Title: Developing a Border Zone-on-chip

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Keywords:
cell and tissue models, cardiovascular science, cardiac ischemia, bioengineering and biotechnology

Abstract
Cardiovascular diseases are among the leading causes of death worldwide, with most of the fatalities linked to cardiac ischemia, specifically sudden cardiac death caused by reperfusion induced arrhythmias. In cardiac ischemia, restricted blood flow causes localized hypoxia. Ischemia border zone (BZ), a steep oxygen gradient between the healthy and ischemic myocardium, has been recognized as arrhythmia sensitive substrate. BZ is a promising therapeutic target as it comprises both healthy and ischemic cardiomyocytes (CMs). Existing *in vitro* ischemia research lacks knowledge, and platforms for the study of the cellular and molecular effects of the BZ. This work aims to advance knowledge of the BZ on CM morphology and functionality. In this work, a novel in house developed BZ-on-chip is used to evaluate the effects of an oxygen gradient and reperfusion on CM morphology and functionality. The chip is validated through ratiometric oxygen measurement, and imaging of a live cell hypoxia-responsive fluorescent dye. Immunocytochemistry is used to assess the expression of cardiac specific sarcomere proteins, nuclei size, and connexin 43 localization. Functionality of the CMs is evaluated by phase contrast imaging CM beating, which is analysed via a novel trajectory analysis. Our results demonstrate formation of an oxygen gradient, reperfusion-induced sarcomere disruption in the region subjected to hypoxia during gradient induction, and differences in beating pattern across the oxygen gradient. We managed to create an oxygen gradient platform to model BZ-on-chip, and a non-perturbative functional analysis, which displayed functional differences in CMs across the oxygen gradient.