q** dead signals at 24 h between the mucosa-chip (first vertical axis) and the well-plate model (secondary vertical axis). **a**–**o** are reproduced from [73], Copyright 2018, with permission from AIP Publishing. **p**, **q** are reproduced from [72], Copyright 2021, under exclusive licence to Springer Science+Business Media

shown that mucosa-on-a-chip models exert higher sensitivity to HEMA than conventional models, especially at lower doses, such as 1.56 mmol/L and 6.25 mmol/L, which is considered to be another potential advantage of in-chip models (Figs. 7p and 7q).

These studies [72, 73] suggest that the mucosal model on-chip is a promising system that contains a multilayered tissue configuration of the oral mucosa, imitates the physiological environment of the oral mucosa interface, and permits direct observation of the responses of mucosal cells to oral bacteria and dental materials.

In another study, in 2016, Kang et al. [74] investigated the interaction between oral cells during differentiation through a microfluidic system for indirect co-culture of stem cells from human exfoliated deciduous teeth (SHEDs) with human gingival fibroblast (hGF) and periodontal ligament stem cell (PDLSC) culture media. This microfluidic co-culturing device was designed for constant perfusion culture and organizing a concentration gradient [74]. The device contains gradient-generating parts, including two reservoirs with a micropump and 32 cell culture chambers that were designed for cell exposure to different culture media (Figs. 8a and 8b) [74]. Multiple cell culture chamber arrays and media reservoirs in this device were formed using a soft lithography technique of PDMS [74]. In this study [74], two groups of microfluidic devices (each containing three devices) were designed, and in all of these devices, SHEDs were cultured in chambers. In one group, reservoir 1 was filled with media cultured with hGFs, and in the other group, reservoir 1 was filled with media cultured with PDLSCs (Figs. 8c–8e) [74]. Reservoir 2 in both groups was filled with fresh media, and in this way, a concentration gradient for culture media was made through all the devices (Fig. 8c) [74]. In each of the

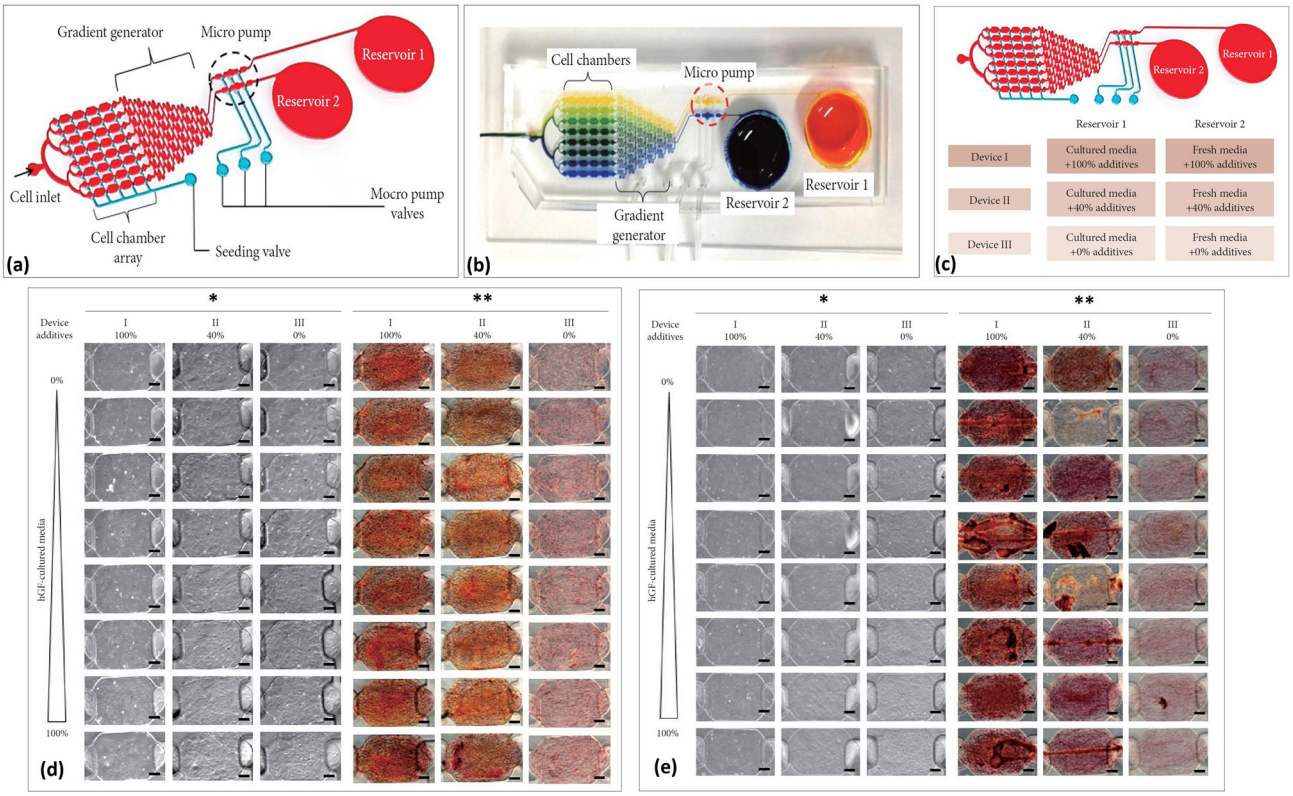


Fig. 8 **a** Schematic diagram of the microfluidic system used for the co-culture method. This device is mainly composed of two parts: multiple cell culture chambers and media reservoirs. **b** An actual photograph of a microfluidic co-culture system. Red and blue dyes in reservoirs 1 and 2, respectively, show a gradient. **c** Media composition used in indirect co-culture. Reservoirs 1 and 2 were filled with media cultured with cells and fresh media, respectively. Three devices were prepared for three different concentrations of mineralization additives. **d**, **e** Mineralization efficiency of stem cells from human exfoliated deciduous teeth (SHEDs) under treatment with mineralization additives and media cultured with human gingival fibroblasts (hGFs) and human periodontal ligament stem cells (hPDLSCs), respectively. One column from each of the three devices was selected, and mineralization efficiencies were compared. *microscopic phenotypes of SHED cultured in microfluidic cell chambers; **alizarin red-S staining to detect mineralization. Reproduced from [74], Copyright 2016, with permission from The Korean Tissue Engineering and Regenerative Medicine Society

three devices in both groups, a specific concentration of differentiation additives (0%, 40%, or 100%) was added to the culture media in reservoir 1 (Fig. 8c) [74]. The study [74] showed that there was no difference in the mineralization activity of SHEDs treated with different concentrations of either hGF or PDLSC culture media. However, the levels of osteoblast gene expression were lower in SHEDs incubated with hGF media than in SHEDs incubated with PDLSC media (Figs. 8d and 8e), suggesting that indirect co-culture of SHEDs with hGF media culture may inhibit osteogenic cytodifferentiation. Moreover, the level of mineralization in SHEDs treated with 100% differentiation additive was higher than 40% and 0%, respectively, in both groups [74]. This co-culture microfluidic system allows the use of small amounts of cells and reagents, and the results can be obtained quickly and easily [74]. In this regard, this device enables high-throughput drug screening by constructing a linear gradient of drugs on a microscale [74].

The utilization of TOOC models compared to conventional models has been justified in various studies [10, 72, 74, 107]. Since these systems can simulate cell–cell interactions much more like body conditions, their results can be thought of as more reliable than conventional models; future studies are required to confirm these outcomes. Moreover, these studies [10, 72, 74, 107] indicate that on-chip models have the potential to assess the effect of several independent factors. For instance, in the study by Kang et al. [74], the optimized concentration of hGFs and human periodontal ligament stem cells (hPDLSCs) cultured with SHEDs was appraised, while the proper concentration of differentiation additives was estimated in the same study. This suggests that we might obtain multiple outcomes as a result of the high potential of on-chip models in a more accurately designed study platform.

Salivary gland-on-a-chip

Salivary gland (SG) dysfunction due to radiation therapy, for instance, leads to several problems, such as xerostomia. Therefore, new in vitro devices that enable investigations on SG functions and drug screening are of utmost importance. SG tissue-on-a-chip by mimicking the function of SGs and allowing high-throughput drug screening helps find new therapeutic strategies for SG dysfunction [75]. Song et al. [75] in 2021 designed a modular SG tissue-on-a-chip platform to evaluate the function and radiosensitivity of acinar cell clusters and intercalated ducts isolated from humans and mice. In this study [75], by using microbubble (MB) array technology with matrix metalloproteinase (MMP)-degradable PEG hydrogels, a supportive microenvironment for SG tissue culture was provided. MB well arrays were fabricated in PDMS by gas expansion molding [75]. In this fabrication method, PDMS was poured on top of a silicon wafer template consisting of etched cylindrical pits and cured at 100 °C for 2 h. MB wells were uniformly formed over each pit by the expansion of air through the pits [75]. The spherical architecture of the MB yields a unique microenvironment for long-term culture, autocrine/paracrine conditioning, and transparency to enable in situ imaging [75]. In a previously mentioned study [75], engineered extracellular matrices were combined with MB arrays to support isolated SG cells. The immunostaining results indicated that this SG tissue-on-a-chip contained all major types of SG cells, including myoepithelial, duct, and acinar cells [75]. It was shown that these cells were viable over the 14 days of analysis, expressed key salivary gland markers, and displayed polarized localization of functional proteins [75]. This SG tissue-on-a-chip can be employed to study radio-protective drugs in a high-throughput manner and enables large-scale studies that currently are prohibitive in in vivo experiments [75].

Recent studies on microfluidic TOOCs have shown that these devices provide a unique microenvironment for more investigations on dental and oral cells and tissues owing to their ability to compensate for several limitations of conventional in vitro and/or in vivo studies.

The limitations of microfluidic TOOCs and their possible solution

Although microfluidic devices offer several benefits over conventionally sized systems, there are some general limitations concerning their usage.

The unnecessary interactions and interventions in construction materials of microfluidics may influence the reaction processes and the related outcomes [9]. In the fabrication process, there are several technical issues causing flow perturbations that can have negative effects on cell culture and cell contamination, especially in porous microfluidic devices. Some solutions, such as gravity-driven flow and rocker platforms, have been suggested to solve issues related to flow perturbations [24, 112]. Additionally, since PDMS is the most commonly used material for the fabrication of TOOCs, another challenge is to control specific gas pressures in the system, as PDMS has high gas permeability properties. Therefore, controlling the gas concentrations is crucial when using PDMS-based devices.

Another challenge is that many physical and chemical factors become dominant due to the dimensions of microfluidics, which may cause some problems [9, 113]. For instance, the dominance of surface effects at the microscale causes the adsorption of molecules of interest on the inner linings, which subsequently may affect the quality of the analysis [9, 113]. This is another important limitation of PDMS-based TOOCs since these devices are selective adsorbents of molecules from the culture media, which in turn reduces their effective concentrations and ability to affect cells. This is of utmost importance in drug efficacy or toxicology studies [114, 115]. The following are possible solutions to overcome this challenge. The surfaces of the PDMS channels were coated and materials other than PDMS (e.g., glass, PS, and COC) were used to prevent adsorption as well as to control the gas pressure [114].

In addition, the size of the TOOCs is generally similar to the standard microscope glass slide, 75 mm×25 mm (3″ by 1″), and the volume of the flow in micrometer channels in these devices is restricted to only 10^{-9} L–10^{-18} L [116, 117]. Therefore, compared to conventional 2D or 3D cell culture systems, the scalability of those microfluidic platforms can hinder their wider applications [116]. Additionally, due to the small number of cells used in microfluidic devices, some choices for experimental assays are limited as well.

Furthermore, in microfluidic dimensions, the flow into the chip is always laminar. Although laminar flow allows for accurate experimental control, the fluid particles in laminar flow follow smooth paths in parallel layers, so they cannot laterally mix with adjacent layers when required [101, 113, 118]. In this regard, changing the mass and concentration of the fluid particles might be a possible solution for mixing the fluid particles, as it leads to diffusion through the interface between the molecules of the fluid layers [24].

Microfluidic TOOCs are also not decent tools for some specific areas, such as responses to endocrine systems or nervous systems [9]. In addition, there are many complexities in tissues/organs that cannot be simulated by microfluidic systems alone. Hence, the integration of other supporting technologies, such as detection systems for electrochemical transductions, electrochemical monitoring, biomarkers, genomics, proteomics, and lipidomics, is essential to support microfluidic TOOCs [9].

Alongside the aforementioned general limitations, there are some specific limitations regarding the application of microfluidic TOOC devices in the field of oral and craniofacial biology. While microfluidic devices are engineering-focused, the simulation of tissues/organs is related to biomedicine [9]. Therefore, researchers will encounter some difficulties in the integration of these two fields [9]. Scientific collaboration between oral biologists and biomedical engineers can be a possible solution to address the associated problems.

These devices also imitate only a subset of histological sections of particular tissues/organs rather than complex microenvironments [119]. Since most interfaces in the oral and craniofacial regions are comprised of soft and hard tissues [120], there is a need for TOOCs that mimic the multi-tissue hard/soft constructs of this region. The potential solution for addressing the aforementioned issues would be the use of advanced human-on-a-chip (HOC) or multi-tissue/organ-on-a-chip (multi-TOOC), which includes several human tissues/organs that are organized in a hierarchical and physiological pattern, making an in vitro human model [121–124]. In this regard, HOC can recapitulate the structure and function of the human system and overcome the limitations of single-TOOC, including the reactions of the immune system and the interactions of the oral and craniofacial regions with other tissues/organs.

Oral tissues/organs are also constantly subjected to mechanical forces due to mastication, speaking, etc. However, the current oral TOOCs do not replicate such important physiological functions. In this regard, other microfluidic TOOCs (e.g., lung-skin-, and gut-on-a-chip) can add mechanical strain to their devices by recreating specific artificial movements [124, 125]. The ability to implement in vivo-like strains in cell culture environments will enable a precise and thorough investigation of the mechanobiology of tissues/organs exposed to strain [126]. Since the types, magnitude, and frequency of these strains are capable of influencing the morphology and metabolism of cells [126], it is of utmost importance that more advanced microfluidic devices that mimic the mechanical strains of natural oral tissues/organs be developed in future studies. Moreover, to imitate the mechanical forces on the natural oral environment, the material used must be flexible enough without fatigue [127]. Elastomeric materials, including PDMS, polyurethane and styrene block copolymers, have been shown to be best suited for such applications [127].

It should also be noted that there are a limited number of studies investigating the application of microfluidic TOOC in the field of oral and craniofacial biology [69–76, 79, 107]; therefore, further studies are needed to explore and define specific problems in this field.

Future perspectives

The goal of these initiatives is to allow robust, precise, and predictable in vitro assembly and functional morphogenesis of 3D human oral microtissues and organs through the development of tools and technology. These microfluidic TOOCs will mimic native oral tissue and organ (tooth-, oral mucosa-, salivary gland-on-a-chip, composite assemblies, etc.) architecture, organization, multi-tissue interphases, physiology, and disease pathology. The mimicking of the main features of native tissues and organs will generate 3D systems that surpass the levels of functionality of conventional 2D monolayer cultures grown on flat surfaces. Co-cultured microfluidic chips can also be a valuable tool for studying the interaction of oral tissues/organs with various dental biomaterials and oral bacteria under combinations of specified parameters to mimic the same conditions of the oral cavity [73]. In addition, microfluidic tissue/organ chips can address mechanistic questions of the health and disease of oral tissues and organs through their functionality. They will also improve the ability to predict the identification of new therapeutic targets for these diseases and the development of precision medicine-based drug efficacy and toxicity testing approaches focused on specific patient populations. This notion further suggests the potential applications of TOOCs for personalized medicine/dentistry. Induced pluripotent stem cells (iPSCs), which can be easily derived from a patient's human oral and craniofacial tissues [128, 129] or be directly obtained as pathogenic cells from patients [130], can be used to develop personalized tissues or disease models. Hence, iPSC-integrated TOOCs would provide a useful tool to create personalized drug testing platforms that can impersonate oral, dental, and craniofacial physiology tuned for specific patient groups/individuals.

Moreover, the dental field could implement or learn from TOOCs designed for other tissues and organs. For instance, in a study [131], a heart-liver-skin-on-a-chip system was designed to assess the toxicity of drugs that are topically administered. Similarly, novel systems could be innovated to assess the safety of topical drugs used for the treatment of oral and maxillofacial diseases. Furthermore, to the best of our knowledge, no specific TOOC has been introduced to investigate mandibular and maxillary bones thus far. Current bone-on-a-chip devices could answer biological questions about bone cell function, bone regeneration, vasculature and innervation, and cancer metastasis to bone [132]. Future TOOCs in the dental field could be developed to investigate craniofacial bones and help to answer similar and further biological questions. Moreover, several organ-on-a-chip devices have been designed to study cancers in recent years [133]. The innovation and application of these devices as cancer modeling tools would be of great benefit to studying oral

cancers and thus decrease the need for using animal models in future research.

Conclusions

Based on the current published studies, the development of 3D culture systems, also known as microfluidic TOOCs, can provide further opportunities to mimic the physiological and mechanical conditions of the body. Therefore, these systems can be employed as standard in vitro models in the future. 3D culture systems can be used to simulate signaling pathways, drug interactions, disease models, and tissue/organ functions more accurately. Likewise, the employment of these in vitro models in dental research can provide new insights for scientists regarding cellular behavior and the precise modeling of the tissue/organ response to new medications and biomaterials.

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Declarations

Conflict of interest LT is an Associate Editor of *Bio-Design and Manufacturing*. The authors declare that they have no conflict of interest.

Ethical approval This study does not contain any studies with human or animal subjects performed by any of the authors.

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