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REVIEW ARTICLE

Fat-On-A-Chip Models for Research and Discovery in Obesity and Its Metabolic Comorbidities

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The obesity epidemic and its associated comorbidities present a looming challenge to health care delivery throughout the world. Obesity is characterized as a sterile inflammatory process within adipose tissues leading to dysregulated secretion of bioactive adipokines such as adiponectin and leptin, as well as systemic metabolic dysfunction. The majority of current obesity research has focused primarily on preclinical animal models *in vivo* and two-dimensional cell culture models *in vitro*. Neither of these generalized approaches is optimal due to interspecies variability, insufficient accuracy with respect to predicting human outcomes, and failure to recapitulate the three-dimensional (3D) microenvironment. Consequently, there is a growing demand and need for more sophisticated microphysiological systems to reproduce more physiologically accurate human white and brown/beige adipose depots. To address this research need, human and murine cell lines and primary cultures are being combined with bioscaffolds to create functional 3D environments that are suitable for metabolically active adipose organoids in both static and perfusion bioreactor cultures. The development of these technologies will have considerable impact on the future pace of discovery for novel small molecules and biologics designed to prevent and treat metabolic syndrome and obesity in humans. Furthermore, when these adipose tissue models are integrated with other organ systems they will have applicability to obesity-related disorders such as diabetes, nonalcoholic fatty liver disease, and osteoarthritis.

Keywords: ADMET, ASC, BAT, fat-on-a-chip, microphysiological system, WAT

Impact Statement

The current review article summarizes the advances made within the organ-onchip field, as it pertains to adipose tissue models of obesity and obesity-related syndromes, such as diabetes, non-alcoholic fatty liver disease, and osteoarthritis. As humanized 3D adipose-derived constructs become more accessible to the research community, it is anticipated that they will accelerate and enhance the drug discovery pipeline for obesity, diabetes, and metabolic diseases by reducing the preclinical evaluation process and improving predictive accuracy. Such developments, applications, and usages of existing technologies can change the paradigm of personalized medicine and create substantial progress in our approach to modern medicine.

Introduction

Obesity is an epidemic that is currently causing significant economic and societal consequences worldwide. The medical costs associated with obesity and obesity-related conditions are estimated to reach two trillion dollars, which comprise 9% of the world's health expenditures. In addition, the high mortality associated with this disease results in $\sim$2.8 million deaths annually.[1,2] Both the high medical costs and high mortality rates associated with obesity are a result of a multitude of health complications/conditions associated with this disease. As the mean body mass indexes in both adults and children continue to increase, obesity-related conditions, such as diabetes, nonalcoholic fatty liver, and osteoarthritis, are becoming more prevalent in society.[3] There is increasing evidence linking

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each of these diseases to the systemic impact of adipose tissue dysfunction due, in part, to the excessive secretion of adipokines, fibrotic inductive factors, inflammatory cytokines, and lipids.[4–9] Industrialization, sedentary lifestyle, easier access to processed foods, and obstacles to accessing healthier foods have all contributed to the global rise of obesity and its associated comorbidities.[4]

Obesity is associated with a persistent inflammatory condition often characterized as a sterile inflammatory process due to the absence of overt infectious agents. Obesity is accompanied by collagen deposition and fibrosis within adipose depots leading to imbalances in cytokine, free fatty acid (FFA), glucose, insulin, and leptin secretion at the level of individual adipocytes.[5] In obese patients, excessive adipose tissue growth and hyperplasia is frequently not accompanied by commensurate levels of angiogenesis and neovascularization, resulting in restricted blood flow to adipocytes and the creation of a hypoxic state that triggers macrophage infiltration of adipose tissues.[5] These macrophages then promote a pro-inflammatory state, increasing secretion of interleukin (IL)-6 and tumor necrosis factor (TNF)-α, while also decreasing secretion of adiponectin.[6] IL-6 promotes hepatocytes to produce C-reactive protein, a marker of chronic inflammation, which is persistently elevated in obese individuals. Independently, TNF-α impedes the storage capacity of adipose tissue by interfering with carbohydrate metabolism, lipogenesis, adipogenesis, and lipolysis.[7] Conversely, adiponectin is responsible for anti-inflammatory effects through M2 macrophage polarization, but its secretion is inhibited by elevated insulin levels seen in the obese.[8]

Moreover, abundant adipose tissue increases the release of FFAs and leptin. Elevated FFA levels decrease insulin sensitivity resulting in elevated glucose levels. The imbalance of cytokines, FFA, glucose, and insulin induces adipocyte enlargement, pancreatic islet β cell death, atherosclerosis, and fatty liver.[9] Macroscopically, these effects result in an array of medical conditions commonly associated with obesity, including increased abdominal fat, high blood pressure, high blood sugar, high serum triglycerides, and low serum high-density lipoprotein. When at least three of these five conditions are present, the diagnosis of metabolic syndrome may be applied.[10] Individuals with metabolic syndrome are at increased risk for heart disease, diabetes, and stroke.[11] Obesity is also highly associated with an increased risk of cancer of the liver, stomach, gallbladder, pancreas, ovaries, thyroid, breast, colon, and brain.[12,13] Obesity-related cancers are responsible for 20% of all deaths from cancer.[14] Although the mechanisms by which obesity increases cancer incidence vary, the chronic inflammatory state induced by obesity, most notably the upregulation of IL-6, is pro-oncogenic.[15]

The primary treatment of obesity is weight loss achieved through a combination of healthy eating behaviors, physical activity, medication, and surgery. The challenges associated with weight loss are numerous and present many risks for patients. For example, increased oxygen demands from exercise can overwhelm the already compromised vascular network of obese individuals, causing further hypoxia and tissue damage.[16] Overly aggressive diets can cause gallstones, nutritional deficiencies, and dehydration if the dieter is not careful. Weight loss medications are limited by their cost and side effects. Although surgery has the greatest potential to decrease weight rapidly, it is expensive and its invasive nature carries the risk of further complications (internal bleeding, perforation, long recovery time, etc.). Thus, responding to a public health threat as prevalent, expensive, and complex as obesity will necessitate better tools built using a new generation of advanced models.

Challenges Facing Adipose Tissue Biology and Obesity Research

Combatting the obesity epidemic has proven to be one of the greatest health challenges of the modern era. Obesity's multifactorial causes and complex effects on the body require further research and understanding the problem before novel solutions for treatment and prevention can be developed. However, this can be difficult to achieve from studies using human subjects since interpretation can be complicated by biological variability due to genetic and epigenetic factor heterogeneity. One solution to this problem has been the usage of animal models. Animal models offer greater control of confounders and can look at systemic effects of changes in adipose tissue on other organ systems. Animal models can be loosely divided into monogenic or polygenic models. Monogenic models refer to animals that either lack a gene or have a dysfunctional gene. These models are used to study a particular absent or dysfunctional gene. In contrast, polygenic models refer to animal models that maintain their genetic integrity but are subjected to different factors to induce a desired condition (i.e., obesity). The factors utilized to induce the desired condition can be surgical, chemical, dietary, or environmental.[17]

Early research into the apparent long-term stability of weight suggests that an axis between body fat and the brain exists to regulate energy intake and expenditure.[18] The majority of animal models used to investigate the causes and treatments for obesity have focused on altering this axis to control satiation and limit energy intake.[17] However, this strategy is limited by the complicated nature of how organisms regulate their eating behaviors; thus, any pharmacologic solution that attempts to target a single pathway faces a substantial challenge.[19] In addition, attempting to increase energy expenditure rather than to limit energy intake is problematic as it is subject to species-dependent translational barriers.[20]

Limitations of animal models

In obesity research mouse models are the most commonly used nonhuman models. Mice provide researchers several inherent advantages over other animals. These include their short life span and breeding cycle, general ease of use, low upfront and maintenance costs, availability of genetically identical strains, well established protocols for genetic manipulation, and historically generated data.[21] However, despite the widespread adoption of mouse models for obesity research, they are suboptimal in several key aspects. One difficulty is inducing obesity in mice. Creating long-standing obesity in mice often requires significant dietary modifications in combination with genetic and chemical modifications. This simultaneous introduction of multiple variables significantly limits the generalizability of mouse-based experiments to human biology.[17] On multiple occasions, drug candidates developed from mouse trials showed either no effect on obesity in humans or, in the instances where an

effect was seen, resulted in significant adverse side effects that were only seen in humans.[22] One particularly notorious example was the appetite suppressant drug, fenfluramine. Fenfluramine was approved for the U.S. market in 1973 after successful mouse testing; however, in 1997, the drug had to be withdrawn from the market after as many as one-third of patients who were taking the drug were diagnosed with pulmonary hypertension and cardiac valvulopathy.[23,24]

While mouse models have taken hold as the premier economical animal obesity model for early stage research, nonhuman primate (NHP) models represent the other end of the spectrum. NHP models have traditionally served as the bridge between mouse and human models for advanced stage studies due to their close resemblance to human models. Unlike in mice, obesity is easily induced in NHPs, even unintentionally, through carbohydrate heavy diets. In general, the comorbidities that accompany obesity in humans such as hypertension, increased visceral adiposity, dyslipidemia, and secondary insulin resistance can be mirrored in NHP models.[25] While NHPs represent a valuable and physiologically relevant model that can serve as an important translational bridge between basic studies performed in rodent models and clinical studies in humans, they are limited by high costs and complicated ethical issues that surround their usage in research.

Advancing knowledge based on adipose structure and function in health and disease

The costs and ethical issues associated with animal testing for new pharmaceuticals have incentivized researchers to utilize *in vitro* experiments to refine, reduce, and replace animal testing whenever possible. Experiments performed *in vitro* instead of *in vivo* are capable of providing faster results, and those using human-derived tissues can yield results with a high likelihood for external validation based on assays and outcomes generated in subsequent clinical trials.[26] Much of this obesity-focused *in vitro* research has centered on the study of intact adipose tissue itself. Of the two major types of adipose tissue found in the body, the most common type is white adipose tissue, a loose connective tissue composed predominantly of white adipose cells which specialize in storing energy as fat. In contrast, the less common brown or beige adipose tissue is composed of brown or beige adipocytes, which consume energy to generate heat.[27] In addition to adipocytes, adipose tissue also contains adipose stromal/stem cells, endothelial progenitors, fibroblasts, vascular smooth muscle cells, B and T lymphocytes, and macrophages which support the tissue.[28] Recently, studies have begun to apply mathematical models to explain the complexity of adipose depots in three dimensions.[29]

Adipose tissue also interacts with the body's immunological and endocrine systems in significant ways. These interactions can involve different compartments both within the adipose tissue and within different organs entirely. Depending on the complexity of an experiment, it can become necessary to simulate these interactions among systems when attempting to use adipose tissue *in vitro*. For example, obese adipose tissue cells are significantly more resistant to insulin and contribute to changes in the overall composition of the tissue. Compartmentalized regions within *in vitro* models may be necessary for the accurate simulation of vascularization, stromal/stem regional activities, and nutrient/waste movement through adipose tissue.

The signaling and transport of factors through the vascular system involve contributions from multiple organs and tissues. In obesity, the signal and transport of factors through the vascular system are challenged by the decreased blood flow, arising from impairment of the vascular system from vasodilation to vasoconstriction, arterial structural changes, and limitations in microvessel function.[16] In addition, the lack of capillaries and blood flow from rapid overgrowth of fat cells can cause adipose tissue to undergo a chronic inflammatory state resulting from hypoxia. This reflects the fact that obesity is a complex disease involving many tissues/cells connected through a vascular network. These components must be integrated into sophisticated *in vitro* models to ensure accuracy. Recapitulating hypoxic environments is also likely to be an important feature in diseased models.

Obesity is associated with elevated circulating levels of pro-inflammatory cytokines, IL-6, TNF-α, and C-reactive protein, due to increased leptin and FFA release from excess adipose tissue.[30] Adipocytes are hypertrophied and hyperplastic in obese tissue, causing microvessels within the adipose tissue to lose the ability to transport oxygen, thereby contributing to a hypoxic state. In turn, hypoxia causes adipocytes to necrose and upregulates expression of inflammatory cytokines while attracting an infiltration of macrophages.[31] In addition to the general infiltration of macrophages, decreased adiponectin levels cause resident and recruited macrophages to be induced to the M1 (classical) phenotype with a pro-inflammatory state.[16] In addition, excess growth of adipocytes causes cellular stress, especially in the form of an unfolded protein response within the endoplasmic reticulum. This leads to more reactive oxygen species release/stress within the tissue.[9] Thus, an accurate simulation of the chronic inflammation component of obesity in *in vitro* models will require the incorporation of an appropriate immune compartment.

Moreover, excess adipose tissue from obesity is accompanied by a change in the adipokine secretome such that normal endocrine function becomes disrupted.[32] These adipokines not only affect the local tissue at which it was released through paracrine actions but also other distant organs using a systemic endocrine response. Therefore, multilevel endocrine functionality in *in vitro* models also must be intact, as insulin and leptin play a pivotal role in fatty acid synthesis and glucose uptake.[33] Leptin's main purpose is to suppress hunger when there is adequate nutrition within the body, but obese patients' resistance to leptin contributes to the need to seek a surplus of energy. In addition, leptin has a reproductive function, as it stimulates the hypothalamus in regulating gonadotrophic releasing hormone, which directly affects luteinizing hormone and follicular stimulating hormone levels.[34]

In vitro models of obesity must exhibit the behaviors of the disease in all stages to further understand the implications of treatment at different time points. The models should also be able to recapitulate patient data, as obesity symptoms vary from person to person. Finally, *in vitro* models must recapitulate the adsorption, distribution, metabolism, elimination, toxicity (ADMET) of drugs that are found *in vivo*, as fat plays a pivotal role in the ADMET of drugs.[35] Adipose tissue compromises anywhere from 20% to

50% of the body weight of an adult acting as a storage and release depot. Thus, modeling the human adipose profile could provide more accurate targeted drug delivery, dosing, and decreased toxicity.

Three-Dimensional Cultures—Adipose on Chip Models

Within the ''organ-on-chip'' field, ''chips'' are presented as either microfluidic or static multiwell cultures and are seeded with cells that take advantage of cell–matrix and cell–cell interactions and/or fluid flow to more accurately simulate physiologic conditions. These chips are also referred to as microphysiological systems (MPS). Chips used for such purposes are either manufactured as multiwell dishes or by photoetching a pattern of channels and chambers onto silicon wafers.[36,37] Both methods are easily adaptable to high throughput applications with sample sizes occurring in the 96-well format or higher. Conventional experimental models culturing animal cell lines or human derived cell lines have been either seeded on plates for two-dimensional (2D) studies or scaffolds for three-dimensional (3D) studies.[38,39] Unfortunately, the utility of traditional *in vitro* pharmaceutical studies has historically been limited by their inability to accurately simulate physiologic conditions. Adipose tissue is exceptionally reactive to the environment around it, as it dynamically stores and releases energy in response to hormonal and energetic cues. As a result, animal cell lines, such as murine 3T3-L1, that undergo adipogenesis during *in vitro* culture lack the single large lipid droplet that is characteristic of ''signet ring-like'' cells *in vivo*.[39]

Even among *in vitro* experiments, results from experiments can differ depending on how closely they emulate an *in vivo* environment. Most models have been found to lack control of major variables, such as fluid volume ratios. Thus, they poorly emulate a physiologic environment. Likewise, in a 2D geometry, cells cannot exhibit many of the behaviors that are normally seen *in vivo*.[38] A common adopted solution to this problem is the replacement of a 2D structure with a 3D structure created using biomaterials such as collagen or silk.[32,33,37,38,40,41] While the difference between 2D and 3D *in vitro* experiments is significant, the applications for such scaffolds also extend to *in vivo* models where the inclusion of biomaterial scaffolds can limit resorption and increase the engraftment of implanted cells.[42,43]

Perhaps the most significant issues associated with *in vitro* models come from their inability to simulate an environment with multiple compartments. This limits the utility of any model using adipose tissue as it is becoming increasingly accepted that adipose tissue has significant endocrine functionality necessary to maintain homeostasis.[44] Such constraints prevent *in vitro* studies from evaluating a drug's interactions with many processes, such as ADMET.[35] To create a better model capable of meeting these requirements, multiple organ systems, or outputs from multiple organ systems, must be simulated simultaneously. While this can be accomplished in a variety of ways, organ-on-a-chip systems are among the most promising.

Mature adipocyte models

Culturing mature, primary white adipose cells poses unique challenges. White adipose cells are characterized as being extremely buoyant, which limits their ability to attach to a surface. Traditional methods have utilized biomaterials to address this challenge by taking advantage of buoyancy as property. Harms *et al.* used a variation on the floating culture model to maintain isolated mature adipocytes from mouse and human tissue.[45] By placing the isolated mature adipocyte fraction released by collagenase digestion beneath a transwell insert, the mature adipocytes remained viable for up to 2 weeks. Based on extensive polymerase chain reaction (PCR) and RNA-Seq analyses, the adipocytes continued to express a panel of adipogenic biomarkers. Furthermore, the adipocytes could be transduced with an adenoviral vector expressing the PPAR gamma coactivator 1 (PGC-1) transcription factor to induce expression of biomarkers consistent with a beige/brown phenotype.[45] Huber *et al.* used a photocrosslinkable methacrylated gelatin scaffold to culture mature human adipocytes for periods of up to 2 weeks, which maintained both viability and expression of lineage specific biomarkers such as the lipid vacuole associated protein, perilipin.[46] The period of UV light exposure could be adjusted to alter the mechanical properties of the scaffold, thereby mimicking the stiffness of both healthy (less stiff) and obese (more stiff) adipose tissue *in vivo*.[46] Louis *et al.* achieved similar results using directly mature adipocytes or by differentiating human adipose-derived stromal/stem cell (ASC) and maintaining them for up to 3 weeks in a collagen scaffold.[47]

Furthermore, they noted more robust adipogenic differentiation in the 3D compared to the 2D culture conditions.[47] Additional alternatives have been introduced to allow direct manipulation of adipose tissue fragments.[48–50] For example, rather than maintain adipose tissue fragments floating in culture, which only maintains viability for several days to a week, the fragments containing mature adipocytes have been sandwiched in the mature adipocytes between tissue engineered sheets of adipose stromal/stem cells. The interest in adding ASC to sandwich the mature adipocytes resulted from two lines of reasoning. First, the ASC provides a structural extracellular matrix framework that can encompass and support the mature adipocytes. Second, the ASC provides paracrine growth factors that promote/retain adipogenic differentiation and functionality. Published models by the Fried laboratory and others have routinely kept adipose organoids in suspension culture although these only maintain robust longevity routinely for periods of up to 1 week in culture.[51] The sandwich approach prevents the adipocyte buoyancy from floating the fragments into the culture media and allows mature adipocytes to remain viable and functional for up to 5 weeks.[49]

Preadipocyte cell line and primary cell models

An alternative that avoids the buoyancy issue of primary adipocytes has been the use of murine preadipocyte cell lines. These cells can differentiate into mature white adipocytes while remaining attached to a plastic surface. Of these murine cell lines, 3T3-L1 and 3T3-F442A have historically been used for modeling white adipose tissue *in vitro* in 3D structures.[32,35,52,53] However, this alternative comes with its own limitations, which include a prolonged incubation period and the fact that they are derived from a single clone.[35,54] It is noteworthy that while murine

preadipocyte cell models display a robust *in vitro* lipolytic response to isoproterenol and glucose uptake response to insulin, these are not necessarily reflective of their human counterparts, which frequently do not exhibit such a pronounced functionality in assays. As a human alternative to these murine cell lines, Simpson-Golabi-Behmel syndrome preadipocyte cells can be used due to their capability of retaining the adipogenic differentiation potential for up to 50 generations.[55] In addition, primary human ASC isolated from human adipose tissue, culture expanded as preadipocytes, and cryopreserved for future use can be used in such cultures.[56] Well established methods and commercially available media reagents are available to promote the adipogenic differentiation of ASC within a 1- to 2-week period, and these approaches have been validated in both 2D and 3D culture models.[57,58] Following exposure to inductive factors, including phosphodiesterase inhibitors capable of elevating cyclic AMP levels and ligands for the glucocorticoid, insulin, and peroxisome proliferator activated receptor γ (PPARγ), human and murine ASCs display a time dependent expression of early (PPARγ, CCAAT enhancer binding protein [C/EBPα]) and late (adiponectin, adipsin, fatty acid binding protein 4, leptin, lipoprotein lipase) genes associated with adipogenesis, as well as vacuolar accumulation of neutral lipid; however, unlike mature adipocytes in the body where lipid bodies coalesce into a single large vacuole occupying >90% of the cell volume, *in vitro* induced adipocytes frequently display multiple, smaller lipid vacuoles, suggestive of a less mature phenotype. Recent studies by Volz *et al.* have compared the adipocyte functionality of mature human adipocytes to differentiated primary ASC when encapsulated in collagen scaffolds.[59] While both models expressed adipogenic mRNA biomarkers at similar levels, the primary ASC model displayed reduced maturity and increased anabolic indicators relative to mature primary adipocytes. Thus, mature adipocyte models remain an important control for future evaluation of adipose-on-a-chip models.[59]

Potential of induced pluripotent cell-derived cells in 3D adipose tissue models

Induced pluripotent cells (iPSCs) hold the promise to advance regenerative medicine through disease modeling, drug development, and organ synthesis. They share the ability of embryonic stem cells (ESCs) to differentiate into all three germ layers. Unlike ESCs, iPSCs do not require embryos as their source. Instead, iPSCs can be derived from a patient's own somatic cells, allowing the individual to serve as his or her own stem cell source with a lower risk for transplant rejection for regenerative applications and the potential for patient specific *in vitro* models. Because of their pluripotentiality, iPSCs hold a unique potential value in allowing investigators to isolate primary adipose-derived cells from patients with common or rare genetic conditions and explore the impact of those genomic mutations or variants not only on adipocytes but also on any cell type in the body.

iPSCs are generated when somatic cells express a panel of transcription factors such as Oct3/4, Sox2, c-Myc, and Klf4.[60] This finding was first demonstrated by Takahashi and Yamanaka after screening different ESC transcription factors based on pluripotency induction.[60,61] There are now several methods to induce pluripotency, including non-integrating viral transfections, chemical agents, and non-viral integration approaches. However, the preexisting variations in the somatic cells themselves, the process of transduction, and the continuing passaging of the cells can introduce a risk of mutation.[62] While the efficiency is very low—about 0.1%—research demonstrates that these odds can be improved through methods such as protein engineering.[63] In addition, iPSCs display pluripotency based on a teratoma assay, which is the standard iPSC validation method. When implanted *in vivo*, iPSCs form tumors that exhibit properties of all three germ layers,[64] based on staining for markers of differentiation (ectoderm, β-III-tubulin; mesoderm, Vimentin; and endoderm, CK AE1-AE3).[65] It is noteworthy that a number of iPSC lines have been successfully generated from both human and murine ASCs with relatively high efficiency.[66,67] Overall, iPSCs hold considerable promise for advancement of regenerative medicine due to their proliferative properties and telomerase activity, patient and disease specificity, and pluripotency.[68]

Beige/brown adipose tissue phenotypic models

These same technologies have been adapted for the creation of beige or brown adipose tissue on a chip (BAT-on-a-chip). Klingelhutz *et al.* demonstrated that spheroids created with murine brown adipose-derived stromal vascular fraction (SVF) cells, but not 2D cultures, displayed robust adipogenic induction of beige/brown biomarkers such as uncoupling protein 1 (UCP1) and cell death activator (CIDEA) based on PCR assays.[69] In comparable studies, Vaicik *et al.* demonstrated expression of UCP1 and CIDEA biomarkers in spheroids prepared from murine SVF cells and encapsulated in a polyethyleneglycol diacrylate hydrogel.[70] They noted that the mechanical properties of the hydrogel modulated the expression of beige/brown biomarkers; as the hydrogel stiffened, the level of UCP1 and CIDEA decreased.[70] Yang *et al.* used both rat and human ASCs to create beige/brown differentiated spheroids in a polyethyleneglycol hydrogel.[71]

In addition to validating differentiation based on PCR detection of biomarkers, they documented altered oxygen consumption rates (OCRs) in response to ATP synthase inhibitors and chemicals known to modify mitochondrial function, consistent with the increased mitochondriogenesis occurring during brown fat adipogenesis.[71] Likewise, Tharp *et al.* used an acrylated hyaluronic acid hydrogel to create spheroids with murine ASC and demonstrated their beige/brown differentiation in 3D cultures, validating this based on both PCR and OCR assays.[72,73] In addition, they demonstrated that the *in vitro* differentiated beige/brown organoids retained their functionality when implanted into live mice for up to 2 weeks based on response to cold exposure, a well characterized physiological feature of BAT.[72,73] Finally, Harms *et al.* have demonstrated the ability to create a beige/brown phenotype following viral transduction of a PGC-1 expression construct.[45] In light of the interest in beige/BAT as a target for drug discovery in the context of obesity prevention and treatment, the development of a robust beige/brown adipose 3D model for drug discovery assays holds considerable interest for biotech and pharmaceutical companies.

Organoid and spheroid models

Daquinag *et al.* used murine 3T3-L1 cells in combination with ferrous nanobeads to develop a spheroid adipocyte model based on magnetic levitation.[74] Upon introduction of an endothelial cell line, these constructs displayed evidence of vascularization *in vitro*.[74] Similarly, Klingelhutz *et al.* have developed hanging drop spheroids created by culturing primary stromal vascular fraction cells from human or murine adipose tissue in low adhesion plates.[69] These organoids displayed robust adipogenesis and exhibited functional outputs, such as secretion of adipokines and pro-inflammatory factors, which could be adapted to high-throughput formats suitable for screening of environmental toxins.[69]

Multicompartment models

A further advancement of this approach has been achieved by seeding different cell cultures into different chambers on the chip, thereby creating a multicompartment system. Having these systems together on the same chip allows for the simulation of complex processes, such as bioaccumulation of hydrophobic compounds in adipose cell compartments. One of the earliest attempts at creating such a system was reported by Viravaidya and Shuler[35] who accomplished this by compartmentalizing different cell cultures into four discrete areas, representing the lung, liver, fat, and other tissues, on a single silicon chip (Fig. 1).[35] This study presented the effects of bioaccumulation of several toxins, such as naphthalene, and demonstrated the direct effects of white adipocytes on the toxicity to other organ systems.[35] The significance of white adipose tissue's ability to modulate toxicity of compounds is great enough to require that it be accounted for when creating safety profiles for drugs. Likewise, Ahluwalia *et al.* developed a three-way perfusion circuit joining hepatocyte, endothelial, and human visceral adipocyte compartments.[26] These investigators noted a correlation between increased adipocyte differentiation with increased secretion of endothelial-derived pro-inflammatory molecules and hepatic-derived biomarkers, such as albumin. This system has the potential to serve as an *in vitro* model of visceral or central obesity.[26]

Other important considerations when attempting to simulate physiologic conditions *in vitro* are the ratio and spatial arrangement of different cell types. For example, if one cell type is responsible for metabolizing a drug to an active form that then exerts its effect on a second cell type, a smaller ratio of the former to the latter than is found *in vivo* would show reduced effectiveness of the drug. One method of addressing this problem utilizes allometric scaling principles. These principles use body size to determine the physiologic parameters needed to simulate physiological conditions. However, this approach carries its own issues as organs and cells change in a multitude of ways as they are scaled, which are not necessarily consistent across organs or cells.[75] Physiologically based pharmacokinetic (PBPK) modeling is a commonly used alternative that modifies classical pharmacokinetic models to be compatible with multicompartment models.[76] Studies evaluating the functional coupling of multiple organ-on-a-chip models in sequence have determined that the metabolism of well characterized compounds (terfenadine, trimethylamine, vitamin D_3) *in vitro* accurately recapitulates clinical *in vivo* data.[77] Nevertheless, while well suited for testing very specific hypotheses, the generalizability of PBPK to organs-on-a-chip may be limited.[75] A third method of addressing this issue relies on the proportions of heterogeneity that are unique to the individual donor of the adipose tissue. These characteristics are thought to be conserved within the SVF of the adipose tissue. Recent studies have begun to combine SVF cells isolated from adipose tissue with human-derived bioscaffolds to recreate the heterogeneity and complexity of the intact tissue *in vitro*.[78,79] These fat-on-a-chip cultures are anticipated to be a reflection of the diseased or nondiseased state of the adipose tissue from which the SVF population originated and hold great promise for mimicking diseased adipose tissue responses.

Microfluidic models

Indeed, simulating physiologic conditions suitable for drug testing requires more complex methods of distributing fluids

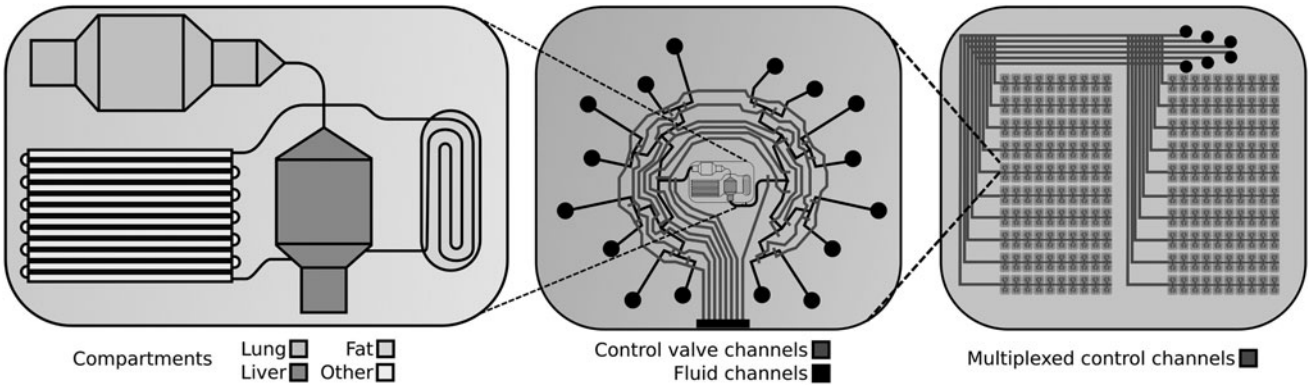

FIG. 1. *Left*: This design represents the microscale compartmentalized cell culture system used in Viravaidya and Shuler[35] in which wells and channels were etched onto a silicon chip. The wells were then seeded with cell types corresponding to different organ systems. The result allows for simulation of complex pharmacokinetic mechanisms, such as bioaccumulation.[35] *Middle*: The structure in the *middle* represents the automated 16-channel microfluidic multiplexer used by Li *et al.* to dynamically stimulate and interrogate cells of interest.[83] The *red lines* represent control channels, which can be used to automatically control exposure to the fluids loaded into the reservoirs (*black circles*). *Right*: Fat-on-a-chip technology allows for multiple instances of an experiment to be run simultaneously with massive parallelization, as seen in Wu *et al.*[84]

than those achieved by simple diffusion alone. For this purpose microfluidics and/or 3D bioprinting are often used to simulate a circulatory system by circulating medium between compartments.[35] In Godwin *et al.* microfluidics were used to allow both culturing and sampling of primary adipocytes.[48] Liu *et al.* extended this approach by combining primary human preadipocytes alone or in combination with mononuclear cells within a perfusion bioreactor construct.[80] The adipocytes could be differentiated *in situ* over a 2-week period, and the combination cultures displayed increased pro-inflammatory cytokine secretion relative to adipocytes alone, indicating a modulatory effect of the mononuclear cells. In comparable studies, Rogala *et al.* maintained primary cultures of mature human adipocytes in a microperfusion bioreactor for up to 5 weeks *in vitro* while maintaining viability and functionality based on fatty acid uptake and lipolytic assays in response to beta-adrenergic agonists.[81] The ability to maintain cultures for such extended periods of time while retaining metabolic functionality and viability are features favorable for drug development screening assays.

Microfluidics may also be used to precisely assay secretions or control delivery of hormones and nutrients to discrete regions on the chip.[82] While most work with such systems have utilized passively controlled fluid distribution systems, it is possible to build systems with actively controlled valves. Such systems would theoretically be capable of greater parallelization and data resolution by virtue of the ability to precisely deliver or sample fluid at a given time to a single region of a crowded chip (Fig. 1).[83,84]

However, the usage of microfluidics to move fluid creates new challenges as the direct flow of media exerts potentially damaging shear forces over the large fragile adipocytes.[50] This effect has wide ranging consequences affecting cell morphology, proliferation, and differentiation that cannot be eliminated even when using a monolayer culture.[85] Adipose cells *in vivo* are protected from shear stresses by the vasculature, which shields the adipose compartment from the bulk flow of fluids. To simulate this *in vitro*, Loskill *et al.* created an endothelial-like barrier that connected the media channels and adipose chambers using micropores, allowing them to maintain functional lipid metabolism for a period of weeks.[33] While this study used 3T3-L1 cells to create a white adipose depot, the same laboratory has previously used primary murine ASC in combination with an acrylated hyaluronic acid scaffold to create 3D beige/brown adipose depots *in vitro* and *in vivo*.[72] This suggests that the microfluidic approach can be adapted to mimic any physiological adipose depot. Another study which used a membrane barrier found it to significantly increase the viability of the cells after several days in culture.[54] Static cultures have the advantage of lower risk for shear stress-induced apoptosis, but would need to address the physiological components associated with nutrient delivery through an active and selective vascular barrier to control nutrients to the adipose compartment.

Potential advantages of readouts from fat-on-a-chip models

Metabolic changes experienced by adipose tissues during an experiment may be quantified using various techniques, which may be broadly classified as either destructive or nondestructive. Destructive methods include immunohistochemistry, electron microscopy, flow cytometry, mass spectrometry, or PCR. Conversely, nondestructive methods have usually focused on measuring the products secreted by cells, such as monitoring endocrine products in the supernatant or radiolabeled CO_2 production after adding labeled palmitic acid to the culture.[27] Newer generations of visual imaging technologies such as two-photon imaging may also be used to nondestructively image tissue function *in vitro*.[86] A significant benefit to nondestructive methods of analysis is that they allow for significantly greater temporal resolution than destructive methods and allow multiple readouts to be correlated on the same chip over time (improving accuracy and reducing the number of samples and thus cost required for each experiment). The goal of such studies is to move toward evaluation methods that provide indications predictive of human clinical responses to drug administration or environmental cues.

Conclusions and Future Directions

New technologies relating to the isolation, characterization, and manipulation of primary human adipose-derived cells and scaffolds have advanced the adipose biology field considerably within the past two decades. There is now ample evidence documenting the superiority of 3D compared to 2D *in vitro* adipose models as a discovery tool for metabolic and obesity research. For example, adipocytes maintained under conditions mimicking their native biomechanical state are capable of enhanced adipokine secretory function.[87] The 3D adipose depots can be generated with either homogeneous or heterogeneous adipose-derived cell populations in native, biological, or synthetic scaffolds. Furthermore, the constructs can be prepared with cells derived from donors in good health, with features consistent with metabolic syndrome, or from individuals with varying levels of severity of diabetes.

Other patient demographics such as age, gender, ethnicity, and so on can also be considered to correlate outcomes as well. The availability of such tools presents novel opportunities for human-based *in vitro* studies. Using such models, it will be possible to compare the relative response of healthy versus diabetic adipose depots to small molecules targeting metabolic disease processes in a manner that has a greater likelihood to be predictive of responses in a clinical setting. Such studies can be conducted in a static system using traditional cell culture methodologies or in a dynamic perfusion system using microfluidic technologies. In addition, the current 3D adipose depots can be adapted to create a more complex microphysiological system.

First, it will be possible to introduce inflammatory and immune cells into the adipose depot during the preparation process or subsequent to full adipogenic differentiation through circulatory delivery. This modification has the potential to mimic the sterile inflammatory changes now associated with the metabolic syndrome and diabetes. Second, it will be possible to link 3D adipose depots to MPS representing other organs, such as cardiac or skeletal muscle, liver, or pancreas. By creating circulatory microfluidic networks linking multiple human derived metabolic organoids, it will be possible to model the impact of obesity and diabetes on cardiac, hepatic, musculoskeletal, and pancreatic function. The outcomes can be quantified using biochemical

assays comparable to those used in a clinical setting. Finally, it will be possible to use adipose 3D constructs alone or in linkage to other organoids to examine the impact of biological aging on metabolic function. With such an approach, it will be feasible to predictably and reliably evaluate rapamycin, metformin, or related agents with respect to their ability to mitigate the effects of chronological aging on the human body. As humanized 3D adipose-derived constructs become more accessible to the research community, it is anticipated that they will accelerate and enhance the drug discovery pipeline for obesity, diabetes, and metabolic diseases by reducing the preclinical evaluation process and improving predictive accuracy. Such developments, applications, and usages of existing technologies can change the paradigm of personalized medicine and create substantial progress in our approach to modern medicine.

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