Adipose-derived cells: building blocks of three-dimensional microphysiological systems

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From the Contents

Introduction	301
Adipose Derived Cells	302
Three-Dimensional Microphysiological Systems for Adipose Tissue	303
Incorporation of Adipose Three-Dimensional Microphysiological Systems into Models of Obesity, Metabolic Disease and Cancer	303
Relative Merits of Adipose-Derived Stromal/Stem Cells and Stromal Vascular Cells for Three-Dimensional Microphysiological Systems Constructs	303
Potential Utility of Adipose Three-Dimensional Microphysiological Systems Models in Regenerative Medicine	304
Conclusions Future and Directions	304

ABSTRACT

Microphysiological systems (MPS) created with human-derived cells and biomaterial scaffolds offer a potential in vitro alternative to in vivo animal models. The adoption of three-dimensional MPS models has economic, ethical, regulatory, and scientific implications for the fields of regenerative medicine, metabolism/obesity, oncology, and pharmaceutical drug discovery. Key opinion leaders acknowledge that MPS tools are uniquely positioned to aid in the objective to reduce, refine, and eventually replace animal experimentation while improving the accuracy of the finding's clinical translation. Adipose tissue has proven to be an accessible and available source of human-derived stromal vascular fraction (SVF) cells, a heterogeneous population available at point of care, and adipose-derived stromal/stem cells, a relatively homogeneous population requiring plastic adherence and culture expansion of the SVF cells. The adipose-derived stromal/stem cells or SVF cells, in combination with human tissue or synthetic biomaterial scaffolds, can be maintained for extended culture periods as three-dimensional MPS models under angiogenic, stromal, adipogenic, or osteogenic conditions. This review highlights recent literature relating to the versatile use of adipose-derived cells as fundamental components of three-dimensional MPS models for discovery research and development. In this context, it compares the merits and limitations of the adipose-derived stromal/stem cells relative to SVF cell models and considers the likely directions that this emerging field of scientific discovery will take in the near future.

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Introduction

Langer and Vacanti's seminal 1993 publication entitled simply "*Tissue Engineering*" launched the arrival of a new interdisciplinary field.[1] By merging concepts from both biology and engineering, these visionaries set the stage for the development of novel therapeutic approaches to human diseases due to aging, metabolism, oncology, and trauma.[1] The field's ultimate goal has been to develop transplantable tissues capable of replacing a patient's failing organ. This is being accomplished by strategically combining biomaterial scaffolds, primary cells, and growth factors to bioengineer a functional tissue or organ with desirable characteristics. While the creation of biomanufactured organs still remains a work-in-progress with limited clinical translatability, Prestwich and others[2-4] in the biomaterials research community had the foresight to recognize the opportunity to achieve short-term goals using tissue engineering technology. They envisioned the ability to employ three-dimensional (3D) tissue constructs for high throughput *in vitro* assays in drug discovery. Such an approach would have the potential to reduce the costs and timeline for small molecule discovery and validation in the pharmaceutical industry.[2-4] Subsequently, there has been growing recognition by regulatory agencies and pharmaceutical companies that the pipeline for new drugs is shrinking. This reflects the fact that less than 1 in 10 small molecules entering Phase I clinical trials ever reaches the marketplace as a U.S. Food and Drug Administration approved medication. The extent to which current *in vivo* testing in animal models

predicts human pathophysiological outcomes remains suspect with respect to drug testing objectives. Indeed, there has been a growing call across multiple sectors of society based on ethical, moral, and scientific grounds to refine, reduce, and replace animal testing altogether. This paradigm shift, coupled with effective policy incentives, has resulted in a reprioritization of preclinical methods. Representatives from the U.S. Food and Drug Administration, the Defense Advanced Research Project Agency, and the National Centre for Advancing Translational Science have begun to promote the use of 3D constructs prepared with biomaterial scaffolds and human-derived cells, known as microphysiological systems (MPS), as an *in vitro* alternative to *in vivo* animal experimentation.[5-7] These compelling arguments for the use of fully humanized MPS in the discovery and development of the next generation of therapeutic drugs paves the way for a new field and industry that will require reliable and robust sources of primary human cells as well as novel biomaterial scaffolds. This review explores the potential advantages and limitations of adipose tissue as a source of primary human cells to address the needs for 3D MPS modelling.

Adipose Derived Cells

Historically, the transplant field evolved using bone marrow and blood as the primary source of therapeutic stem cells.[8] Indeed, bone marrow transplantation has reigned for decades as the "gold standard" for all cell therapies. Nevertheless, advances in the field of tissue engineering and regenerative medicine over the past two decades have been fuelled, in part, through new insights regarding the capability of cells derived from subcutaneous adipose tissue. The landmark publication by Zuk et al.[9] in 2001 described the enzymatic isolation of multipotent stromal cells from waste human adipose tissue discarded after lipoaspiration procedures. Initially identified as processed lipoaspirate cells,[9] they have subsequently been identified by multiple equivalent acronyms including: adipose derived stromal cells (ADSC), adipose mesenchymal stem cells (AMSC), adipose-derived stromal/stem cells (ASC);[10] henceforth, they are referred to as ASC. Routinely, the isolation process calls for the release of cells from the adipose tissue extracellular matrix by digestion with collagenase type I alone or in combination with dispase followed by a low-speed centrifugation step. The pelleted cell population recovered immediately following enzymatic digestion and centrifugation is known as the stromal vascular fraction (SVF) and consists of heterogeneous mix of endothelial, fibroblast, lymphoid, myeloid, pericytic, and stromal/stem cells.[10] These populations have the potential to contribute to angiogenesis, innate immune function, and vascularization as well as mesenchymal lineage differentiation. Flow cytometric characterization of both freshly isolated and cryopreserved SVF cell immunophenotypes has demonstrated the presence of both CD34 and CD45 as well as lymphoid and myeloid lineage biomarkers.[10] The SVF cells can be used directly at the point of care. Alternatively, they can be cultured on a plastic surface to isolate and expand the ASC population which are enriched based in part on their adherent properties. The ASC are noteworthy first and foremost for their functionality as adipogenic progenitors. Additionally, they display mesenchymal lineage potentiality as chondrogenic and osteogenic progenitors.[10] Unlike SVF cells, the immunophenotypic profile of ASC based on flow cytometry displays lower levels of CD34 and CD45 while uniformly exhibiting high levels of CD10, CD13, CD73, CD90 and CD105.[10] The SVF cell and ASC proteomes show substantial overlap based on mass spectrometry profiles.[11-13] Likewise, in addition to cytokines and tetraspanin membrane proteins, the SVF cells and ASC secretome contains microRNAs known to impact the trilineage differentiation pathways (adipogenic, chondrogenic, osteogenic).[14] **Table 1** briefly summarizes the distinguishing features of ASC and SVF cells.[10]

The vast majority of the scientific literature reports on SVF cells and ASC isolated using methods optimized by individual research laboratories and performed using a "hands-on" approach in biological safety cabinets or equivalent levels of sterility and safety. Nevertheless, for purposes of biomanufacturing and reproducibility, forward-looking academic laboratories and biotech companies have advocated for the development and use of closed-system devices to process adipose tissue for cell isolation.[15-19] These investigators consistently argue that standardization of the dissociative enzyme cocktail composition as well as the length of time and physical/mechanical steps, along with containment of all steps and media within a closed container, will yield improved reproducibility and uniformity of the final cell product. Indeed, several studies have performed side by side comparisons between individual devices and manual separation of SVF cells. Güven et al.[20] reported increased yields of nucleated (62%) and clonogenic (24%) cells using the Biosafe Sepax device (Biosafe, Eysins, Switzerland) accompanied by reduced

Table 1. Features of adipose-derived stromal/stem cells & stromal vascular cells.

	Adipose-derived stromal/stem cells	Stromal vascular cells
Shared features	· Isolated by enzyme digestion and centrifugation	
	· Multilineage differentiation potential (adipogenic, chondrogenic, osteogenic)	
Distinguishing features	Culture adherent & expanded	Not exposed to plastic or expanded
	Depleted of Hematopoietic Lineages ($CD45^-$)	Contains endothelial progenitors, fibroblasts, pericytes, lymphoid and myeloid cells ($CD45^+$)
	Higher colony forming unit – fibroblast frequency (> 5%)	Lower colony forming unit – fibroblast frequency (1%)

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variability in a study including 11 donors. SundarRaj et al.[21] reported equivalent viability and yields in SVF cell recovery between the Stempeutics Research device (Stempeutics Research Pvt. Ltd., Bangalore, India) and manual procedures. In contrast, Hanke et al.[22] reported lower SVF cell yields with reduced enrichment of $CD34^+$ cells using the Neogenesis device (NeoGenesis Co., Ltd., Seoul, South Korea) relative to manual procedures. Despite this variability among devices, since SVF cells will be isolated routinely at the "point of care" in a surgical setting, the process will rely on operating room nurses or equivalent biomedical/healthcare personnel rather than dedicated cell biologists. By automating the isolation process in a quality controlled and quality assured manner, the opportunity for human/operator errors in the operating room will be reduced. Furthermore, the automated device is argued to be more cost efficient compared to the maintenance of a fully operational tissue processing and cell isolation laboratory in the operating suite. Multiple manufacturers now offer their closed system devices to the research and clinical community with some advancing to regulatory approval. Using such devices, autologous SVF cells can be obtained within ~1 hour of lipoaspirate collection in the operating room. In contrast to ASC, SVF cells do not require multi-day expansion in a cell culture media containing foetal bovine serum, human platelet lysate, or equivalent recombinant growth factors. For these reasons, there is growing enthusiasm among researchers and clinicians to exploit SVF cells as an investigative and therapeutic resource.

Three-Dimensional Microphysiological Systems for Adipose Tissue

The "ideal" 3D MPS should display the following desired features: (a) cost effective; (b) biomanufactured with reagents of human origin; (c) adaptable to high throughput screening assays; (d) adaptable to microfluidic substrates, perfusion bioreactors, and other fluid dynamic-based systems; (e) validated for multiple functionalized assays reflecting tissue or organ specific function; and (f) yielding reproducible and quantifiable outcomes.[2, 3] The emerging MPS field has focused substantial attention on cardiac, hepatic, neuronal, and oncology models, each of which represents a competitive opportunity for drug development; however, studies focusing on adipose tissue depots have been relatively limited.[6, 23-32] This reflects, in part, the perception by many that adipose tissue plays a more "passive" role in metabolism as compared to the liver or skeletal muscle. This viewpoint may be undergoing a paradigm shift due to the growing obesity epidemic and growing understanding of the role of adipose tissue in metabolic dysregulation. The U.S. Centre for Disease Control and Prevention has calculated that the age adjusted obesity prevalence (body mass index > 30 kg/m^2) in 2017–2018 was 42.4% for American adults. It is likely that the value is even higher in 2021. In light of this data, it can be argued that adipose tissue now constitutes the largest organ by weight within the human body of the average adult American. Consequently, there is a need for increased attention to the role of adipose metabolism in all drug discovery efforts.[33] It is well established in the pharmaceutical and toxicology literature that adipose tissue can act as a reservoir or "sink" for lipophilic chemical compounds. Presumably, weight loss programs associated with lipid turnover can result in the rapid release of stored lipophilic chemical compounds into the systemic circulation. Likewise, adipocyte metabolism of drugs targeting cardiac, hepatic, or neuronal tissues can conceivably alter their pharmacotoxicological profile. Furthermore, triglycerides and other compounds released by adipocytes can interact with circulating drugs, thereby influencing their circulatory half-life and downstream receptor interactivity. While these relationships may have less influence in lean individuals, their importance is compounded with obesity and increased adipose tissue mass.

Incorporation of Adipose Three-Dimensional Microphysiological Systems into Models of Obesity, Metabolic Disease and Cancer

Ultimately, 3D MPS *in vitro* models will need to include an adipose tissue "fat on a chip" component in addition to heart, liver, and brain in order to fully incorporate the complexity of the obese individual's pathophysiology within the context of drug discovery. Studies have developed 3D MPS models mimicking adipose hypertrophy and hyperplasia suitable for diabetes and obesity related studies.[24, 34] Comparable hepatic steatosis models have already proven valuable for ex vivo drug validation in the fibrotic liver.[35] Similarly, there is a need for adipose components in 3D MPS oncology models. There is a substantial body of work demonstrating that adipocytes and adipose tissue influence the growth of tumours. An increased incidence of breast, colon, and prostate cancers has been correlated with elevated rates of patient obesity. Studies have linked adipocyte secretion of adipokines such as leptin with enhanced growth and invasion rates for breast cancer models *in vitro*.[36-39]

Relative Merits of Adipose-Derived Stromal/Stem Cells and Stromal Vascular Cells for Three-Dimensional Microphysiological Systems Constructs

At present, there is a compelling argument favouring the selection of SVF cells rather than ASC to create pathophysiological relevant 3D MPS adipose constructs for discovery research. First, SVF cells display greater heterogeneity relative to ASC. While ASC represents a relatively homogeneous population of adipogenic stromal cells, the SVF cell has greater heterogeneity with inclusion of endothelial, fibroblast, lymphoid, myeloid, pericytic, and stromal cells, consistent with the composition of the intact native tissue. Consequently, 3D MPS constructs prepared with SVF cells are capable of displaying spontaneous vascularization-like morphology in the presence of standard growth medium as well as time dependent maintenance of a cell population reflective of the native adipose tissue. Second, the time required to obtain the SVF cell specimen is substantially shorter than that required to isolate ASC. Using a closed system device at point of care, SVF cells can be processed within 90 minutes. In contrast, ASC will require adherence and seeding in a culture flask or equivalent, expansion over a 4- to 10-day period,

and enzymatic harvest/release prior to use. Nevertheless, the expansion process provides an advantage by ensuring that a greater number of ASC can be available compared to the number of SVF cells isolated from the same starting volume of lipoaspirate tissue. The relative value of a greater number of 3D MPS constructs that can be generated with ASC relative to the heterogeneity of SVF cells is a judgement that investigators need to make in the context of their individual experimental design. Both ASC and SVF cells are equivalent with respect to their biocompatibility with multiple biological hydrogels. Obatala's scientists have observed that ASC and SVF cells are viable and capable of adipogenic or osteogenic differentiation in both human blood-derived (ObaGel) and human adipose-derived (AdipoGel) matrices.[23, 40, 41] Others have reported that ASC are compatible with non-human derived hydrogels such as collagen type I (rat-tail) and Matrigel (murine Engelbreth-Holm-Swarm sarcoma).[42-44] Likewise, ASC have been shown to be compatible with bacterial- or plant-derived nanofibrillar cellulose scaffolds which are capable of supporting adipogenesis or osteogenesis.[45, 46] Additionally, ASC and SVF cells are readily available from donors reflecting a wide range of demographics based on age, body mass index, endocrine disease background, ethnicity, and family medical histories. The cell populations can be sourced from multiple adipose depots, including subcutaneous, omental, visceral, peri-renal, peri-cardiac, infrapatellar, retro-orbital, mammary, and other locations.

Potential Utility of Adipose Three-Dimensional Microphysiological Systems Models in Regenerative Medicine

Adipose 3D MPS models have additional value in the context of soft tissue regeneration for cosmetic, plastic, and reconstructive surgery. Currently, plastic surgeons employ fat grafting of autologous lipoaspirates as a routine method to address body contour and soft tissue defect issues. While this approach can be effective, volumetric loss over time due to cyst formation, fibrosis, and necrosis can lead to multiple procedures as well as sub-optimal tissue tactile features and morphological outcomes. The concepts incorporated into 3D MPS adipose tissue modelling have the potential for scale up and clinical translation. Theoretically, it will be feasible to biomanufacture a fully mature adipose tissue with pre-specified volume and dimensions in a bioreactor from autologous or allogeneic SVF cells or ASC. Plastic and reconstructive surgeons could use non-invasive imaging techniques to design an adipose tissue construct with patient-specific dimensions for direct implantation. While such an approach remains speculative for soft tissue repair, it has been implemented for bone and hard tissue regeneration.[47, 48] Culture expanded ASC have been employed to bioengineer bone/cartilage grafts customized to fit critical sized mandibular defects in porcine models with the intent of using such data to support U.S. Food and Drug Administration authorization of safety and efficacy clinical trials.[47, 48]

Conclusions Future and Directions

In summary, adipose tissue is a robust source of cells for advancing 3D MPS models biomanufactured in combination with human and synthetic biomaterials. Investigators should routinely consider SVF cells as an alternative to either culture expanded ASC or bone marrow-derived mesenchymal stem cells when designing an experiment. In contrast to the relatively homogeneous ASC or bone marrow-derived mesenchymal stem/stromal cells, the heterogeneous SVF cells are particularly appropriate for studies evaluating immunomodulatory, inflammatory, infection, or oncological aspects of adipose depots *in vitro*. In order to maximize the growth of the 3D MPS field, it will be necessary to advance several objectives. Investigators will need to have improved access to affordable, reliable, cryopreserved human SVF cells harvested and processed in accordance with international recognized standards with respect to biological safety and patient-oriented ethical concerns. Information regarding the donor demographics and cell product validation (viability, immunophenotype, lineage differentiation) will need to be provided in an anonymous but rigorously authenticated manner. There will need to be a body of peer reviewed literature from multiple laboratories in academia, biotech and pharma validating the relative utility of both ASC and SVF cells in 3D MPS for use in drug discovery screening and target chemical identification for cardiovascular, metabolic, and oncological diseases. Easy access to human adipose-derived cells will enhance the scientific community's experimental toolkit and impact its future research discovery and development capabilities.

Author contributions

Review design TF, JG; 1st draft of review JG; 2nd draft of review JG, TF; final edits of review KH, ER, XW, MH, OM, JR, HL; graphical abstracts JR, HL. All authors read and approved the final version of manuscript.

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Conflicts of interest statement

All authors except OM are employees of Obatala Sciences where TF, XW, and JMG are also co-founders and co-owners. XW and JG are co-founders and co-owners of Talaria Antibodies Inc.

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