Diabetic Complications Consortium

Application Title: Development and functional analysis of a human adipose tissue chip

Principal Investigators: Patrick Seale, Ph.D. & Dan Huh, Ph.D

1. Project Accomplishments

We developed a PDMS-based 3D microfluidic device containing *in situ* differentiated human adipocytes and perfusable interconnected vasculature formed by human endothelial cells (hEC). We are able to assay functional parameters of adipocyte function in our devices, including hormone secretion and lipolysis.

2. Specific Aims

Aim 1: To develop a 3D microfluidic device engineered for long-term co-culture of human stem cells and vascular endothelial cells in a physiological 3D microenvironment which replicates the microarchitecture of human adipose tissue.

Several iterations of microfluidic devices (Fig. 1) were developed and tested to obtain a practical design. Some of the engineering objectives included: 1) an interface for microfluidic pump-driven perfusion, with distinct in- and out-flow ports for sampling of pre- and post-adipose-conditioned medium; 2) a biocompatible substrate for three-dimensional co-culture of adipocytes and vascular endothelium in a biological extracellular matrix (ECM); 3) optical clarity to allow live imaging of cultured cells; 4) support for in situ differentiation of intermixed tissue precursors; 5) adequate culture volume to provide material for functional assays; 6) ease of device assembly; 7) simplicity of tissue insertion/assembly into device.

A polydimethylsiloxane (PDMS)-based device attached to a glass microscope slide was chosen as the basis for the design. PDMS has been widely used in such devices due to its favourable material characteristics for this application, including biocompatibility, stability, optical clarity, and ease of handling. Due to the design flexibility of this material, we were able to generate a number of device variants to address practical issues that arose as we developed our system. Although the initial design ("phase-guide device", Fig. 1A-C) was compatible with many of our objectives, our early tests revealed that it was very technically challenging to insert cell/ECM mixture into the device. Surface tension effects were used to constrain the injected mixture to the central channel; however, the high viscosity of gelling ECM and decreased attachment surface tended to make the format very sensitive to handling for successful loading. "Embolisms" of cell/ECM mixture tended to intrude into the exterior channels during culture, disrupting or preventing the microfluidic flow through them.

The early phase-guide device was redesigned in a "peapod" format (not shown) to trap the cultures in serial chambers between the flow channels. Once again, surface tension effects allow the unpolymerized ECM/cell mixture to move into and be retained in the chambers. After gelling of the ECM, the "peas" of 3D matrix are trapped. Flow across the device between the adjacent flow channels occurs through the vasculature formed by the differentiated endothelial cells, driven be a pressure gradient across the device. However, although this format was more successful at retaining the tissue in the correct locations

than the earlier version, it remained challenging to correctly load the device. Furthermore, the small culture volume was a potential barrier to being able to assay biological function due to sensitivity limits.

Our current design bypasses the phase-guide approach and separates the manufacture of the culture chamber and flow channels into distinct steps. The flow channel are preserved but physically obstructed by inserting syringe needles along their paths, and the upper surface of the device is left open for simple access to the culture chamber (Fig. 1D). A cell/ECM mixture is loaded by pipetting into this chamber; after gelling of the 3D matrix, culture medium is overlaid to provide nutrients to the cells by diffusion into the ECM. Endothelial and adipose cells are differentiated in static culture under the pool of medium. After several days, the syringe needles are withdrawn and the flow channels are seeded with additional vascular endothelial cells to coat their surfaces. These EC-lined "vessels" anastomose with the capillaries formed within the central chamber, producing perfusable connections between the two channels across the device.

Aim 2: To characterize and validate the fat-on-a-chip model through quantitative analysis of tissue specific hormone secretion, lipolysis, and glucose uptake.

In addition to addressing the manufacturing and design challenges of the mechanical device, we also examined interactions with the biological components going into it. As a three-dimensional culture model, the cellular constituents are embedded within a biological extracellular matrix (ECM) to provide cytoskeletal attachment and support. A tumor-derived ECM (Matrigel/Geltrex) was initially used for ease of handling. However, the undefined nature of this product was a concern moving forward. Therefore, we transitioned to a collagen/fibrin scaffold that produced a reproducible and stable gel that adhered well to the underlying PDMS structure and was compatible with the growth and differentiation of the adipose and endothelial progenitors. Our current working mixture uses 0.15% collagen and 1.3% fibrinogen cleaved *in situ* with thrombin.

Several approaches were used to populate the devices with human adipocytes. Initially, human adipose stem cells (hASC) were mixed with ECM components and flowed into the devices as a suspension, after which the ECM would gel and restrain the hASC in the culture channel. Treatment with our standard cocktail of adipose inducers (insulin, T3, dexamethasone, IBMX, rosiglitizone) in static culture produced a low level of differentiation into lipid droplet-containing adipocytes after several weeks. Compared with standard tissue culture (2D) or droplet (3D) culture, the efficiency of differentiation was lower than desired.

Pre-differentiation of hASC outside of the device was considered as a way to obtain a high density of mature adipocytes. Following maturation of the adipocytes in 2D culture on plastic, cells were trypsinized and resuspended in ECM for injection into devices. This resulted in a device containing a high density of lipid droplet-rich adipocytes. Although successful in this respect, this approach was less desirable than in situ differentiation since the goal was a co-culture device with endothelial cells for vascularization. The idea may be applicable for introducing tissue-derived primary adipocytes from biopsy samples, and we are continuing to examine this. However, changes in device geometry and culture conditions have improved our results in hASC/endothelial cell co-culture.

Vascular differentiation was also tested independently. Early attempts with human umbilical vascular endothelial cells (HUVEC) successfully produced an interconnected web of capillaries after 1-2 weeks of culture. Reports in the literature of variation in endothelial cell phenotype between tissues led us to test human adipose microvascular endothelial cells (hAMEC) for compatibility with our system. hAMECs differentiated more efficiently into a denser network of vessels than the HUVECs. In addition to being derived from the "appropriate" tissue source (adipose) for our device, this improved differentiation led us to use hAMECs in our subsequent work.

In order to achieve a function fat-on-a-chip system, we co-cultured hASC and hAMECs in ECM in devices for differentiation and visualization. Differentiated adipocytes were be visualized by microscopy by immunostaining for the lipid droplet protein Perilipin-A (Fig. 2A), while the vasculature was observed by immunostaining for the endothelial cell surface protein CD31 (Fig. 2B). Anastomosis and perfusability of the capillaries across the device is illustrated by following fluorescent microbeads as they pass through the vascular network from one flow channel to another (Fig. 2C). Co-culture of differentiated adipocytes and endothelium in devices is shown by co-staining for both Perilipin-A and CD31 (Fig. 2D). This design fulfills our major design objectives and represents a prototype for culturing hAMEC-derived vasculature and hASC-derived adipocytes in a perfusable and optically accessible microfluidic device.

We have demonstrated several assays of adipose function for the phase-guide variant of our system. For example, we showed by human leptin ELISA that our differentiated hASC secrete detectable levels of leptin into the culture medium (Fig. 3A). As well, our early tests using a phase-guide device populated with pre-differentiated mouse adipocytes and attached to a microfluidic pump demonstrated that we can assay isoproterenol-stimulated lipolysis in the microliters quantities flowing through the chip (Fig. 3B). These levels are comparable to our measurements in human adipocytes in 3D droplet culture (Fig. 3C). Therefore, we conclude that the volumes and sensitivities are applicable on this device scale. We expect our current device to function within a similar range as these earlier tests.

Ongoing work is oriented towards refining our methods for further improving the efficiency of adipose and endothelial cell differentiation. Adipose differentiation using our existing stock of cells is a languid process, with good morphology achieved in about one month. Recently, we've gained intermittent access to surgical adipose tissue from which we can derive a primary human stromovascular fraction (hSVF). These freshly derived cells differentiate substantially more robustly and quickly (~2-3 weeks) than passaged cells, which require 1-2 months to achieve a robust differentiated phenotype (Fig. 4). We anticipate that using these primary hSVF will improve the adipose character of our devices by producing more high-quality adipocytes among the vessels.

Aim 3: To establish a microengineered disease model of adipose tissue inflammation for the study of obesity-relevant macrophage biology.

Now that we have achieved a functional device design, we are anxious to begin manipulating the components to ask biological questions about tissue function, including the interactions between cell types. Adding macrophages to the hASC/hAMEC/ECM mixture during the initial device seeding, or adding macrophages to the culture medium after differentiation will be a priority. We are also interested

in developing a model of adipocyte insulin-resistance by using hASCs from insulin-resistant subjects and/or genetic/pharmacological manipulations.

3. Publications

A publication describing the model is planned in the next 2-3 months.

Figures

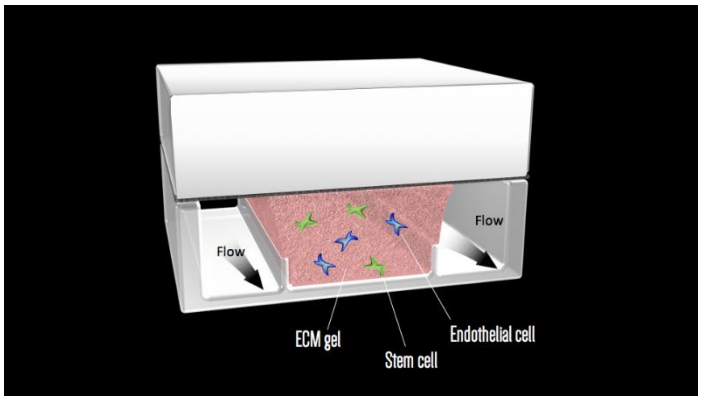


Fig. 1A: Conceptual design of phase guide device filled with cells and ECM.

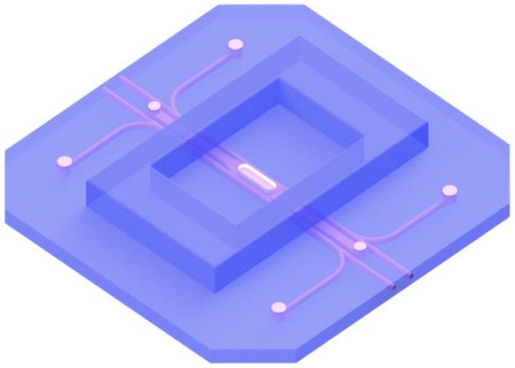
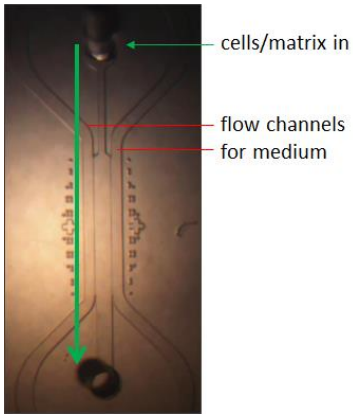


Fig. 1B: Conceptual model of phase guide device.



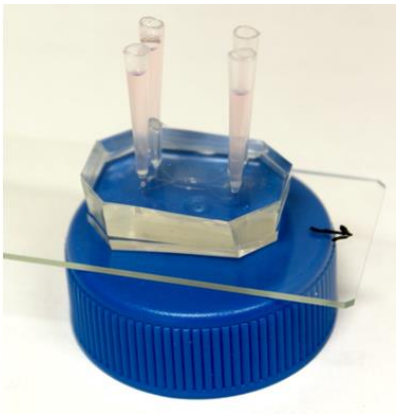


Fig. 1C: Implementation of phase guide device for static culture.

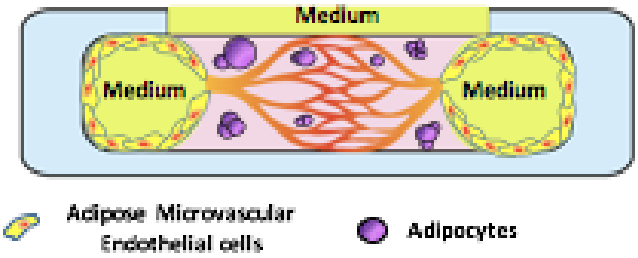


Fig. 1D: Conceptual design of channel/chamber device

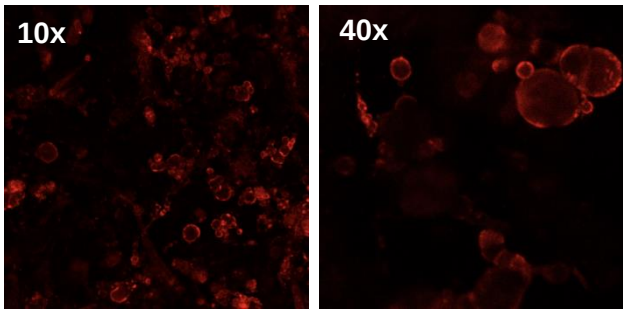


Fig. 2A: Differentiated adipocytes in device, visualized with anti-Perilipin A staining

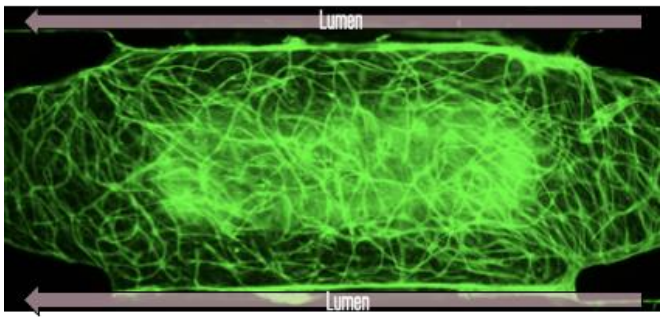


Fig. 2B: Anti-CD31 immunostaining of differentiated endothelial vasculature in device.

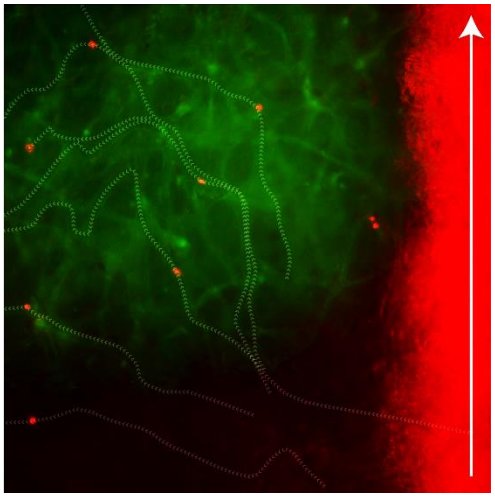


Fig. 2C: Anastomosis and perfusability of the capillaries illustrated by flowing fluorescent microbeads through the vascular network from one flow channel to the other.

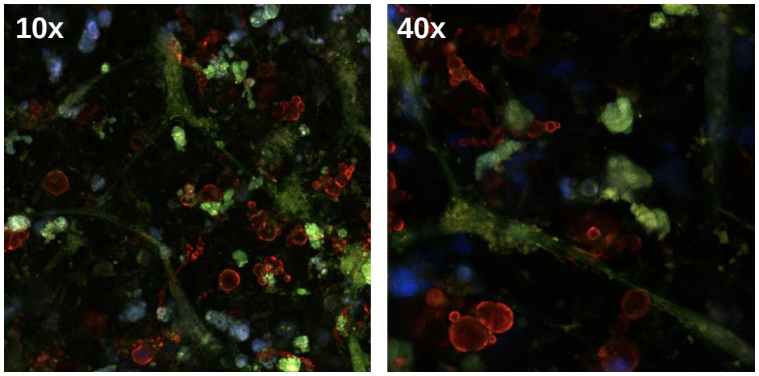


Fig. 2D: Immunofluorescence in device using anti-CD31 (green) and anti-Perilipin A (red) to visualize vasculature (CD31+) and adipocytes (Perilipin A) in co-culture. (Hoechst, blue).

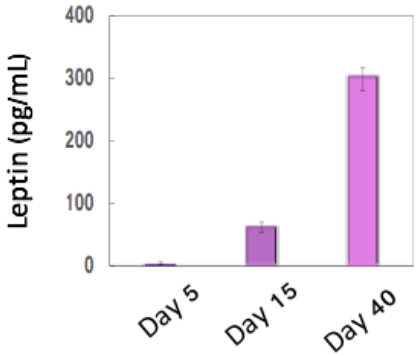


Fig. 3A: ELISA for leptin secretion from human adipocytes

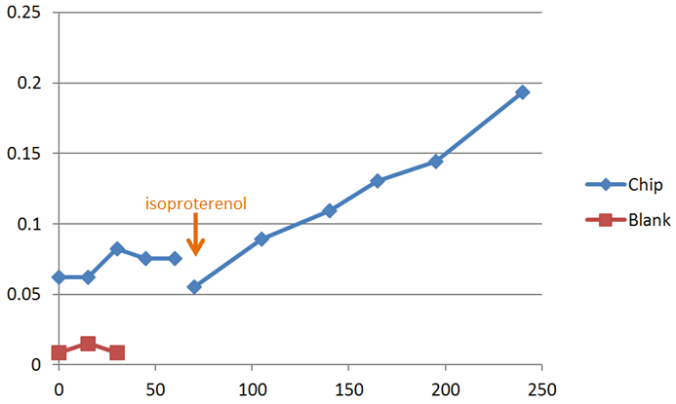


Fig. 3B: Isoproterenol-stimulated lipolysis in mouse adipocytes in phase-guide device.

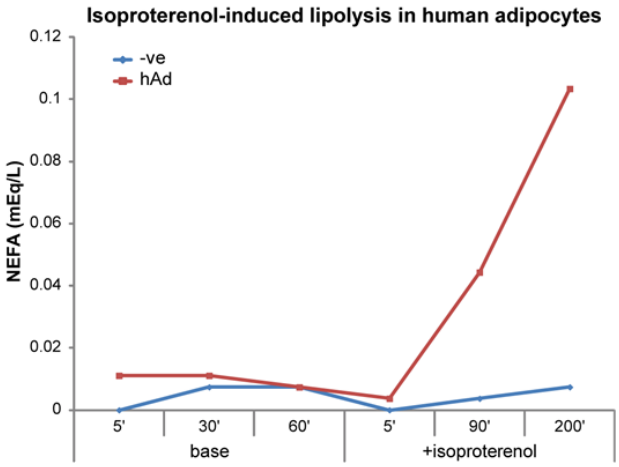


Fig. 3C: Isoproterenol-stimulated lipolysis in mouse adipocytes in phase-guide