BODY-ON-A-CHIP: ON-CHIP HEART RECEIVING METABOLITES FROM ON-CHIP LIVER

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ABSTRACT

We report on the development of an integrated microfluidic and multi-electrode array (MEA) system housed in a microbioreactor for Body-on-a-Chip applications. On a single chip, we successfully demonstrate the use of this system by connecting an on-Chip Liver and an on-Chip Heart. We demonstrate that acylated dihydropyrimidinone (DHPM), when added to the on-Chip Liver, is deacylated and results in DHPM, a well-known calcium channel blocker, delivered to the on-Chip Heart by on-Chip organ-to-organ interconnectivity. Deacylated DHPM delivery to the cardiomyocytes is verified by MEA measurements of cardiomyocyte electroactivity and Liquid chromatography–mass spectrometry (LCMS) of the cardiomyocyte medium.

KEYWORDS: P19 Stem Cells, Cardiomyocytes, Hepatocytes, Organ-on-a-Chip

INTRODUCTION

As we recently reviewed [1], with significant advancements in technology focused on Organ-on-a-chip continuing, it is feasible to consider the future of Body-on-a-chip technology. With serious work being done to realize functioning artificial livers, kidneys, hearts, and lungs on chips, the next step is to interconnect these organs to create an integrated on-Chip Body. Such a Body-on-a-chip requires a sophisticated set of tools for micropattering cell cultures in 3D to create interconnected tissue-like organ structures.

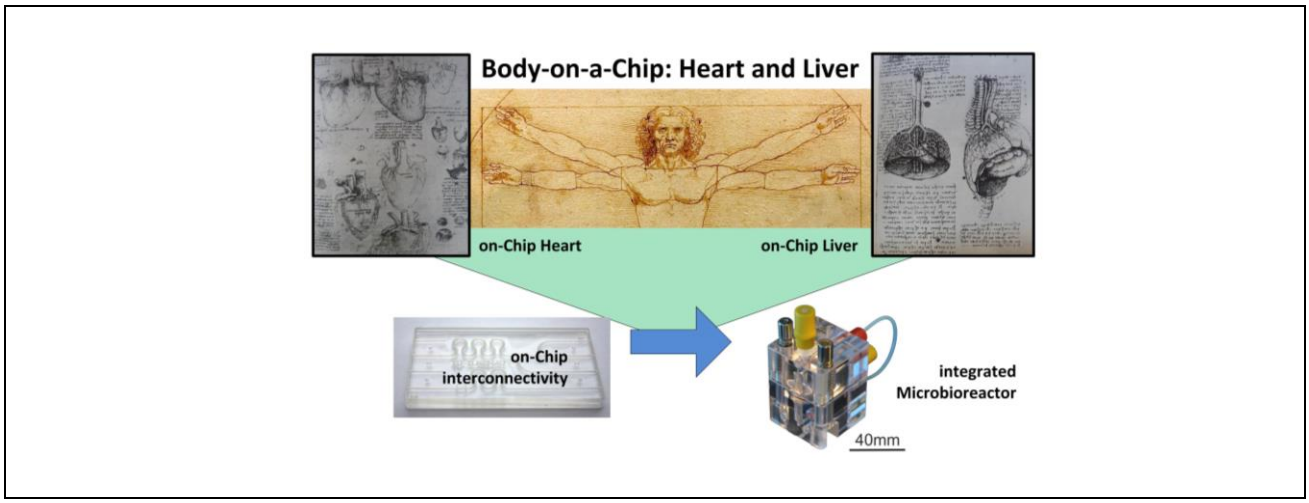

Figure 1: System Overview. The system is an integrated microfluidic chip and multi-electrode array (MEA) for the cultivation of both cardiomyocytes and hepatocytes. The chip itself is housed in a microbioreactor for controlled dose delivery and perfusion. Chemicals of interest are added to the on-Chip Liver. Processed metabolites from the liver are subsequently delivered to the on-Chip Heart. We report here on the use of calcium channel blocking by use of an interconnected heart and liver.

This work demonstrates advanced methods of interconnecting on-Chip heart and on-Chip liver cultures, complex 3D patterning of cultures, and state-of-the-art scaffolding, for the future of Body-on-a-chip. We anticipate such a technology will have a wide area of application, primarily benefitting drug development, chemical safety testing, and disease modeling.

THEORY

The process of adding an acyl group to a compound in chemistry is called acylation. Compounds which provide an acyl group are called acylating agents. We opted for DHPM as a candidate for calcium channel blocking activity. In our work, the acylation of DHPM is performed in two step process by the acylating agents, Tetrahydrofuran (THF) and n-Butyllithium (n-BuLi), as seen in Figure 2. The left side of the molecule is identified as the active site, however, the right urea moiety possess significant importance due to it hydrogen bonding and binding to active sites. Therefore, we distorted the structure on the right side of the molecule by acylation using low temperature organolithium chemistry. By using 2 equivalents of n-BuLi and acetic anhydride we obtained the required structure.

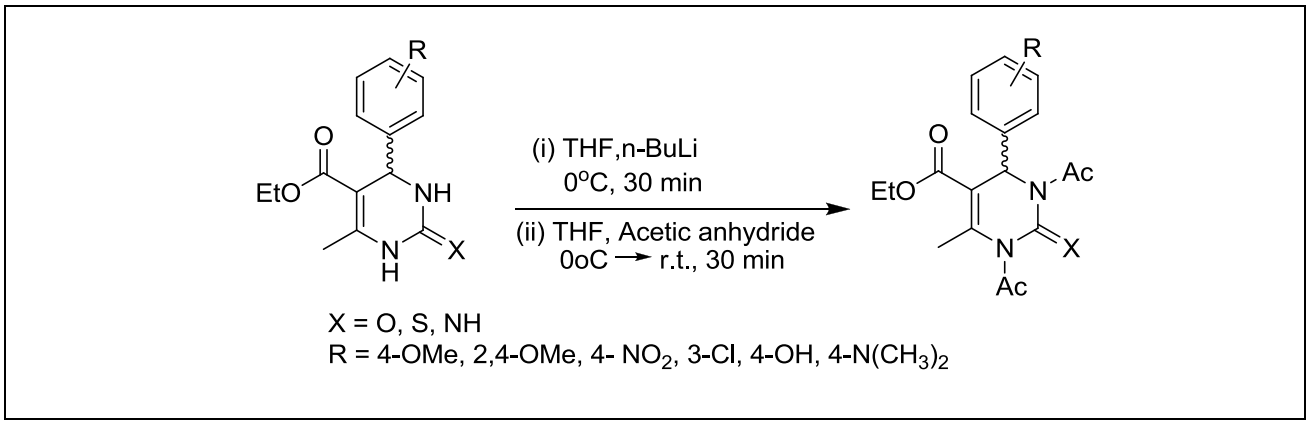

Figure 2: Acylation reaction utilized to prepare compounds for metabolization by Hep G2 liver cells

EXPERIMENTAL

Silicone frames are vacuum cast and mounted on standard MEAs from Multi Channel Systems (MCS) GmbH, as seen in Figure 3. Hep G2 cells are cultured in the multiple semicircular wells of a Matrigrid, a thermoformed polycarbonate scaffold for 3D cell culturing. The large square chamber of the silicone frame houses the Matrigrid and is connected via a microfluidic channel to a smaller square chamber located directly above the electrodes for culturing the cardiomyocytes.

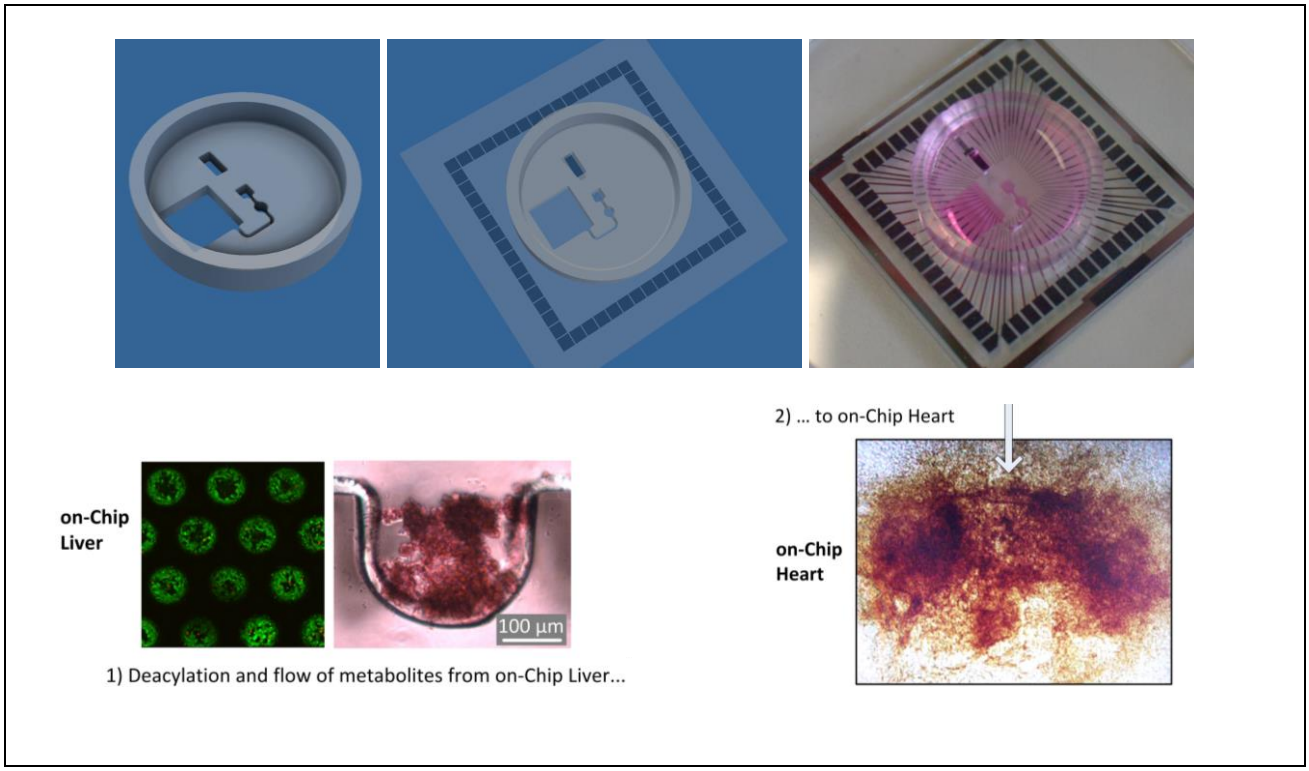

Figure 3: On a single chip 3D cultures of Hep G2 cells (on-Chip Liver) are connected to cultures of P19 derived cardiomyocytes (on-Chip Heart) via microfluidic channels. Acetylated Dihydropyrimidinone (DHPM) is added to the on-Chip Liver where deacylation, the removal of all acyl groups, is performed. The deacylated DHPM products are then delivered to the on-Chip Heart

RESULTS AND DISCUSSION

Acylated DHPM is added to the scaffolded Hep G2 cells, as seen in Figure 3. The Hep G2 culture removes acyl groups and delivers DHPM directly to the P19 derived cardiomyocyte culture. DHPM is an ideal candidate to demonstrate the interconnectivity of the on-Chip organs as acylated DHPM has no noticeable effect on the electroactivity of the cardiomyocytes, Figure 4 (left panel). However, as deacylation continues, the calcium channels are blocked and the beating of the cardiomyocytes stops, Figure 4 (right panel). Beating of the cardiomyocytes is restored by continued perfusion of the cultures with fresh medium.

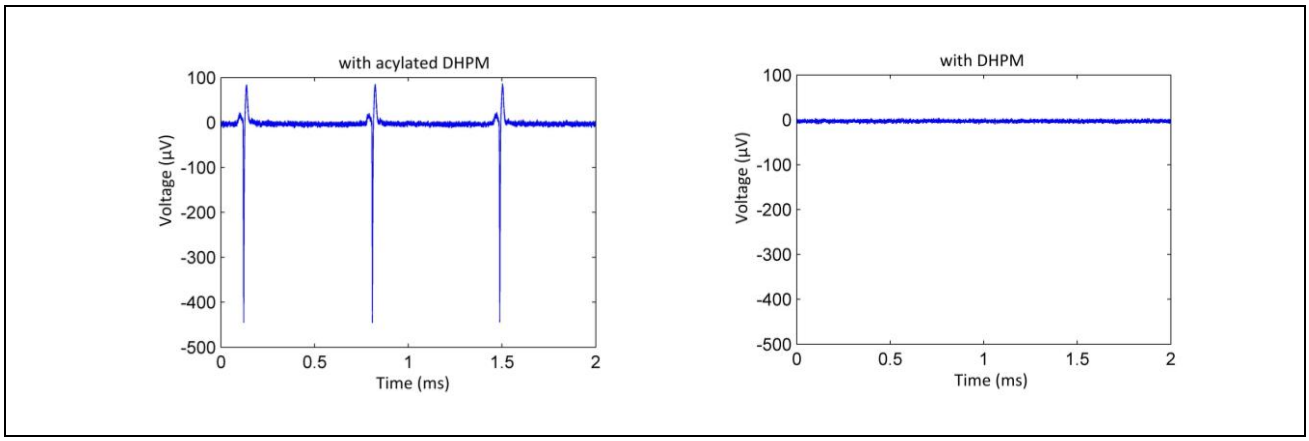

Figure 4: DHPM is a known calcium channel blocker however, in its acetylated form, the bulky acetyl groups on the 1 and 3 positions prevent the DHPM from fitting into the active site of calcium channels. As seen in the top panel, the addition of acetylated DHPM to the cardiomyocyte medium has no effect on electroactivity. However, as seen in the bottom panel, the addition of acetylated DHPM to the Hep G2 medium results in deacylation and delivery of pure DHPM to the cardiomyocyte culture, blocking calcium channel activity.

CONCLUSION

We have functionally demonstrated an interconnected on-Chip Heart and on-Chip Liver. This is a significant step for the future of Body-on-a-Chip. We also plan neuronal cultures as an on-Chip Brain and blood-brain-barrier to distinguish between metabolites destined for Heart and for Brain compartments.

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