PERSONALIZING ORAL HEALTHCARE: THE FUTURE OF DENTISTRY ON A CHIP

An Undergraduate Research Scholars Thesis

by

SANA ANWAR, SOPHIA JANG, DIANE TANG, AND ANNA TRUJILLO

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Approved by
Faculty Research Advisors: Faizan Kabani, BSDH, MHA, MBA, PhD, FAADH
Eric Fox, RDH, MS
Maureen Brown, RDH, MS-HIED

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This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

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ABSTRACT

Personalizing Oral Healthcare: The Future of Dentistry on a Chip

Sana Anwar, Sophia Jang, Diane Tang, and Anna Trujillo
Caruth School of Dental Hygiene, College of Dentistry
Texas A&M University

Research Faculty Advisor: Faizan Kabani, BSDH, MHA, MBA, PhD, FAADH
Caruth School of Dental Hygiene, College of Dentistry
Texas A&M University

Research Faculty Advisor: Eric Fox, RDH, MS
Caruth School of Dental Hygiene, College of Dentistry
Texas A&M University

Research Faculty Advisor: Maureen Brown, RDH, MS-HIED
Caruth School of Dental Hygiene, College of Dentistry
Texas A&M University

Healthcare professionals may experience challenges with the efficacy of treatment as results could fluctuate depending on a patient's genetic disposition. Personalized dentistry may present an impact and optimizes patient care by adapting and personalizing treatment to the individual. The genomic revolution redesigns clinical care from being imprecise to making allowance for individuals variables in exogenous factors, genetics, and lifestyle. Personalized dentistry, medicine and healthcare is employing the use of genomic revolution to understand the human body to challenge and become the new gold standard approach to clinical trials rather than randomized controlled trials. One of the first successful microfluidic devices, Organ-on-a-

chip (OOC) enabled researchers to study human physiology as it assisted in the progression of personalized medicine and dentistry leading to the creation of the Tooth-on-a-chip (TOC) device. The OOC device controls and replicates the model system and measures cell behavior, improving the process of treating specific organs and diseases. TOC provides an in-depth view on the arrangement of the tooth organ, improves the understanding of the inner processes of dental cells in their natural environment, and assists in understanding their reactions to biomaterials. Standard dental procedures involving restorative biocompatible materials such as adhesives, acid etches, and composite resins degrade over time and may lack biocompatibility depending on the patient's oral microbiome. The TOC device has the potential to aid clinicians in making evidence based decisions for patient care based on their genetic makeup.

DEDICATION

To our friends, families, instructors, and peers who supported us throughout the research process.

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INTRODUCTION

Innovations in personalized health care have progressed rapidly due to research advancements in decoding the human genome.[1] Personalized health care has the potential to significantly improve patient care and yield better health outcomes compared to randomized controlled clinical trials, the current gold standard in evidence-based care.[1] Healthcare professionals persistently aim to have a deeper understanding of the human body and devise treatment that focuses more on the prevention of disease itself rather than a cure. The inception of Organ-on-a-chip device (OOC) paved the way for microfluidic devices, such as Tooth-on-a-chip (TOC), to be explored.[2] TOC could be the first device of its kind to aid in preventative treatments by improving the clinician's ability to predict an individual's susceptibility to oral diseases. This narrative review will explore personalized medical treatment and its applications in dentistry specifically through TOC. TOC could provide insight on how the dentition develops, how teeth react to biomaterials, and how the teeth interfere with other organ systems.[3] Research on this topic supports the current NDHRA priority area of clinical decision support tools. The purpose of this narrative review is to describe the clinical and public health significance of personalized medicine, recognize the application of personalized medicine in practice, inform about the history of TOC and how it relates to personalized dentistry, and discuss the scientific and technological aspects of TOC.

1. PRECISION MEDICINE AND PERSONALIZED DENTISTRY

The successful completion of the Human Genome Project in 2001 was a landmark in the field of medicine.[4] The international research program first started in the 1990s. It was a collaborated effort initiated with the intention of mapping and sequencing all the human genes to be able to understand inherited traits.[4] Collectively, the genes would be called a genome. Once entirely decoded and understood, the genome could act as a detailed manual that would enable clinicians to provide patients with a superior level of treatment, especially preventative care. With the involvement of rapid technological advancements and increased research, the genomic revolution has now influenced clinical practice. Allowing a customized diagnosis and treatment, genome sequencing and data analysis became the key elements of precision medicine.[4]

Relying on information that is sourced from an individual's genetic make-up and environmental factors, personalized medicine has the ability to map a patient's journey from health to disease.[4] Precision and personalized medicine are interchangeable terms; however, precision medicine is sometimes used to describe the new approach towards medicine. This approach refers to the way health care providers will create a specific treatment plan catering to an individual's needs, based on their genes. The rationale is to consider the variations amongst humans instead of following treatment strategies for the average person.[4] The use of genomics to practice personalized medicine thus marks a shift in approach from offering generic health solutions to targeted treatments via a deeper understanding of disease and molecular processes.[5]

The genomic revolution, also implicated in personalized dentistry, employs the TOC model to provide customized dental solutions, insight into rare diseases, and drug effects on dental tissue. The TOC could serve as a unique model for research to predict, prevent, personalize, and participate in improving dental health care.[4] While the TOC could be a breakthrough in personalized dentistry providing detailed analyses of individual oral health and disease, its application can be limited due to a number of reasons. Some possible limitations may include the engineering cost, funds needed for research and usage safety, and accessibility to people of all socioeconomic backgrounds. Therefore, it is imperative that practitioners are aware of the various limitations of the TOC device.

Human DNA is the instruction manual that guides development form a single cell to a complex being. The entire DNA is composed of six billion base pairs, which collectively forms the genome.[6] The genome carries 20,000 genes and is responsible for many human traits, such as eye color and ear lobe shape. Additionally, it guides human interaction with the environment and behavioral responses, including complex diseases, which differentiates populations.[6]

Personalized medicine utilizes data made available after the interpretation of these sequences encoded in the genome to predict, prevent, and treat diseases on an individual basis.[6] Launched in 2015, the Precision Medicine Initiative focused on acquiring enhanced knowledge about health and disease so as to exceed treatment expectations.[4] Advanced technologies like Illumina's Core and Oxford Nonapore Technologies are being employed for sequencing as part of the next generation sequencing and have significantly increased the rate of diagnosis.[4] This process involves a depiction of the microbiota using multi-omics applications without having to culture microbes individually and thus providing in-depth understanding of the microbiome.[5] The time and cost associated with sequencing have both decreased, allowing more efficiency and precision. From an economic standpoint, this is crucial. The use of genomics to practice precision medicine marks a shift in approach from offering generic health solutions to targeted treatments via a deeper understanding of disease and molecular processes.[4]

The heart of biomedical research has focused on tracking disease-causing agents and mapping a pathway for therapeutic strategies, which is an important part of precision medicine.[5] The discovery of the microbiome, a combination of microorganisms within the human body, presents itself as an integral element.[5] The genomic revolution facilitated the understanding of the microbiome by enabling DNA-based identification of uncultured bacteria living in different hosts.[5] The significance of the microbiome in human and host cells, inter-individual variability or patterns of variation among individuals, as well as plasticity asserts that the microbiome be integrated in the evolution of precision medicine.[5] The ability to track infectious diseases and prevent potential outbreaks by using genomic data has prompted further research. Moreover, the genomic data could reveal characteristics of an infection and may identify antibiotic resistant genes and susceptibilities in resistant pathogens, including those of idiopathic origins. The microbiome acts both as a modulator and target for therapeutic strategies.[5]

The increase use of personalized data through biotechnological advancements may create more efficient evidence-based practices.[1] For example, Polymerase chain reaction, or PCR, is a technique that was a direct result of researching complex proteins using biotechnological advancements.[1] PCR is utilized to boost DNA fragments to more efficiently detect specific proteins that can indicate chromosomal abnormalities, hereditary diseases, and genetic defects in an individual.[1] PCR could also be applied in dentistry to detect periodontal and cariogenic pathogens, viruses, and oral cancer.[1] With the increase in research on personalized treatments, oral infectious diseases may become more preventable as dental professionals could understand the genomic interaction of a disease within an individual; as a result, dental professionals could potentially determine the most optimal strategy to specifically treat a patient.[7] Currently, there are limitations to personalized dentistry as the encouraging results from most systems and techniques have only been produced in pre-clinical studies and has translated poorly as an effective treatment in clinical settings.[8] The TOC is a device that is currently in development and could be utilized to improve the quality of restorative care.[9]

2. THE APPLICATION OF PERSONALIZED MEDICINE ON OOC MODEL SYSTEMS IN PRACTICE

Personalized medicine is an ongoing development that may provide different clinical applications in assisting for successful treatment of the individuals of human organ and diseases. In 2018, Jodat et al. described that the creation of microfluidic chip may limit the error in the study of OOC device by controlling and replicating the model system in relation to any human organs using microfluidic devices.[10] In the research of personalized medicine, microfluidic devices called OOC may be seen promising and it mimics the human pathophysiology in approaching the limitations of the human cells and animal models.[2]

The OOC device is a mixture of cell biology, engineering, and biomaterial technology that displays the structural and functional components of human tissue, inciting the organ of tissue interfaces and mechanical stimulants.[11] This displays the prediction in response to drug feedback and environmental effects as well as the structural and functional aspects of human tissue.[11] The applications of the OOC device are in the development of physiological models, drug development, and toxicology in the viewpoint of other specific human organs.[11] OOC device has been developed in treatment of specific human organs and diseases, applying human cells or human induced pluripotent stem cells (hiPSC) of a 2D or 3D biocompatible set.[9]

The OOC device research may be a direct approach in human study or the model system of physiology in a *in vivo* experiments.[11] The research of OOC device has much potential, such as it can imitate the surroundings of a physiological organ, with intelligence to control key features such as shear force, patterning of the cell, tissue-boundaries, and relation of tissue-organ.[11] OOC devices may be useful in testing the organ responses to, and interactions with, clinical drugs.[9] The results from the experiments with the OOC device may assist researchers study organ cell behavior with various drugs and treatments.[10]

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In preclinical testing, lack of information of human tissue environment may lead to defective testing of tissue function, compromise the research design and ultimately fail the results of the study.[11] However, with the implementation of other physiological model systems, the development of OOC device may be used to identify its capability in reducing the problems and limitations.[11] With the incitation of physiological surroundings of human organs in in vivo testing, OOC may be a replacement of future technology focused on serving the purpose of OOC within the study of human physiology and animal models.[11] The OOC device research of the functions were tested with a variety of drug assays and organs tested with the OOC device are liver, kidney, gut, central nervous system (CNS) and peripheral nervous system (PNS), blood brain barrier, heart, muscle, and many others.[10]

Recent studies have focused on the regulation of airway mechanical pressure, the blood-blood barrier, and the action of force of pathophysiological processes.[11] In 2012, Huh et al. developed the lung-on-a-chip, also known as LOC device, was the first OOC device to be researched and showed evidence of success in the behavior of the organ.[2] The LOC device consists of two compartments, which resembles a functioning lung organ.[11] The LOC device is divided by a layer of human alveolar epithelial cells to create the two compartments.[2] Either sides of the compartments are affected by two different environmental factors like the lung organ; one compartment is in contact with air, while the other was able to portray vascular perfusion.[2] LOC device have been discovered to be functional as implantable respiratory assistance devices.[11]

In 2015, Huh et al. developed a LOC device, dividing the chip into parts by 10 μm polydimethylsiloxane (PDMS) membranes with the extracellular (ECM) using soft lithography.[10] The PDMS was divided to mimic the alveolar- capillary barrier in which the upper PDMS consist of alveolar epithelial cells and the lower PDMS consist of human pulmonary microvascular endothelial cells.[11] The study of LOC device may show success that encourage advanced research of current *in vivo* study.[11]

The success of LOC devices enabled the development of additional devices that may be successful in treating other health conditions.[11] For example, the lung assist device (LAD) is designed by Peng et al. in 2015 to aid the respiratory failure of preterm infants by enabling more gas transfer within the placenta.[11] Microfabrication for microfluidic blood oxygenators was done using double-sided gas transfer, hoping to provide better gas exchange.[11] The results indicated that the oxygen uptake improved to 343%, when comparing it to a single-sided devices.[11] LOC device was also helpful in being able to study the microenvironment of cancer cells in lung cancer, and may help future research in providing treatment for cancer patients.[11]

3. THE SCIENCE OF TOC

The response of the dentin matrix to biomaterials may vary depending on the microbiome and genetic disposition of an individual.[9] This issue could be present when treating dental caries and sensitivity as the biomaterials used for restorative care could experience degradation over time.[9] By directly observing the cells inside the dentin matrix when exposed to different biomaterials, critical information could be found on the morphological and metabolic events that occur overtime; however, existing models that perform this observation are ineffective in doing so.[9]

Given the limitations of existing models, the TOC was developed to improve the direct observation of biomaterials on the dentin matrix by providing real time data and live imaging.[9] When examining an in-vitro model study, in 2020, França, et al, used the OOC model as a basis to replicate the structure of the dental pulp interface with dentinal tubules and two chambers; the pulp and cavity side.[9] Cells cultured from the third molar are placed on the pulp side of the device and establishes a stable cell-dentin interface. The cell-dentin interface monolayer then interacts with the dentin wall to replicate the reaction of stimuli similar to a natural tooth.[11] The TOC simulates the anatomical structure of a natural tooth to study the relationship between biomaterials and dental matrix interface using live-cell imaging.[9] In 2020, França, et al, compared the TOC against an ISO-10993-1 in-vitro model control referred to as an off-chip control.[9] França, et al, measured the reaction of 2-hydroxyethyl methacrylate (HEMA), phosphoric acid (PA), and Adper-scotchbond (SB) on both the TOC and off-chip control using three criterions: cytotoxicity, cell morphology, and metabolic activity.[9] The results were statistically significant as the materials had cytotoxic effects in both the TOC and off-chip control ($p<.05$) and cells had a higher metabolic activity on the TOC ($p<.05$).[9] The near physiological reproduction of a real tooth could potentially be utilized to efficiently assess the reaction of biomaterials against the dental pulp using cultured cells.[9] The TOC's ability to better track the morphological and metabolic events that occur could provide valuable information that could potentially predict a patient's biocompatibility to biomaterials.[9]

To make up for the gaps between *in vivo and in vitro* conditions, researchers that engineer tissues have applied microfluidic devices to culture cells since the beginning of the 21st century.[12] The combination of microengineered substrates and microfluidic technologies allow duplication of tissue performance levels that are strenuous to get with conventional 3D cell culture models.[1] Because of this microfluidic devices are expected to be stages for cell based assays during the discovery of drugs and disease study.[12]

In 2019, Niu et al. used a microfluidic device to grow odontogenic processes(OP), which are found in dentin tubules.[13] The OP's growth was achieved by using a microchannel length of 2 μm and applying an influential hydrostatic pressure of 260 μL, which was respectfully divided to create a pressure difference.[13] Niu et al. recognized AQP4 as a marker protein to monitor odontoblast.[13] AQP4 was identified in the chip and the comparing petri dish culture, demonstrating that the chip can mimic an in vivo process.[13] Although Niu et al. did not provide statistical significance, the research does provide preliminary work for future studies of OP's in dentin tubules.[13] In 2019, França et al. used stem cells from apical papilla and placed them on the pulp side of the microfluidic device, these cells produced a monolayer that interacted with the dentin to mimic the reaction of dentin to biomaterials.[9] Three dental materials were tested after the monolayer formed, HEMA in cell culture medium (at a cytotoxic concentration), phosphoric acid (used to etch dentin), and Pa plus Adper single bond.[9] A dentin-pulp interface similar to a restored tooth formed after the materials were placed to the 'cavity side'.[9]

França et. al suggested that the longevity of resin restorations depend on the solidarity of the hybrid layer, the hybrid layer is the system of collagen fibers and adhesive resin.[9] It is thought that this hybrid layer is degraded by proteases.[9] The degradation by proteases of the hybrid layer can be measured by gelatinolytic assays.[9]Using this novel TOC device allows for measurement of the gelatinolytic assays. Using a confocal microscopy, images of the proteolytic activity can be captured.[9]

França et. Al found uncoordinated metabolic activity in tooth cells that were treated with phosphoric acid (PA), single bond, and HEMA.[9] Off chip untreated cells presented with a spontaneous decrease after treatment of HEMA, PA, and SB as soon as twenty-four hours after treatment.[9] To address the discrepancy with metabolic activity a duplication of the clinical application of a dental adhesive system was performed.[9] Off chip showed differences in cell cytotoxicity mechanisms because HEMA acts fast and Single Bond releases toxins more slowly.[9]

To finalize the study a functional assay was performed with TOC by exploring the performance of cellular MMPs (Matrix metalloproteinase) in hybrid layer degradation, which eventually leads to failure of dental restorations.[9] TOC allows a view to record cellular pulp and subcellular responses in surroundings like the *in vivo* situation. A limitation of this includes the impracticality to cover all conditions of the TOC at a single time.[9]

CONCLUSION

A person's biology and genetic profile could often affect the efficacy of treatment and presents a challenge to health care professionals.[6] Personalized medicine presents a potential new opportunity to improve the quality of patient care by customizing treatment to the individual.[6] New models and systems that are currently being developed could accelerate the adaptation of personalized dentistry.[7] The microfluidic device, OOC, has produced successful results and has laid the framework for the TOC device.[9] The tooth's reaction to biomaterials could differ depending on the person's biocompatibility and could benefit from personalizing treatment.[7] As a result, the TOC is currently being developed to improve knowledge on how biomaterials react with the dentin matrix.[9] TOC has the potential to improve the knowledge needed to personalize dental treatment.[9] While promising, the existing systems that personalize treatment are limited in their capacity for clinical applications and further research is required on the clinical practicality of these systems. With continual development through research, personalized dentistry could be integrated into clinical practice, improve the overall quality of patient care, and improve the efficiency of diagnosing diseases.

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