Design, fabrication and characterization of microphysiological systems to study drug toxicity in cardiac and adipose tissue

Peter Loskill[1, 2], Anurag Mathur[1, 2], SoonGweon Hong[1], Bruce R Conklin[4, 5], Luke P Lee[1, 3], Andreas Stahl[6], Kevin E Healy[1, 2]

1. Department of Bioengineering, University of California at Berkeley, CA, United States.
2. Department of Materials Science and Engineering, University of California at Berkeley, CA, United States.
3. Department of Electrical Engineering and Computer Science, University of California at Berkeley, CA, United States.
4. Gladstone Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, United States.
5. Department of Medicine, Division of Genomic Medicine, University of California at San Francisco, CA, United States
6. Department of Nutritional Sciences & Toxicology, University of California at Berkeley, CA, United States

Statement of Purpose: Drug discovery and development is hampered by high failure rates attributed to the reliance on non-human animal models employed during safety and efficacy testing [1,2]. A fundamental problem in this inefficient process is that non-human animal models cannot sufficiently represent human biology, and more importantly, they cannot adequately recapitulate human disease states. With the discovery of patient-specific human induced pluripotent stem (iPS) cells, the tissue engineering community is now in position to develop in vitro disease-specific model tissues and organs to be used for high content drug screening and patient specific medicine. By mimicking the dimensions and cellular arrangement of "minimal elements" of human tissue, we designed, fabricated and characterized two microphysiological platforms that accurately represent human cardiac and adipose tissues, and will be amenable to drug screening.

Methods: For the molding of the microfluidic devices, negative photoresist (SU-8) on a silicon wafer was employed. First, a thin SU-8 layer was patterned on a silicon wafer to define: (i) high fluidic resistance endothelial barrier channels that connect a flow channel with the tissue channel; and, (ii) a gap between the sealing and the weir. A second layer of SU-8 was then spin-coated on top of the thin layer. The transport and tissue channel, including the weir, were then photo-lithographically defined. The complete master was used for silicone replica molding. In the case of the cardiac system mechanosensing pillars were introduced into the cell chamber using the same approach as for the weir. Final PDMS devices were bonded to glass, coated with ECM molecules and filled with human cardiomyocytes and adipocytes. Nutrient diffusion properties were determined using fluorescent dye. Oxygen concentration was modeled using COMSOL Multiphysics®.

Results: The developed microfluidic three-dimensional culture devices consist of three functional components: a tissue channel (100-400 µm); an artificial endothelial barrier with rectangular "pores" (2 µm high and 2-5 µm length) spaced 10-20 µm apart; and, a nutrient transport channel acting as a capillary. This setup allows the diffusion of nutrients and drug compounds to the tissue, while protecting it from shear stress and enables a sufficient oxygen supply in the tissue channel. One end of the cell culture pocket was blocked by a weir, which enabled the loading of cells at high density (> 5000 cells). Since the design of the tissue channel also allows for unloading of cells post screening, ex situ analysis tools

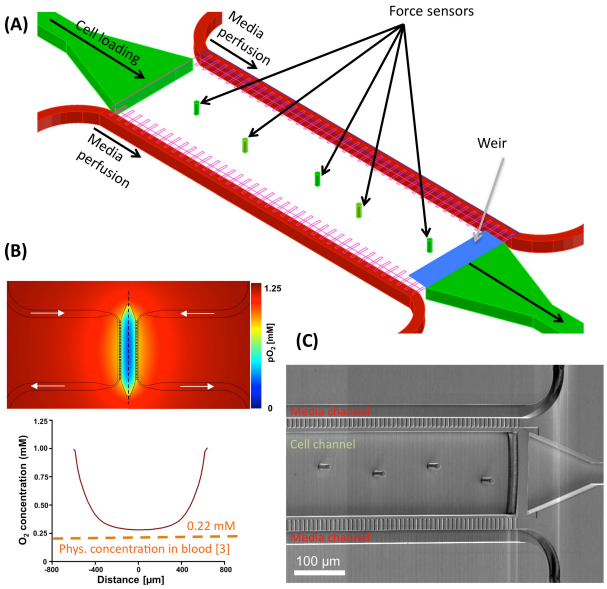

Figure 1: (A) Scheme of the microphysiological systems, (B) Model of the oxygen distribution in the channel and (C) SEM image of the developed platform.

(e.g. flow cytometry, qPCR) can also be employed. In the case of the adipose chip, a modified more permeable version of the weir was introduced enabling an easier loading of the fragile and larger cells. For the cardiac chip, the tissue channel was equipped with pillars enabling an in situ time resolved characterization of the forces exerted by beating cardiac cells. Live/dead staining and functionality monitoring demonstrated the ability of the devices to keep the tissue viable over multiple weeks.

Conclusions: We designed, fabricated, and characterized microfluidic devices with various in and ex situ monitoring tools, which can be used to study drug toxicity. When fed with continuous flow our configuration and control over media perfusion enables a high density of human cardiomyocytes and adipocytes to be maintained extended time in physiologically relevant morphology. To further facilitate drug screening and assessment of direct on off-target response, multiple units of the microfluidic three-dimensional culture devices can be assembled into an array, and also combined together and with further organ on-a-chip modules, such as the liver or lung.

References:

[1] Ledford HH. Nature. 2011;477:526.
[2] Kola I. Nat Rev Drug Discov. 2004;3:711.
[3] Radisic M. Biotechnol Bioeng. 2006;93:332.