NIH Diversity Supplement Professional Development Workshop

August 29th – August 30th 2023

Book of Abstracts

In vitro 3D human gingival tissue model to study oral microbiome: computational model to estimate long-tern culture conditions

Anyelo Diaz, University of Massachusetts Lowell

NIDCR

anyelo_diaz@student.uml.edu

Introduction: In the field of oral tissue engineering, few advances have been achieved in terms of understanding the synergistic effects of host-pathogen interaction in oral flora. Current approaches lack the three-dimensional traits and bio-mechanical behaviors of human gingiva, thus resulting in the need to develop a physiologically and human relevant in vitro oral model to successfully comprehend host-pathogen imbalances and providing better insight into patient health, resulting in improved prevention strategies and clinical treatment for better ways for oral dysbiosis. Towards this goal, we have designed a 3D gingival tissue model composed of i) silk-based sponge to mirror the architecture of gingiva, ii) human primary cells to mimic the cytology of the gingiva, iii) an Arduino-controlled closed-loop bioreactor system to represent the oral cavity environment while circulating artificial saliva. Taken together, this model serves as a human-based in vitro model of gingiva which can be further implemented with microbiome and serve as a platform to study gingivitis and periodontitis. However, salivary flow (0.3-0.4 mL/min) is essential to ensure continuous lubrication of the oral mucosa, mechanical clearance that helps balance the oral microbiome, and ensures physiological host-pathogen interactions. Altered salivary flow can lead to dysbiosis by increasing the risk of dental caries, gingivitis, or periodontal disease (2). Thus, we focused on the development of the computational model to support and predict the bioreactor design for the long-term culture of the tissue model.

Methods: Shear stress assessment was carried out by Digital Particle Image Velocimetry (DPIV), fluorescent red microspheres were dispersed in artificial saliva solution within the customized bioreactor chamber while a camera captured the motion of the particles. A silk-based anatomical replica of an adult lower gingiva was placed in the chamber for an accurate estimate of the shear stress profile, while the chamber was connected to a peristaltic pump and different flow velocities were tested. Additionally, three inlet positions in the bioreactor chamber

were screened, as the inlet position affects the velocity vectors at the gum-tooth level. The DPIV analysis was then conducted using a script/code written in MATLAB (MathWorks)- PIVlab2. To assess the shear stress, a shear rate was first computed as S= v/âˆ†h, where ???? (s-1) is the shear rate, ???? is the fluid velocity (m/s) obtained from the PIV experiment and Î”â„Ž (m) is the thickness of the artificial saliva film above the rim of the sponge. Then, shear stress was calculated as Tw=6Î¼S, where ???????? (dynes/cm2) is the shear stress, ???? is the dynamic viscosity (cP) and ???? (s-1) is the shear rate. Porosity of the sponge was assessed through SEM and image analysis.

Results: For the humanized tissue model, the final flow rate was set at 1 mL/min, corresponding to a shear stress of 0.40 dynes/cm2. To further validate the dimensionality and chosen parameters of the bioreactor, we profiled velocity, pressure, and vorticity in the simulated (FEATool Multiphysics - Finite Element Analysis Toolbox for Multiphysics; MATLAB) sections of the bioreactor perpendicular to the inlet-outlet axis. This 2D section is the portion of the sponge that most directly receives flow from the inlet; it follows that the resulting values are the upper limits. The computational simulation replicated the bioreactor geometry, the artificial saliva properties, the liquid height above the rim of the sponge, the permeability of the sponge, and the inlet flow rate. The velocity was profiled along the width of the sponge, at 4 mm above the rim of the sponge, and used to compute shear rate, viscosity, and shear stress. The results showed that all the parameters were within the physiological range. In addition, we computed the pressure at the two sides of the sponge and obtained the vorticity profile at different liquid heights along the width of the bioreactor. As expected, the pressure on the inlet side of the sponge was greater than that on the outlet side. Little vorticity was found except on the liquid above the sponge and near the outlet. Numerical discrepancies between the DPIV data and the simulation may be stemming from the approximations adopted including, laminar and Newtonian fluid flow, continuous flow media, 2D rather than 3D geometry, and pressure at the outlet set at 0 kg/m2. Despite those limitations, the numerical simulation was found in agreement with the experimental results.

Conclusion: Overall, the computational model was critical to finalize the bioreactor design, as a closed-loop circuit with circulating artificial saliva with a force profile compatible with native forces. The simulation supported the choice of geometry and parameters to approximate physiological values within the oral cavity.