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Engineering Oral Microenvironments Using Microphysiological Systems

Yuji Nashimoto,[1*] Takeshi Hori,[1] Serge Ostrovidov,[1]
Sayaka Katagiri,[2] and Hirokazu Kaji[1**]

[1]Institute of Biomaterials and Bioengineering (IBB), Tokyo Medical and Dental University (TMDU),
2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, Japan
[2]Department of Periodontology, Graduate School of Medical and Dental Sciences, TMDU
1-5-45 Yushima, Bunkyo-ku, Tokyo 113-0034, Japan

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A deeper understanding of oral homeostasis and a precise evaluation of the effects of foreign materials are crucial for the oral health and total quality of life. Microphysiological systems (MPSs) or organs-on-chips have emerged over the past decade as attractive tools for emulating the physiological functions of organs *in vitro*. An MPS can pattern cells and matrices in a tiny microfluidic chip, which realizes *in vivo*-like cellular interactions and biochemical and physical forces. From this review, we summarize recent developments in MPSs that mimic the oral microenvironment. Oral MPSs can effectively evaluate the toxicity of dental materials in the oral cavity and recapitulate morphogenesis in the oral microenvironment. Oral MPSs are valuable tools for screening dental materials and understanding the development of oral tissues.

1. Introduction

The oral cavity is a unique microenvironment in the body. It is constantly exposed to foreign substances, including bacteria and viruses, and there are many dental care materials for oral diseases including dental caries. Since oral health is a key factor in total health and the quality of life, it is important to study the homeostasis of the oral microenvironment and the effects of foreign materials on the oral cavity.

Oral dysbiosis is an imbalance of oral flora. The breakdown of the oral epithelial barrier causes the breakdown of systemic homeostasis. Oral dysbiosis due to periodontal disease has been correlated with type 2 diabetes and obesity, suggesting that oral dysbiosis may affect metabolism. We previously reported that mice infected with *Porphyromonas gingivalis*, a representative periodontopathic bacterium, showed increased body weight, impaired glucose tolerance, insulin resistance, liver steatosis,[(1)] and lipid accumulation in skeletal muscles.[(2)] In addition, pregnant mice infected with *P. gingivalis* delivered low-birth-weight pups compared with uninfected control mice.[(3)] However, the mechanism by which oral bacteria invade vessels and reach other organs remains unclear.

*Corresponding author: e-mail: nashimoto.bmc@tmd.ac.jp
**Corresponding author: e-mail: kaji.bmc@tmd.ac.jp
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Microphysiological systems (MPSs) or organs-on-chips are attractive tools for studying physiological and pathophysiological mechanisms in the oral microenvironment.[4] MPSs can incorporate cell-cell interactions and physical forces, including physiological levels of fluid shear stress, cyclic strain, and compression. MPSs are often made of polydimethylsiloxane (PDMS), which is optically transparent with no apparent cytotoxicity. PDMS has functional elastic properties and can be applied to accurately replicate molds up to the nanoscale for molding during microfabrication.[5,6] Using materials such as PDMS, it is possible to fabricate MPSs with microscale-width channels and posts. MPSs with perfusable 3D vascular networks have been constructed using endothelial cells.[7,8] In addition, the use of two-stage MPSs including a sandwiched porous membrane allows the culture of different types of cells on the upper and lower surfaces of a membrane. This enables the recapitulation of the functional interfaces of organs, such as the alveolar–capillary interface and the blood–retina barrier interface.[9,10]

In this review, we introduce the latest MPSs that mimic the oral microenvironment. We consider MPSs from two aspects (Fig. 1): the effects of dental materials and inflammation in the oral and physiological environments, especially morphogenesis in the oral microenvironment. Although commercially available Transwell and other mechanical simulation systems have been developed as oral models, they are not the focus of this review. Other recent reviews detail these systems.[11–13]

2. Effects of Dental Materials and Inflammation in Oral Microenvironment

Biomaterials used in tooth treatment are in contact with mineralized and soft tissues such as dentin (mineralized), enamel (mineralized), pulp (soft), and mucosa (soft, Fig. 1). An MPS allows the direct observation of morphological and metabolic events that occur as cells inside the tooth are exposed to biomaterials over time. In this section, microfluidic systems for testing the biocompatibility of biomaterials in the oral mucosa and dentin–pulp interface are introduced.

2.1 Oral mucosa

The oral mucosa is a layered tissue that is challenged by various stimuli including dental materials and oral bacteria. Rahimi *et al.* reported an oral mucosal model constructed using a

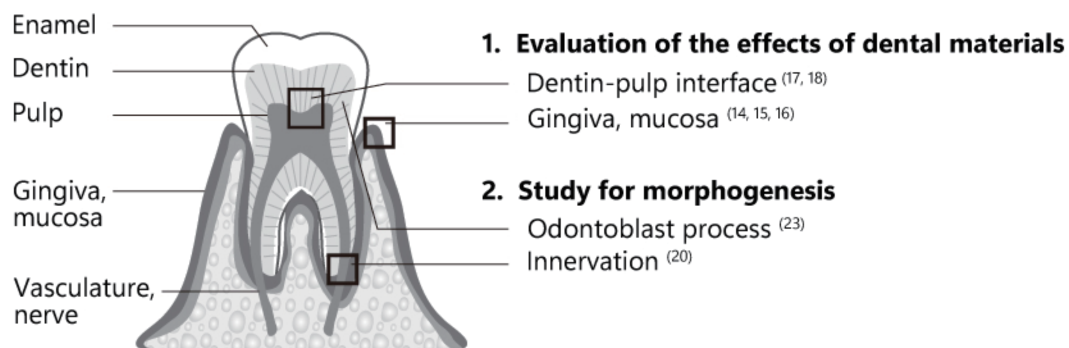

Fig. 1. Schematic diagram of a tooth and summary of the overview of the review.

microfluidic device.[14] The device was composed of three microchannels separated by microposts [Fig. 2(a)], allowing the micropatterning of cells and cellular interactions between channels. Human gingival fibroblasts (hGFs) encapsulated in type I collagen were introduced into the central channel and the surface was covered with human oral keratinocytes [Figs. 2(b) and 2(c)]. Using this oral mucosa model, the toxicity of the dental material 2-hydroxyethyl methacrylate (HEMA) was evaluated. Exposure to 25 mM HEMA significantly reduced cell viability in an oral mucosa model. Moreover, the authors evaluated the effects of *Streptococcus mutans* in this model [Fig. 2(d)]. During 24 h of co-culture, most *S. mutans* remained on the surface of the keratinocytes, whereas some bacteria invaded collagen, resulting in a 64% decrease in transepithelial electrical resistance. More recently, the same group has detailed the effects of HEMA on the model.[15]

The gingival epithelium–capillary interface was also mimicked in a microfluidic device containing two channels separated by a porous polyester membrane with a pore diameter of 1 μm.[16] Epithelial and endothelial cells derived from healthy human gingival tissue were seeded in the upper and lower channels, respectively [Fig. 2(e)]. The levels of the inflammatory markers intercellular adhesion molecule 1 and human beta defensin 2 increased following the addition of the inflammatory factors lipopolysaccharide and tumor necrosis factor-alpha. Furthermore, the pretreatment of cells with pyrrolidinedithiocarbamate ammonium prevented increases in the levels of inflammation markers in the model.

2.2 Dentin and pulp

Dental materials affect the oral mucosa and cells in the dental pulp through the dentin matrix. To evaluate the effects of biomaterials on the dental pulp in a more physiological manner, França

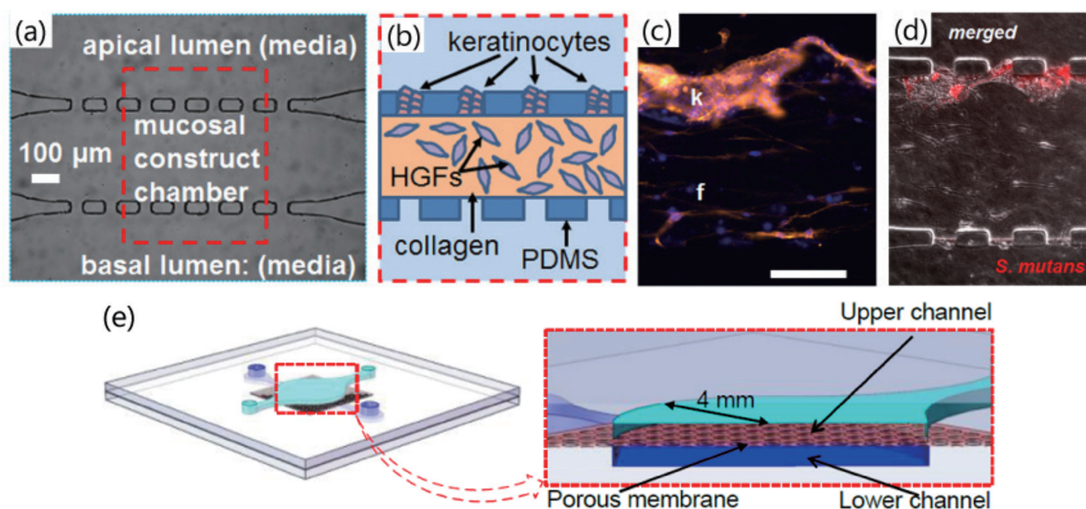

Fig. 2. (Color online) Oral mucosa-on-a-chip. (a) Representative bright-field image of the platform for oral mucosa-on-a-chip. (b) Schematic of the cell configuration in the microfluidic device. (c) Reconstructed mucosal model in the chip. Orange: F-actin; blue: nuclei. Scale bar: 100 μm. (d) Inoculation of S. mutans (red) in the oral mucosa model. (e) Microfluidic model of gingival epithelium–capillary interface. The information shown in panels (a)–(d) was adapted with permission from Ref. 14 (Copyright 2018 AIP Publishing) and that in panel (e) from Ref. 16.

et al. developed a microfluidic system that integrates cells cultured directly on a dentin wall [Fig. 3(a)].(17) Human teeth were cut into pieces (*L* 4.5 mm × *W* 0.5 mm × *H* 1.0 mm). A microstructured PDMS layer was fabricated from a PMMA mold. This PDMS layer has two chambers separated by a small space used as a holder in which a tooth piece was placed. Each side of the tooth piece faces one chamber through a window (L 2.0 mm × W 0.5 mm × H 1.0 mm). Stem cells from apical papilla (SCAPs) were seeded in one chamber defining the pulp side, and the other chamber was the cavity side. The device was closed by a glass coverslip bonded to the PDMS layer. Dental materials that included 2-HEMA, phosphoric acid, and Adper Scotchbond were individually added to the opposite side of the tooth piece in the other chamber (cavity side) and their effects on SCAPs through the dentin matrix were evaluated [Fig. 3(b)]. The microfluidic device allowed the real-time tracking of the cell number, contraction, and metabolic activity, and the visualization of the activity of matrix metalloproteinases (MMPs) in SCAPs. More recently, the same group has reported the evaluation of calcium silicate cements (CSCs) in dentin and pulp.(18) Human dental pulp stem cells (hDPSCs) were cultured on a dentin wall. Cell morphology, pH change, and the release of transforming growth factor-beta following the addition of CSCs to the cavity side were examined. The findings demonstrated that the platform allowed the monitoring of *S. mutants* biofilms following the introduction of CSCs to the cavity side.

3. Morphogenesis in Oral Microenvironment

An MPS allows the manipulation of biological specimens in entirely new ways. Chemical and biomechanical stimulations can be precisely controlled at spatiotemporal resolution, revealing mechanistic insights that would otherwise remain hidden. The features of the MPS have been actively utilized in developmental biology. In this section, we introduce the MPS simulating tooth innervation and odontoblast process.

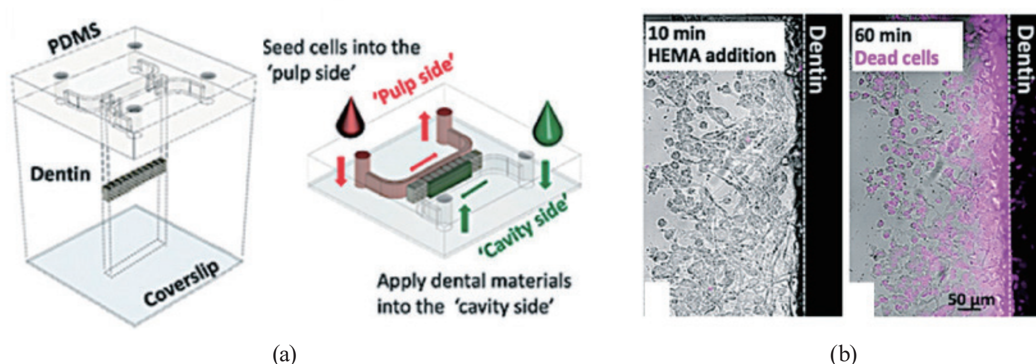

Fig. 3. (Color online) Tooth-on-a-chip. (a) Schematic of the construction of the chip. Dentin materials compartmentalized the channels (pulp and cavity sides). (b) Real-time live-dead assay in tooth-on-a-chip after HEMA addition to the cavity side. These figures are adapted with permission from Ref. 17 (Copyright 2020 RSC Publishing).

3.1 Tooth innervation

During tooth development, trigeminal nerve fibers navigate and establish axonal projections to the developing tooth in a spatiotemporal manner. A co-culture system is a valuable method for investigating the interactions between trigeminal nerve fibers and teeth. However, this conventional system is not optimal for mimicking *in vivo* development because nerves and teeth are cultured in the same culture medium. A microfluidic culture platform can culture neurons and target tissue separately in different compartments while allowing the growth of axons from the neural cell bodies through microgrooves toward the target tissue.(19) Pagella *et al.* used a microfluidic system to study tooth innervation.(20) They co-cultured trigeminal ganglia with embryonic and postnatal molars in a microfluidic device [Fig. 4(a)] and evaluated the formation of axonal projections toward the molars [Fig. 4(b)]. The growing axons were repelled by embryonic molars [Fig. 4(c)]; in contrast, they were promoted and attracted by postnatal molars [Fig. 4(d)). These events faithfully reproduced tooth development *in vivo*. Recently, the authors have employed the same system to study the neurotrophic properties of ameloblastomas,(21) hDPSCs, and human bone marrow stem cells.(22)

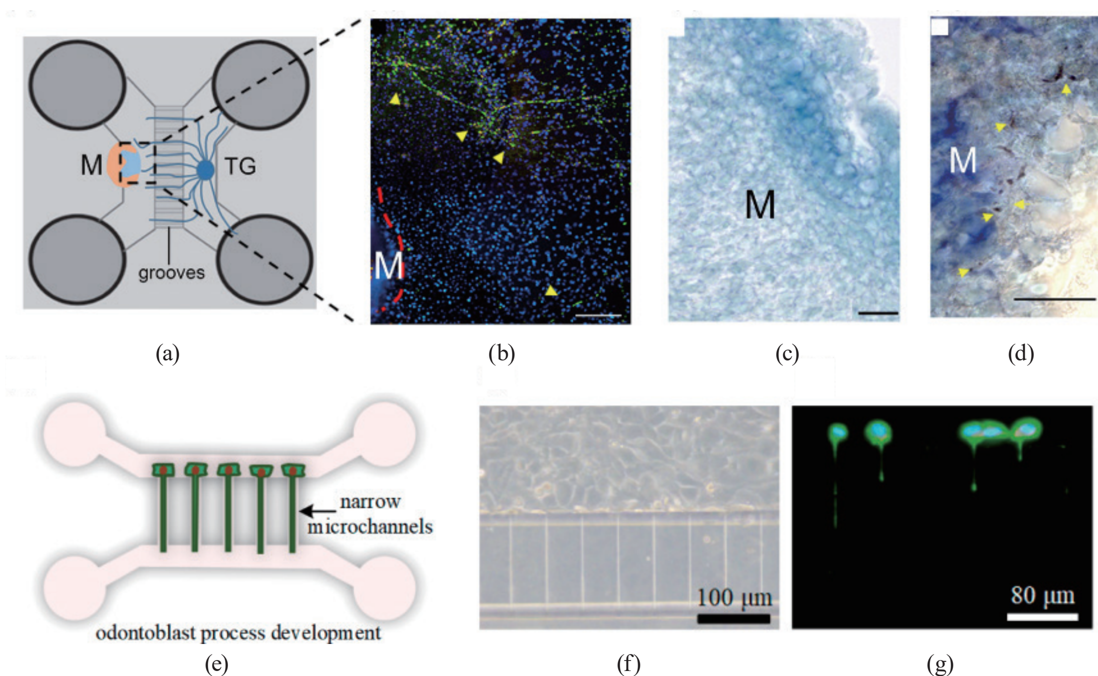

Fig. 4. (Color online) Tooth morphogenesis-on-a-chip. (a) Microfluidic co-culture system for studying tooth innervation. A trigeminal ganglion (TG) was cultured on one side and a developing molar (M) was cultured on the other side. (b) Representative image of axons and molar in the microfluidic device. Green denotes neurofilaments, blue denotes cell nuclei, and the red dashed line is the interface of the molar. Scale bar: 200 µm. (c) and (d) Immunohistochemical analysis of the embryonic (c) and postnatal (d) molars after culture in device. The contact of neurites with the molar is indicated by yellow arrowheads. Scale bars: 20 µm. (e) Schematic of the microfluidic chip for odontoblast process growth. (f) and (g) Representative phase contrast (f) and fluorescent (g) images of the growth of odontoblast process after 5 days of culture in the device. Green: F-actin; blue: nuclei. (a)–(d) were adapted from Ref. 20. (e)–(g) were adapted with permission from Ref. 23 (Copyright 2019 American Chemical Society).

3.2 Odontoblast process

Niu *et al.* studied the formation of dentin tubules in a similar microfluidic device that contained microgrooves.(23) The study involved odontoblasts, which are dentin-forming cells that line the periphery of the pulp and extend their cytoplasmic processes into dentin tubules. The authors seeded odontoblasts in one chamber and evaluated whether odontoblast growth was observed in the microgrooves [Fig. 4(e)]. By optimizing the microgroove size, odontoblast processes were successfully induced in the microfluidic device [Figs. 4(f) and 4(g)]. The findings indicated the value of this system for studying odontoblastic biology and dental diseases.

4. Conclusions and Perspectives

In this paper, we consider recent MPSs that emulate oral microenvironments *in vitro*. These innovative systems enable investigations of physiological and pathophysiological phenomena in the oral microenvironment. Biomechanical stimulation (vascular flow and motion in oral flow) and the oral microbiome will be incorporated in the near future. These approaches have the potential to advance the dental field while promoting innovation and reducing dental healthcare costs.

Acknowledgments

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