Revolutionizing Drug Testing Platforms by Leveraging Angiochip and Flight Data Recorder Technologies

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For hundreds of years, animal models have been used as pre-clinical drug testing platforms to ensure drug safety and efficacy prior to administration to patient populations (1). However, the distinct physiology of animal primates renders this practice unsuitable for large-scale, translatable drug testing studies and has driven the use of human tissues as its own platform. At the University of Toronto, Dr. Milica Radisic's research group has designed a nano-scale "organ on a chip" called the Angiochip (2). Combining this novel technology with the Flight Data Recorder, which was recently developed by Dr. Samantha A. Morris at the University of Washington, will allow for independent testing of novel drugs while tracking the lineage and identity of individual cells in human tissues cultured on the Angiochip (3). This study could have great potential to increase the accuracy of current drug testing platforms and further advance our knowledge of cellular responses to novel drugs.

The Angiochip is a nano-scale chip that cultures endothelial and parenchymal cells to simulate human heart function (Figure 1) (4). Its unique host-vascularized network is created upon implantation using bioprinting techniques, hydrogels and various sacrificial materials (2). A well-established vascular system is essential for providing oxygen and nutrients while sustaining surrounding cells. Forming such a network while simultaneously replicating human tissue function and physiology has been challenging. Here, a host-vascularized tissue was developed to overcome the obstacle of creating a separate vascular system. Furthermore, the Angiochip has been shown to surgically anastomose, broadening its potential applications in the field of regenerative medicine to include implantation of the tissue scaffold. A bottom-up nanotechnology fabrication method with a synthetic biodegradable material called POMac was utilized to create this platform. This allows for permeability of molecules, vascular sprouting and cell migration through microholes in the channel wall, all permitting the parenchymal space to effectively simulate tis-

sue of the human heart. POMac layers were stacked through patterning and bonding to create a 3D structure. Subsequent UV cross-linking bonded the layers together to create a multi-layer microchip into which cells can be implanted. Specifically, endothelial cells were embedded along the luminal wall of the chip whereas parenchymal cells were encapsulated in the hydrogel surrounding the vascular network for easily accessible remodeling. Importantly, 100-micrometer wide channels functioned as blood vessels following the integration of cells in the device. This unique combination of culturing and fabrication techniques allows for the chip to simulate cardiac muscle contractions.

Figure 1. Diagram of Angiochip scaffold (a) Diagram of Angiochip tissue depicting parenchymal cells in the outer layer of the chip allowing easy accessibility. (b) Model of Angiochip scaffold showing microchannels imbedded with stacked POMac layers (4).

Epinephrine, a heart rate accelerating drug, was administered to the chip to determine its efficacy as a drug testing platform (2). As expected, epinephrine induced quicker electrical impulses and contractions along the entire Angiochip, mimicking physiological responses. Interestingly, it was also found that patient-specific responses can be simulated by injecting endothelial and parenchymal cells from the patient into the chip. These findings highlight the feasibility of using the Angiochip as a drug testing platform that can accurately recapitulate human cellular responses.

Cellular reprogramming is a vital component of regenerative medicine, allowing scientists to control cellular differentiation. It has great potential to improve treatments such as cellular therapies by providing wider accessibility to different cell types as they can be interconverted between specializations. However, the molecular stages that cells undergo for differentiation during cellular reprogramming are relatively unknown. To address this concern, the Flight Data Recorder has been used as a tracking method throughout experiments of reprogramming mouse fibroblast cells to induced endoderm fibroblasts, bypassing pluripotency (3). The technique functions similarly to a virus wherein tiny DNA barcodes named CellTags were inserted into each cell. As these cells divided, their unique barcodes were passed down to descendant cells that were monitored over a 28-day period. Throughout this period of time, the lineage of cells was tracked and identified through each stage of reprogramming. Various factors mediated the trajectory of successfully reprogrammed cells and created “pre-flight” conditions which were later added to the original cellular reprogramming cocktail. This approach improved the efficacy of cellular reprogramming, raising the possibility of achieving a near 100% accuracy rate. More specifically, the initial experiment utilizing the Flight Data Recorder tracked over 100,000 cell lineages and identified 2 trajectories. The first trajectory was a route towards successfully reprogrammed cells and the second was a path to a “dead-end” state meaning they did not reprogram effectively. The cell size of each clone was determined by the 28th day and more importantly, the gene *Mett17a1* was identified as a pre-flight condition. The presence of *Mett17a1* was shown to give a three-fold increase in the yield of successfully converted endoderm progenitors as compared to cell lines lacking the gene. It was then revealed that a combination of single-cell RNA sequencing and computation trajectory reconstruction can predict the outcome of reprogrammed cells as early as two days after CellTags are injected. Ultimately, this method is shown to be very effective, as CellTag expression was revealed in 99% of cells. In the future, this cell tagging method shows great potential as a cell tracking and identification method. Further, the Flight Data Recorder was able to reveal previously undiscovered characteristics of cell reprogramming and shows great utility towards improving the way that this is accomplished in the

future.

If used together, these technologies could hold great potential in the field of drug testing and design. Specifically, the molecular and cellular changes of individual cells embedded on the Angiochip platform upon drug administration can be tracked by the Flight Data Recorder to advance our understanding of drug effects on various cellular types (ref). The first step would include labeling endothelial and parenchymal cells with CellTags before they are implanted into the Angiochip. An in-depth analysis of the associated cells could reveal important information such as what creates the intended outcome of the drug to improve its effectiveness, the exact duration of a new drug, and fluctuations in cell sizes and drug targets. Through drug testing on the Angiochip, lineages of cells can be tracked in drug-induced and drug-free controlled conditions. Comparing both lineages would help to determine what factors lead to successful biological responses to the drug. These factors may act as pre-flight conditions and can then be accounted for in the initial drug formula so that a longer duration of its effectiveness can be achieved. Additionally, drug efficacy and effectiveness can be tracked over long periods of time at a cellular level. Tracking cells with CellTags and thorough analysis of cells at various waypoints could be helpful to see how the drug function changes over the 10 weeks in which CellTags are expressed. Fluctuations in cellular size can also be characterized; Bidy et al. have shown that a clone of cells defined by the library of CellTags called CellTagMEG increased in size by day 28 using the Flight Data Recorder (2). Additionally, as cells are reacting to a new drug in the Angiochip, clones of cells can be compared to each other at different time points to determine the minute effects of the drug which may not be found otherwise. Through comparing the biological reactions or imaging of the CellTag-labelled clones, the cell types that are targeted by the drug can be revealed. The factors that lead to a successfully induced drug response, or pre-flight conditions, may be found through a comparison of cell lineages, and this can also be an indicator of which cell types are affected by the drug. Moreover, cells can easily be taken as a sample from the Angiochip for analysis as the parenchymal cells are easily accessible from the outer layer of the chip, as shown in Figure 1 (4). Lastly, this method of combining both technologies can be effective, as CellTags are expressed in

99% of cells over 10 weeks meaning the drug can be tested for this duration with incredible accuracy (3).

In summary, it is evident that two revolutionary platforms, the Angiochip and the Flight Data Recorder, can create a unique platform for drug testing and potentially reveal biological factors that may improve drug safety and efficacy. Additionally, other technology, such as the Angiotube, may present a viable alternative to the Angiochip due to its more efficient fabrication process, begging the need for further research on this platform. Continuous experimentation is being done on the Flight Data Recorder to improve the accuracy of cellular reprogramming to liver tissue and create a further understanding of cellular reprogramming mechanisms. Furthermore, interdisciplinary collaborations are increasingly common and should be strongly encouraged to maximize innovation in technologies such as the Flight Data Recorder and the Angiochip. Thus, in order to prevent harm caused due to testing on human patients or inaccurate animal testing, platforms that culture human tissue, like the Angiochip in combination with the Flight Data Recorder, present an exciting opportunity. This technology could be highly beneficial to understand minute and crucial mechanisms of new drug reactions *ex vivo* and even accelerate the rate of success for drug efficacy.

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