The Oral Microbiome Impact on Chemotherapy-induced Oral Mucositis (CIOM) *In vitro* Modeling Using Liquid Scaffold Co-culturing System

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ABSTRACT

A painful side effect of chemotherapy is chemotherapy-induced oral mucositis (CIOM), which 20–40% of cancer patients experience. Cancer patients with CIOM develop mouth ulcers and experience severe pain that often makes them unable to eat, drink, talk, and sleep (together with the other side effects of chemotherapy). With no effective means of prevention and only limited supportive treatments (such as ice chips and numbing gels), the quality of life for cancer patients with CIOM decreases. Recently, the role of the oral microbiome on CIOM has gained attention(1-3). Chemotherapy cause changes in the oral microbiome(2, 4–6). Since antibiotic therapy did not improve CIOM(7–9), the oral microbiome role in CIOM should be beneficial rather than harmful. Another study exhibits that a probiotic decreases CIOM in patients who had nasopharyngeal carcinoma treatment(10). However, which microbial species prevent CIOM development is still unknown. So, we plan to identify the beneficial microbiome species for CIOM and underlying mechanisms. **We propose investigating the impact of microbes on oral epithelium tissue to determine how different microbial species and oral cells interact during chemotherapy.** The study requires co-culturing microbe and mucosa cells, which is experimentally challenging. We will **directly grow bacteria over mucosa cells with a unique aqueous two-phase system (ATPS) bioprinting technique**. Identifying microbes with a protective impact on CIOM and the underlying mechanism will offer treatments for CIOM and improve cancer patients' quality of life.

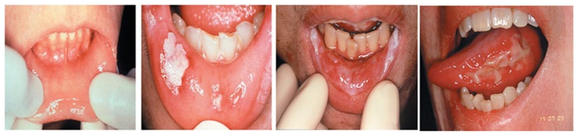

Figure 1: Toxicity scale of OM. Adopted from Maria, OM., et al. Frontiers in oncology 7 (2017): 89.

OBJECTIVES

1. To determine whether ATPS maintain growth of two bacteria strains.
2. To determine the stability of ATPS over 3D oral tissue model.
3. To determine impact of *Staphylococcus aureus* (SA) on the 3D oral tissue model's viability.

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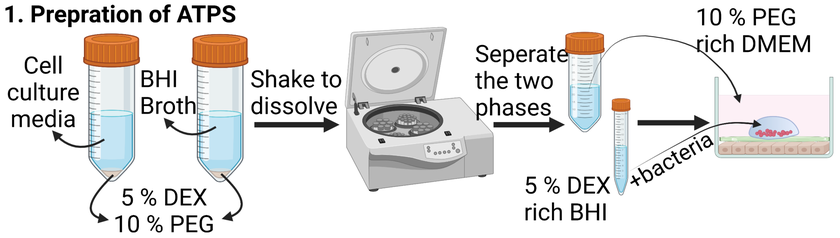
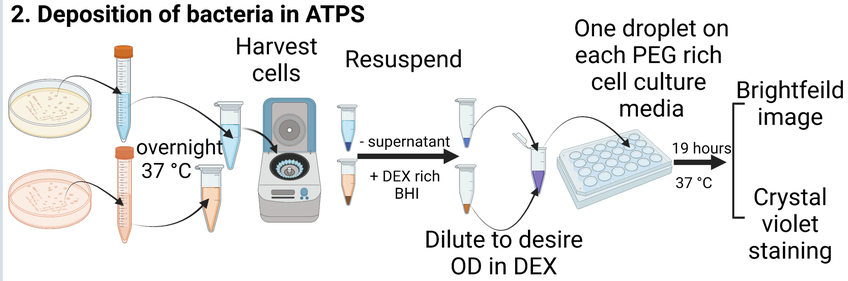
METHODS

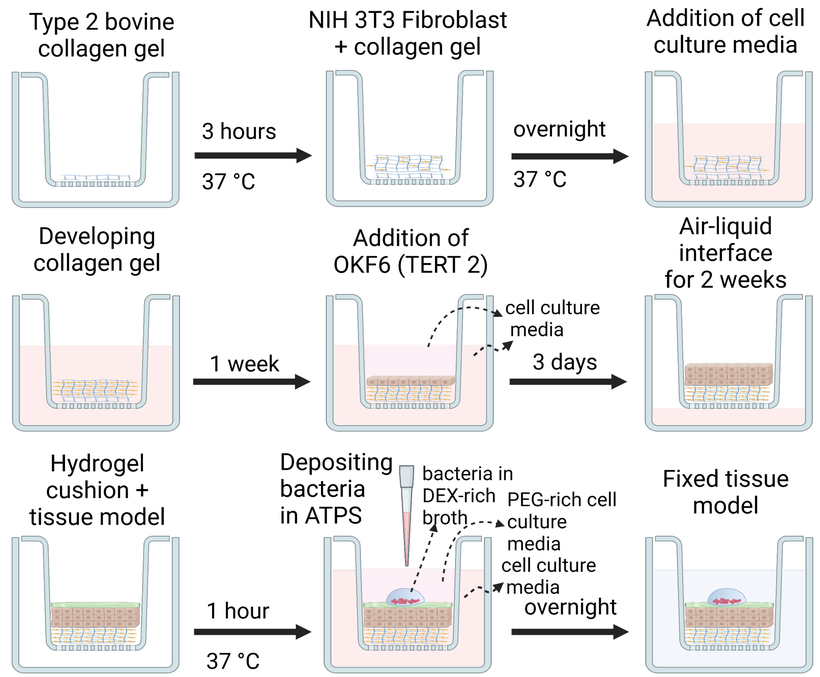
1. Prepration of ATPS



2. Deposition of bacteria in ATPS



3. Prepration of 3D oral tissue and deposition of bacteria using ATPS



4. Viability assay

A live/dead assay and Hoechst stain from Thermo Fisher Scientific were used. Live cells counts based on the total number of the cells count (based on the Hoechest stain) minus dead cells counts (based on the Ethidium homodimer-1 stained).

RESULTS

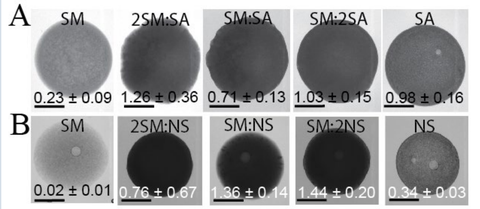


Figure 2: *Co-culturing two bacteria species in ATPS is feasible.* A) *Streptococcus mutans* (SM) and *Staphylococcus aureus* (SA) co-culture in ATPS of 10 % PEG DMEM media, 5 % DEX Brain Heart Infusion broth (BHI) for 22 hours at 37 °C. B) SM and *Neisseria sicca* (NS) in ATPS of 10 % PEG DMEM, 5 % DEX BHI for 19 hours incubation at 37 °C. The scale bar is 650 mm. Ctrl is the bacteria-free DEX droplet which is our negative control. The crystal violet staining value after subtraction of the Control value is reported on the bottom right side of each image. **The DEX droplets with one bacteria species exhibit less growth than those with mixed species.**

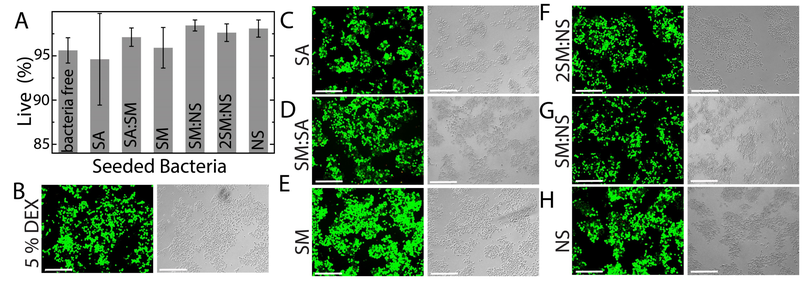


Figure 3: *Co-culturing OKF6 cells with mixed bacteria in ATPS for 19 hours shows cell viability is 95 % for all samples.* Cells were cushioned by 1% alginate hydrogel. ATPS was 10 % PEG in DMEM and 5 % DEX in BHI. A) the percentage of live cells after 19 hours based on 5 samples presented in graph. B-H) representative samples of live cells in green and dead cells in red in the fluorescent images on the left and the corresponding brightfield images on the right side. Images are at 10X magnification. **OKF6 cells are compatible with ATPS, and bacteria species has no impact on OKF6 viability.**

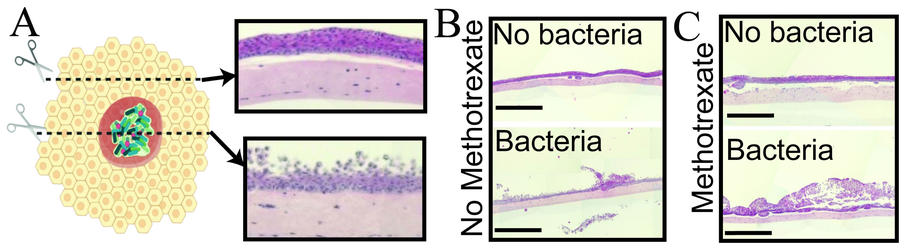


Figure 4: *Histology images of 3D oral epithelial tissue model.* A) Histology image of 3D oral epithelial tissue model co-cultured with SA in ATPS for 19 hours. Physically separated but chemically connected image is on top and physically connected image is on the bottom. **Tissue model that is chemically connected but physically separated from SA is not damaged.** B) Histology images of tissue model exhibit significant difference between incubation with and without bacteria. C) Histology images after exposure to methotrexate with and without bacteria. The scale bar is 1 mm. **Oral tissue express damage in the presence of chemotherapy and the damage is more significantly in the presence of SA.**

CONCLUSIONS

1. Co-culturing two bacteria strains in ATPS is feasible and mixed species exhibit more growth than one bacteria species.
2. The 3D oral tissue model and ATPS are compatible.
3. *Staphylococcus aureus* (SA) have little effect on the 3D oral tissue model's histology when it is not physically connected but it is chemically connected. The oral tissue experience damage in the presence chemotherapy and the damage is more dramatic with SA.

ACKNOWLEGMENTS and CONTACT

Sarah Spencer, Dr. Said Daboor, Ashley Kervin, Dr. Morgan Langille and Dr. Zhenyu Cheng for their support and assistance in this research.