

DESIGN AND DEVELOPMENT OF A MILLIFLUIDIC PLATFORM FOR SLOW POSITRON SPECTROSCOPIC STUDIES IN WET LIFE SCIENCE

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Introduction

Positron Annihilation Spectroscopy (PAS) is a technique with the unique capacity to provide information about the structural and chemical environment of matter. When applied in material science, the approach reveals the presence of nanoscale defects [1,2] and recently provided a novel way to characterize biological tissues. For example, alterations in oxygen concentration influence the positron lifetime in cellular organoids [3]. A common PAS approach utilizes a polyenergetic radioactive source, i.e. emitting positrons in a wide energy range. This implies a high particle implantation depth of millimeters, making single-cell analysis impossible. In this work, we developed a millifluidic culture system providing optimal cell culture conditions and optical access for microscopy while coupling wet living matter with the high-vacuum chamber needed for a monoenergetic positron beam. This bioreactor platform will pave the way for cell monolayer analysis and axial sectioning of 3D cellularized constructs with PAS.

Methods

The bioreactor prototypes were 3D printed using biomedical resins (Form 3B+, Formlabs). The system consists of two parts: the upper one with a Ø12 mm coverslip glass for optical access and the bottom one bearing a 200 nm thick silicon nitride (SiNx) membrane suitable for positron-based inspections. The coupling of the two components forms a millifluidic culture chamber where we seeded endothelial and epithelial cells (bend.3, H5V, HepG2). We used the software Abaqus CAE to predict the mechanical resistance of the SiNx membrane during coupling with the high vacuum chamber and during culture chamber perfusion. Furthermore, we computed the oxygen consumption of a cellular monolayer and the shear stress inside the culture chamber using COMSOL 6.1. Doppler broadening and positron lifetime measurements were conducted using a positron beam [4].

Results

The 3D-printed bioreactor complied with the design specifications for coupling with the high vacuum chamber of a positron beam. In fact, the mechanical analysis predicted the ability of the SiNx membrane to withstand pressures of up to 1 atm. The LIVE/DEAD assay (ThermoFisher Scientific) validated the ideal conditions preventing cell hypoxia and compatible shear

stress identified by CFD analysis (Figure 1a-1b). The optical window of the system allows for cell inspection with epifluorescence and two-photon microscopy at the same point of antiparticle measures. The results from the PAS studies revealed differences between endothelial and epithelial monolayers of immortalized cells.

Figure

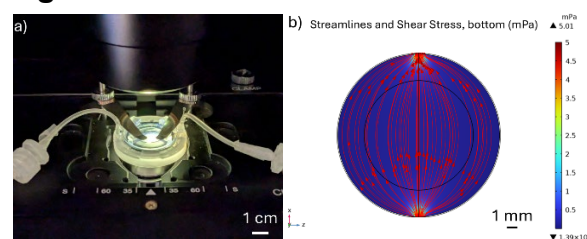


Figure 1: a) The bioreactor platform positioned on the confocal microscope Nikon A1-R performing acquisitions after LIVE/DEAD assay. b) Shear stress values in mPa on the bottom chamber of the bioreactor.

Discussion

We developed a novel bioreactor platform with a compact design, integrating both optical access and a positron-permeable membrane for PAS. Additionally, the watertight culture chamber of the bioreactor can sustain cell viability throughout the measurements and withstand high vacuum as predicted computationally. The preliminary results in positron lifetime components and Doppler broadening highlight the ability of PAS to investigate the chemical environment and nanoscale structure of live cellular monolayers. Furthermore, optical access allows for real-time monitoring and the assessment of radiation effects on cell constructs. This device opens new avenues to analyze the chemical environment in physiology, in the presence of pathologies or even changes in tissue structural characteristics in response to drug stimulation.

References

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