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Review

Breaking barriers in obesity research: 3D models of dysfunctional adipose tissue

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Obesity is a global health crisis characterised by excessive accumulation of adipose tissue (AT). Under obesogenic conditions, this metabolically active tissue undergoes fibrosis and inflammation, leading to obesity-linked comorbidities. Modelling AT is essential for understanding its pathophysiology and developing treatments to protect against metabolic complications. 3D *in vitro* AT models are promising tools that address the limitations of traditional 2D *in vitro* models and *in vivo* animal models, providing enhanced biomimetic and human-relevant platforms. 3D models facilitate the study of AT pathophysiology and therapeutic screening. This review discusses the crucial role of AT in obesity-linked comorbidities, its dynamicity and complexity, and recent advances in engineering 3D scaffold-based *in vitro* dysfunctional AT models, highlighting potential breakthroughs in metabolic research and beyond.

Highlights

Adipose tissue (AT) dysfunction plays a crucial role in the development of pathologies associated with obesity.

3D models mimicking AT dysfunctions are promising tools to study AT pathogenesis and test therapeutic strategies. They can overcome translational and ethical limitations of animal models and the lack of tissue complexity of 2D cultures.

Engineering AT 3D models involves mimicking its structural and functional changes by choosing biomaterials replicating AT extracellular matrix (ECM) and its mechanical properties, promoting adipocyte hypertrophy, adding immune cells to replicate inflammation, and incorporating vasculature to vascular/oxygen changes in fibrotic dysfunctional AT.

3D AT models advance reduction, refinement, and replacement principles; elucidate cell–biomaterials interactions; facilitate AT scaffold development; and provide insights into the cultivated (animal) meat sector.

The central role of AT dysfunction in obesity complications

The development of advanced *in vitro* culture systems has revolutionised our ability to study complex biological processes in a controlled environment. Among these, 3D culture platforms have emerged as superior models for replicating human tissue architecture. In obesity research, 3D culture systems are valuable tools for studying the relevance of white AT – the main energy storage organ of the body – in the onset of obesity-associated complications and the development of therapeutic approaches.

Obesity has reached epidemic proportions worldwide [1]. Obesity is characterised by an excessive and unhealthy amount of AT, typically diagnosed via >30 **body mass index (BMI)** (see Glossary). This condition significantly increases the risk of severe health sequelae, including type 2 diabetes, cardiovascular diseases, and various cancers [2,3].

AT functions as a dynamic 'bio-mechano-sensing' organ, continuously monitoring and responding to changes in the body's energy demands and supplies. White AT is the most abundant in human adults. It is composed of adipocytes, which store lipids as triglycerides, and various other cell types within the **stromal vascular fraction (SVF)**, including vascular cells, adipocyte progenitors, fibroblasts, and immune cells. These cells are embedded within a complex **extracellular matrix (ECM)** that provides structural support and engages in biochemical signalling (Box 1). As obesity progresses, AT must expand to accommodate increased energy storage demands. This expansion involves both **hypertrophy** and **hyperplasia**. Excessive expansion can lead to hypoxia and immune cell infiltration. Circulating monocytes penetrate AT and differentiate into macrophages, forming 'crown-like' structures around hypertrophic adipocytes. Both hypertrophic adipocytes and immune cells contribute to an inflammatory secretome [**adipokines**, including tumour necrosis factor (TNF)α, interleukin (IL)-6, monocyte chemoattractant protein-1 (MCP-1)], perpetuating AT inflammation and promoting insulin resistance [4]. AT progenitors, influenced by macrophages, acquire a profibrotic profile (excessive ECM deposition) and lose their ability to differentiate into adipocytes.

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AT fibrosis is linked to cellular dysfunctions, leading to metabolic complications [5]. Consequently, adipocytes in obesity are exposed to a variety of stressors, including hypoxic, oxidative, metabolic, inflammatory, and mechanical stress [6]. Changes in the extracellular environment are detected by adipocytes, triggering intracellular pathways involved in functional regulation and adaptation to their environment (Box 1). Understanding the underlying mechanisms of AT expansion and dysfunction in obesity is critical for developing and screening effective treatments to protect against and reverse the severe metabolic consequences of obesity.

The importance of 3D models in AT research for metabolic disorders

Obesity, a complex disease affecting multiple organs, necessitates advanced and sophisticated models to study its underlying mechanisms and metabolic complications. Given that AT plays a central role in the development of complications, understanding its biological role is crucial to identifying causes and developing therapeutic approaches. However, traditional *in vivo* **animal models** and **bi-dimensional (2D) *in vitro* models** are affected by limitations that may hinder progress in understanding and treating AT-related metabolic conditions. Therefore, reliable biomimetic models of AT in obesogenic conditions are required to facilitate our understanding of AT dysfunctions and our capability of screening treatments (Figure 1).

Traditionally, researchers have relied on mammalian *in vivo* models [7], particularly mice, due to their short lifespan, well-characterised obese strains and diet-induced models, and advanced genetic tools [8]. The use of animal models has been invaluable in advancing our understanding of obesity and metabolic diseases. However, no animal model perfectly replicates human obesity, presenting limitations such as physiological differences in energy partitioning and fat deposition, dissimilarities in the pathophysiological background with different trends in AT browning [9] and hypoxia [10], inaccessibility of early developmental stages, and the high cost of facilities hindering

Box 1. Adipocytes: the hyper-sensitive players

Within AT, adipocytes detect changes in the extracellular environment through mechano-sensors, which activate mechanotransduction pathways to adapt to the microenvironment. These pathways convert physical signals into biological responses by regulating membrane and cytoskeleton integrity (Figure I). Five key pathways translate mechanical forces into biological responses:

(i) Integrins are transmembrane receptors composed of α and β subunits that anchor cells to the extracellular matrix (ECM) and mediate bidirectional communication [74]. Integrin signalling regulates adipogenesis, insulin action, and metabolism. Loss of integrin activity causes lipodystrophy and insulin resistance in mice [75]. During adipogenesis, reduced interaction of FN-integrin α5 aids in cytoskeleton reorganisation and differentiation [76]. Many integrin genes are differentially regulated in insulin-resistant and hypertrophic adipocytes, indicating a role in pathological processes [77]. Specific integrins (e.g., αvβ3, αvβ5, α5β1) activate profibrotic cytokine cascades, affecting signalling networks [78]. Neutralising integrin α5 reduces proliferation and profibrotic profiles in adipocytes exposed to inflammatory media [79].

(ii) Discoidin domain receptors (DDRs) are tyrosine kinase receptors activated by collagen that regulate adhesion, proliferation, and ECM remodelling [80]. The role of DDRs in adipocyte function is not well-studied. High-fat diets induce DDR1 expression in AT, which is linked to expansion and fibrosis. Deleting DDR1 reduces fibrosis, promotes beiging, and enhances metabolism in mice [81].

(iii) Ion transient receptor potential (TRP) channels. TRP channels respond to stimuli like voltage and pressure, regulating intracellular Ca^{2+} dynamics essential for adipocyte function. Proper Ca^{2+} levels maintain normal adipocyte function, while imbalances lead to dysfunction, excessive fat deposition, and obesity [82]. Increased Ca^{2+} deposition occurs around lipid droplets in hypertrophic adipocytes [83]. Ca^{2+}-dependent signalling also regulates inflammatory gene expression [84]. Calcium levels also regulate the nitric oxide (NO) pathway and have been shown to drive wound inflammation via reactive oxygen species (ROS)-associated mechanisms [85].

(iv) Lipid rafts and cell traction forces. Lipid rafts in the plasma membrane mediate mechanotransduction. Caveolae, specialised lipid rafts, require Caveolin-1 (Cav-1) for structure and interact with cytoskeletal filaments to regulate tension. Caveolae modify the ECM through remodelling and chemical changes. Loss of Cav-1 reduces lipolytic activity, increases lipid droplet formation, and heightens susceptibility to fibrosis [86,87].

(v) G protein-coupled receptors (GPCR). Some GPCRs act as mechanoreceptors binding collagens or responding to stress, like CXCRs [88–90]. In adipocytes, GPCRs regulate adipogenesis and metabolism [91].

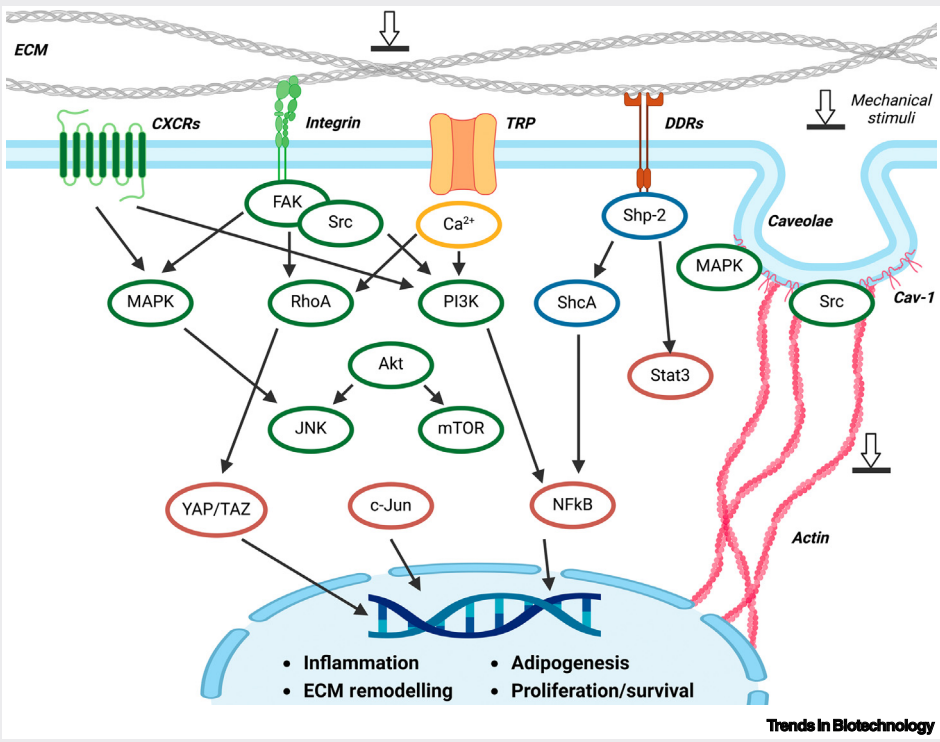

Figure I. Adipocytes respond to mechanical changes in their microenvironment through several mediators: integrin–matrix interactions, discoidin domain receptors (DDRs), G protein-coupled receptors (GPCRs) like CXCRs, stretch-activated ion channels (e.g., Ca^{2+}), and cytoskeletal strain responses via caveolae. Mechanical forces transmitted over the cell membrane activate multiple signalling pathways, including calcium-dependent targets, mitogen-activated protein kinases (MAPKs), RhoGTPases, and phosphoinositol-3-kinase (PI3K). These pathways lead to transcriptional changes, modulating cell phenotypes such as inflammation, fibrosis, and adipocyte functions. Created with BioRender.com. Abbreviations: ECM, extracellular matrix; TRP, transient receptor potential.

high-throughput studies, which altogether pose limitations to their translation to human studies [11]. Moreover, the use of animal models may raise ethical considerations, and non-animal alternatives are required to advance the **3Rs principles** (reduction, refinement, and replacement) and reduce reliance on animal research, ensuring ethical and responsible use of animals in research. Promoting the 3Rs principles in metabolic research would not only lead to ethical benefits associated with avoiding animal testing, but it would also provide scientific benefits, such as increasing accuracy, consistency, rapidity, cost-effectiveness, and human relevance [12].

2D *in vitro* models rely on the culture of adipocytes, preadipocytes, and other cell types on traditional 2D plastic substrates. In recent decades, 2D cell culture models have been critical platforms in driving significant progress in AT biology. These models have led to pivotal discoveries, including the identification of the key regulators of adipogenesis, such as PPAR-γ [13] and C/EBP family members [14]. These 2D models are currently commonly used for adipogenic differentiation due to their simplicity and cost-efficiency. Research priorities with classical 2D models include investigating adipogenesis, adipocyte hypertrophy, and lipolytic functions to understand obesity mechanisms better and test anti-obesity drugs using classical 2D culture systems [15,16]. While these models remain widely used, particularly in the preliminary stages of research projects, they fall short of accurately representing the pathophysiological conditions, complexity, and dynamicity of AT. Limitations include their single-layer cell structure, lack of tissue three-dimensionality, absence

Glossary

Adipokines: bioactive proteins secreted by AT playing roles in regulating metabolic processes (e.g., leptin and adiponectin) and inflammation (e.g., cytokines).
Alginate: natural polymer, linear polysaccharide, derived from algae and composed of units of mannuronic acid and guluronic acid.
Animal models: non-human species used in biomedical research to mimic aspects of a biological process or disease of humans.
Bi-dimensional 2D *in vitro model*: cells grown on bi-dimensional (2D) plastic surfaces to replicate physiological or pathological processes.
Body mass index (BMI): index used to screen weight categories and defined as a person's weight in kilograms divided by the square of height in meters.
Collagen: natural polymer, most abundant protein in mammals, characterised by three polypeptide chains forming a triple helix.
3D *in vitro model*: cells grown in 3D structures to replicate physiological or pathological processes.
Deformation: change of material shape, size, or volume when a force is applied.
Extracellular matrix (ECM): network of proteins and molecules surrounding and supporting cells and tissues.
Fibrinogen: natural polymer, glycoprotein that coagulates in presence of thrombin.
Gelatin: natural polymer, protein, isolated from collagen (typically bovine, porcine, fish) that partially resembles collagen properties.
Hyaluronic acid: natural polymer, glycosaminoglycan, present in the extracellular matrix.
Hydrogel: 3D polymeric networks that hold large quantities of water or biological fluids in their structure.
Hyperplasia: increase in number of fat cells (adipocytes).
Hypertrophy: increase in size of fat cells (adipocytes).
Organoid: a laboratory-grown complex clusters of organ-specific cells or tissue resembling parent organs.
PEGDA: poly(ethylene glycol) diacrylate, synthetic polymer that can be crosslinked in presence of a photoinitiation and light.
3Rs principles: cells grown on bi-dimensional (2D) plastic surfaces to replicate physiological or pathological processes.

of other cell types/blood vessels, and limited biomimicry of cell–cell and cell–ECM interactions [17]. Of note, fully differentiated white adipocytes, characterised by a unilocular lipid droplet, have yet to be fully achieved in 2D *in vitro* cultures. Moreover, the high triacylglycerol content of adipocytes makes them buoyant and fragile, posing specific challenges in culture [18].

The demand for more biomimetic, translational, and accurate pathophysiological models that can replicate the AT environment has driven the advancement of **3D *in vitro* models**. These models enable human cells to interact within a 3D scaffold that mimics elements of the ECM, achieving non-animal, physiologically relevant cell–cell and cell–ECM interactions. Adipocytes grown in 3D biomaterials exhibit different morphologies compared with those cultured in 2D, with a rounded morphology in 3D versus a more stretched spindle-like morphology in 2D [19]. Moreover, 3D cultured cells also differ in their biological functions, response to pharmacological compounds, and maintenance of cell–cell and cell–ECM interactions [20].

Over the past decade, various 3D culture systems have been developed for white adipocyte cultures. These systems can be broadly classified into three categories [21]: (i) scaffold-free systems, including **spheroids** and **organoids** [22,23]; (ii) scaffold-based systems, such as cell/spheroid/

Spheroid: a laboratory-grown simple cluster of broad-ranging cells.
Stiffness: material resistance to deformation in response to an applied force.
Stromal vascular fraction (SVF): heterogeneous mixture of cells of AT excluding mature adipocytes.

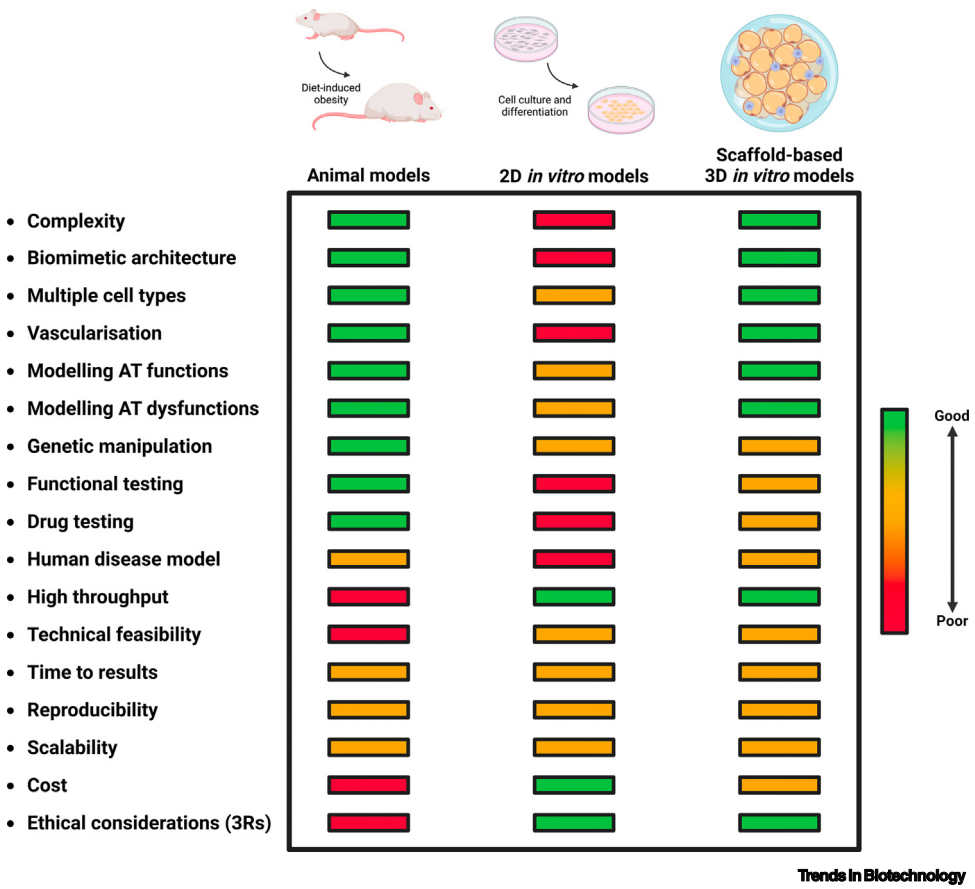

Figure 1. Modelling dysfunctional adipose tissue: *in vivo* animal models, *in vitro* 2D cell cultures, and scaffold-based 3D *in vitro* models. Factors influencing the development and success of a model of dysfunctional adipose tissue (AT) and evaluation of their suitability (poor: red; average: yellow; good: green). Created with BioRender.com. AT, adipose tissue; 3Rs, reduction, refinement, and replacement.

organoid-laden **hydrogels**; and (iii) hybrid models including microfluidic systems and organ-on-chips [24,25].

While scaffold-free and hybrid systems have been previously reviewed thoroughly [26–28], this review emphasises scaffold-based 3D *in vitro* models of dysfunctional AT. These systems offer unique advantages compared with animal models and 2D *in vitro* models (Figure 1), including support of cells in realistic 3D architectures, promoted differentiation, and facilitated cell migration. 3D models are highly customisable, allowing for the inclusion of ECM components, adjustment of matrix **stiffness**, and variation in cellular composition (e.g., addition of immune and vascular cells). In the context of AT, these features enable the development of 3D platforms that simulate the fibro-inflammatory environment of AT in obesity and related cellular dysfunctions (Box 2). Furthermore, these systems can enable high-throughput screening, making them ideal for studying mechanisms associated with AT dysfunctions (e.g., OMICs) and screening pharmacological treatments. Later, the latest advances in scaffold-based 3D *in vitro* models for studying human AT in the context of obesity and metabolic diseases are described. We will discuss the various engineering approaches used to develop these models, their applications in obesity research, and the challenges they face. Furthermore, we will explore how 3D models are overcoming existing barriers and the challenges they face in transforming the investigation, drug screening, and the development of effective patient-specific therapies in the treatment of obesity. This review serves as an essential guide for the development of next-generation biomaterial-based AT models, offering crucial insights for advancing non-animal models in the study of metabolic diseases. The unique role of scaffolds in achieving biomimetic and transferable 3D *in vitro* models of dysfunctional AT are discussed while outlining key design criteria that enhance the biomimicry of these models to human AT and their translation to AT discovery science, identification of pathophysiological AT processes, and drug screening.

Engineering approaches for 3D *in vitro* models: the sweet spot between animal models and 2D *in vitro* models?

The successful development and application of a model for (dys)functional AT strictly depends on its capability to replicate the structure and function of human AT accurately. This capability allows for the

Box 2. Impact of the biomaterial and associated mechanical cues on adipocyte functions

AT cells are subject to various mechanical loads, including tensile, compressive, and shear strains, due to body weight and weight-bearing activities. Additionally, AT fibrosis, characterised by excessive and abnormal ECM production, increased tissue stiffness and mechanical **deformation**, affecting adipocyte differentiation and function. These mechanical factors must be carefully considered when developing *in vitro* culture systems to replicate a fibrotic AT environment accurately.

(i) Stiffness. In conditions such as obesity, fibrosis, diabetes, and adipocyte hypertrophy, AT stiffness increases [56,92,93]. *In vitro*, microenvironment stiffness can be controlled by designing the biomaterial and scaffold. Stiff biomaterials inhibit adipogenesis and induce fibrotic characteristics in adipocytes, while soft matrices (~2 kPa, like native AT stiffness) promote adipogenic commitment [94]. Increased AT stiffness results from reduced remodelling capacity and plasticity, characterised by increased collagen crosslinking and decreased degradability. Tuning the biomaterial mechanical properties by engineering the scaffold design offers the unique advantage of mimicking AT fibrosis.

(ii) Deformation. AT fibrosis imposes biophysical constraints that limit adipocyte hypertrophy [95]. *In vitro* exposure to fibrotic extracts induces adipocyte deformation, reducing their sphericity and leading to metabolic dysfunctions [36]. Modelling these constraints through mechanical compression replicates these metabolic alterations. Additionally, compressive loads inhibit adipogenesis *in vitro* [96]. Designing the biomaterial mechanical properties allows a gradual replication of fibrosis-induced adipocyte deformation to be mimicked.

(iii) External mechanical forces. Dynamic loading modes, such as cyclic stretching or vibration, generally suppress adipocyte differentiation in both *in vitro* and *in vivo*/human studies, whereas static stretching may promote these processes *in vitro* [97]. Incorporating mechanical forces, such as tensile or compressive strains, can mimic obesity-associated weight bearing and influence adipocyte responses to treatment approaches for obesity and related conditions.

study of architectural changes in AT under obesogenic conditions, including cell phenotype and composition, tissue dysfunctions, and response to pharmacological treatments. 3D *in vitro* models are a promising alternative to animal and 2D *in vitro* models, capable of overcoming their limitations while combining their advantages. However, mimicking the complex and dynamic AT microenvironment and functions in a non-animal 3D artificial model presents several challenges that still need to be addressed to engineer a biomimetic AT model [29]. Multifaceted criteria and design elements should be incorporated in the model (Figure 2), including but not limited to (Figure 2A) a suitable biomaterial supporting the 3D culture of viable cells displaying proper phenotype, (Figure 2B) biomimetic mechanical properties guiding the desired cell response via mechanotransduction, the ability to replicate obesity-associated AT alterations including adipocytes (Figure 2C) hypertrophy and (Figure 2D) coculture with immune cells to replicate AT chronic inflammation, and (Figure 2E) vascularisation to promote oxygen and nutrient delivery for cells inside the 3D construct and mimic oxygenation changes in obesogenic conditions. In addition to these criteria related to the resemblance of native AT, the

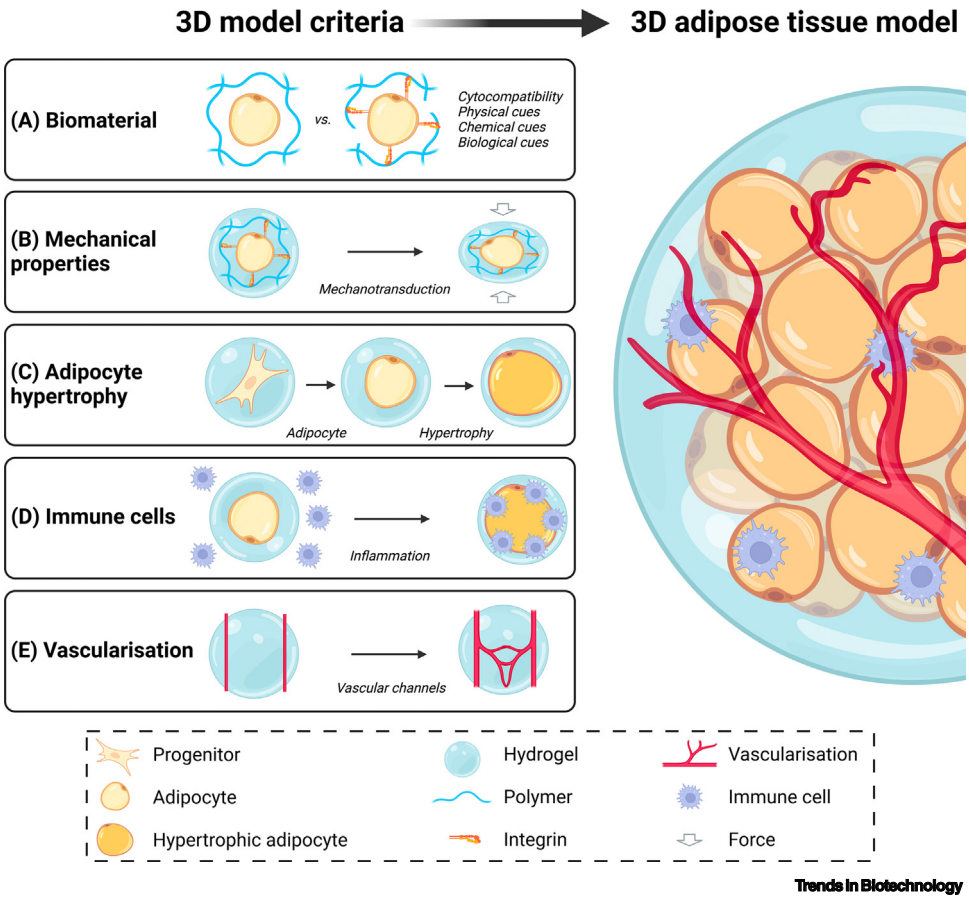

Figure 2. Criteria to engineer a scaffold-based 3D *in vitro* model of dysfunctional adipose tissue in obesity. (A) The selection of the biomaterial (polymer, crosslinking strategy) determines the cell–biomaterial interaction, biomaterial remodelling, and cytocompatibility. (B) The mechanical properties are determined by the biomaterial and scaffold design and they influence the response, fate, and functions of cells embedded in the model via mechanotransduction. (C) Adipocyte hypertrophy mimics the adipocyte phenotype in obesogenic conditions and the release of proinflammatory signals found in the adipose tissue of individuals living with obesity. (D) Immune cells are required to mimic the chronic inflammation of adipose tissue in individuals living with obesity and its relationship with obesity-linked comorbidities. (E) Vascularisation is essential to allow the transport of nutrients and oxygen in the 3D structure of the scaffolds and to mimic oxygen changes of adipose tissue in obesogenic conditions. Created with BioRender.com.

optimal 3D system should also be compatible with a wide range of technical assays, imaging techniques, long-term monitoring, and high-throughput assays. Although a translational model incorporating all these elements has yet to be developed, pioneering research has been conducted in the last decade to deliver scaffold-based 3D *in vitro* models of dysfunctional AT, advancing the field of 3D *in vitro* modelling in metabolic research and elucidating how these criteria are fundamental for the design of a translational model (Table 1).

The biomaterial (Figure 2A) constitutes the matrix, allowing the culture of cells in a 3D microenvironment mimicking AT ECM and overcoming the limited biomimetics of cells cultured on 2D substrates. Culturing adipocytes in 3D matrices is critical, given the delicate adipocyte culture procedures affected by buoyancy and de-differentiation when performed on traditional 2D substrates [18]. In fact, mature adipocytes can display a fibroblastic spindle morphology with few fat vesicles during 2D cultures, indicating de-differentiation (Figure 3A, right), while they exhibit typical unilocular lipid droplets when cultured in 3D collagen microfibre scaffolds, indicating proper phenotype maintenance (Figure 3A, left) [30]. Moreover, the differentiation of adipose stem cells has been shown to be more efficient when cells are cultured in 3D scaffolds compared with 2D substrates, as observed by increased expression of adipogenic markers and lipid droplet size in 3D cultures [30]. As the biomaterial plays a crucial role in transitioning from 2D to 3D cultures, its properties must be properly engineered to develop a biomimetic AT model. While biomaterial stability over the relatively long-term cultures required for adipogenic differentiation (typically >2 weeks [31]) and cytocompatibility are imperative to guarantee 3D cell culture, survival, and growth, other properties should be finely designed to provide the desired physical cues (e.g., surface texture, architecture, and mechanical properties discussed later), chemical cues (e.g., surface chemistry, degradation), and biological cues (e.g., growth factors/small molecules) which directly influence and determine cell fate and functions [32,33]. To date, hydrogels have been the most desirable class of biomaterials to fabricate AT models owing to the possibility of embedding cells and ECM-mimicking motifs in hydrogel precursors [34] and crosslinking them to obtain 3D cell-laden hydrogels [35,36]. Moreover, the wide diversity of polymers and crosslinking strategies available increases the versatility of these platforms, allowing them to tune the hydrogel properties on demand, which critically determines the overall model properties [37]. Despite different polymers from both synthetic [38,39] and natural origin [40,41] and their combinations [42,43] having been described to fabricate scaffolds for AT regeneration, only a limited range of these materials have been proposed so far to develop 3D *in vitro* models of dysfunctional AT. These include **alginate** with **gelatin** and **collagen** [44–47], collagen and **fibrinogen** [30,48–50], ECM-derived proteins and decellularised matrices [51,52], and a commercially available hydrogel composed of gelatin, **hyaluronic acid**, and poly(ethylene glycol) diacrylate (**PEGDA**) [24]. For instance, the printability of alginate has enabled the bioprinting of preadipocytes and macrophages to obtain *in vitro* models of insulin-resistant AT. At the same time, gelatin and collagen were added to improve cell adhesion owing to the presence of cell-adhesive ligands (e.g., RGD motifs) [44]. Based on these biomaterials, the authors fabricated hydrogel beads loaded with human adipose-derived stem cells (ADSC) and set up indirect 3D co-cultures with immune cells to investigate the different cell contributions to inflammation and insulin resistance [45]. The authors also used a similar biomaterial to fabricate mature AT 3D *in vitro* models, maintaining the adipocyte specificity and functionality with respect to the patient's BMI (spanning from <18 to >30). This constitutes a promising platform for personalised AT drug screening [48]. This model was also shown to support the direct co-culture of preadipocytes and immune cells capable of responding to anti-inflammatory drugs in high-throughput platforms [46].

Biomaterial architecture and stiffness are crucial elements to be incorporated in the design of AT *in vitro* models, as adipocytes and other AT cells are mechanosensitive and biomechanical

Table 1. Scaffold-based 3D *in vitro* models of dysfunctional adipose tissue in obesity and metabolic disease

Biomaterial	Fabrication	Adipocyte source	Other cells	Major outcomes	Refs
Alginate + gelatin + type 1 collagen	Bioprinting	3T3-L1[a]	RAW264.7[b]	Viable preadipocytes printed and differentiated into adipocytes Increased insulin resistance in adipocyte-macrophage co-cultures	[44]
Alginate + gelatin + type 1 collagen	Beads	hADSC[c]	U937[d], RAW264.7	Viable hADSC encapsulated in microspheres and differentiated into adipocytes Indirect 3D co-culture platform to distinguish cells' contributions	[45]
Alginate + gelatin + type 1 collagen	Beads	3T3-L1	RAW264.7	Optimisation of 3D co-culture of adipocytes and macrophages Anti-inflammatory drug screening on the 3D co-culture model	[46]
Type 1 collagen	Mold casting	Mouse mature adipocytes, hADSC	not present	Maintenance of mature adipocyte phenotype in 3D culture and acceleration of ADSC adipogenic differentiation	[30]
Type 1 collagen + fibrinogen	Mold casting	Human mature adipocytes, hADSC	HUVEC[e]	Hydrogel formulation optimised for long term adipocyte culture and maintenance Insulin resistance and correlation with patient's BMI replicated in the model	[48]
Type 1 collagen	Mold casting	hBMMSC[f]	not present	Collagen stiffness and architecture tuned by varying biomaterial design Stiffer altered ECM induces fibrotic gene expression in dysfunctional fibrotic state	[49]
Fibrinogen + basement membrane proteins	Casting on cellulose scaffold	hADSC	hPBMC[g]	Optimisation of adipogenic differentiation in the scaffolds Adipocyte hypertrophy induced via fatty-acid culture medium mimicking caloric overload	[51]
Alginate + AT decellularised ECM	Bioprinting	Human subcutaneous preadipocytes	hPBMC	Optimisation of bioink formulation and bath bioprinting Densely packed adipocytes bioprinted and hypertrophic adipocytes obtained in obesogenic cultures	[47]
Type 1 collagen	Microfluidic chip	Human mature adipocytes	not present	Culture and maintenance of mature adipocytes in 3D vascular-like perfused chambers	[50]
AT decellularised ECM	Microfluidic chip	Murine primary adipocytes	not present	Validation of the decellularised ECM to maintain mature adipocyte in *in vitro* culture The composition of the ECM (lean vs. obese) influences cell fate and functions	[52]
Hyaluronic acid + gelatin + PEGDA	Microfluidic chip	Human mature adipocytes	Human SVF cells[h], endothelial cells, and immune cells	Integrated modular chip replicating AT heterogeneity, including mature adipocytes, endothelial barrier, and SVF cells Immunocompetency replicated, including immune cells	[24]

[a]Murine preadipocyte cell line.
[b]Murine macrophage cell line.
[c]Human adipose-derived mesenchymal stem cells.
[d]Human myeloid leukaemia cell line.
[e]Human umbilical vein endothelial cells.
[f]Bone marrow-derived human mesenchymal stem cells.
[g]Human peripheral blood mononuclear cells.
[h]Stromal vascular fraction.

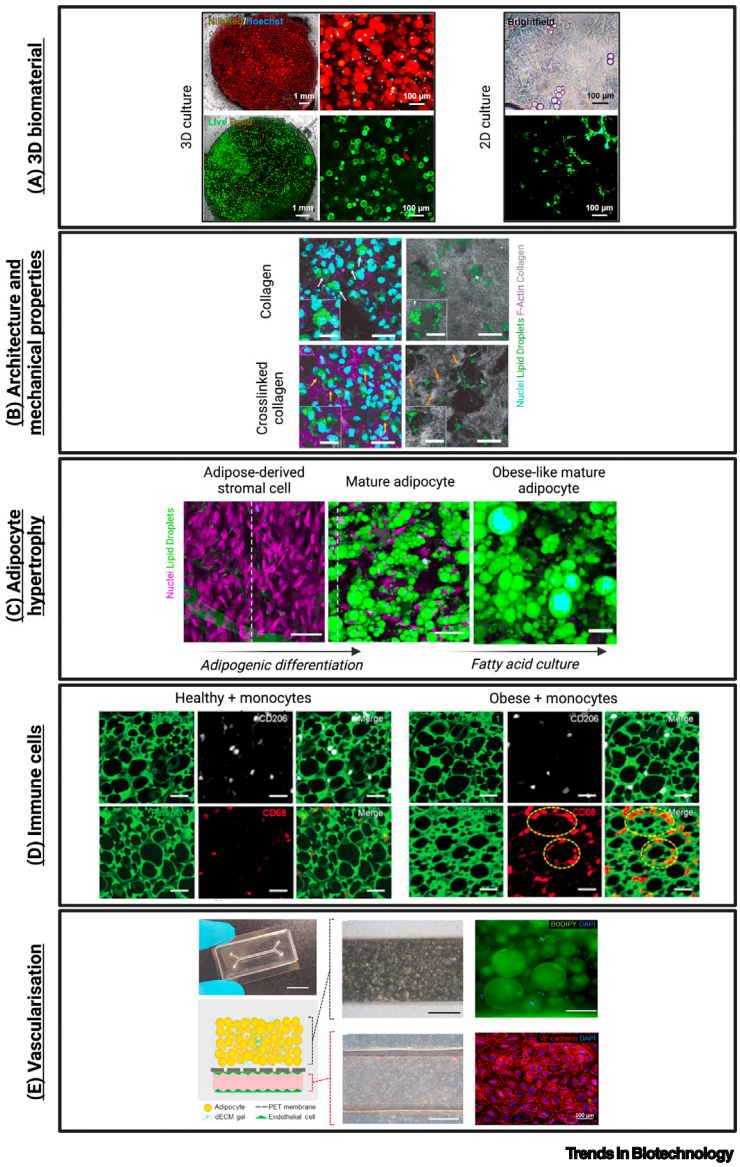

Figure 3. Applications of 3D scaffold-based *in vitro* platforms to mimic dysfunctional adipose tissue in obesity and metabolic disease. (A) Comparison of viability and functionality of 3D mouse mature adipose tissue and 2D cultures (low magnification scale bar = 1 mm; high magnification scale bar 100 µm). Reproduced, with permission, from [30]. (B) Influence of the biomaterial structure and mechanical properties on adipocyte cell response, fate, and function. Adipocytes encapsulated in collagen and crosslinked collagen; white arrows indicate low actin formation, yellow arrows high actin fibre formation, and orange arrows indicate areas of high fibre accumulation (image scale bar = 50 µm, insets scale bar = 25 µm). Reproduced, with permission, from [49]. (C) 3D *in vitro* cell culture of adipocytes in obesogenic-mimicking conditions to obtain hypertrophic adipocytes. Adipose-derived stem cells were cultured on cellulose scaffolds, differentiated into mature adipocytes, and cultured in fatty acid-enriched medium to obtain hypertrophic adipocytes (scale bar = 50 µm). Reproduced, with permission, from [51]. (D) Replicating adipose tissue inflammation via co-culture of adipocytes and monocytes. Representative images of healthy (left) and obese (right) adipocytes co-cultured with monocytes (yellow dotted lines represent crown-like structures; scale bar = 50 µm). Reproduced, with permission, from [47]. (E) Fabrication of adipose tissue microvasculature in a microfluidic chip. Microfluidic chip device and schematic (left), brightfield image of adipocytes (top centre) and endothelial cells (bottom centre) (scale bar = 500 µm). Adipocytes cultured in the decellularised matrices (top right; scale bar = 200 µm) and endothelial cells in the channels (bottom right; scale bar = 100 µm). Reproduced, with permission, from [52]. Created with BioRender.com. Abbreviations: dECM, decellularized extracellular matrix; PET, polyethylene terephthalate.

features directly influence their cell fate, functions, and dysfunctions (Figure 2B). The challenge in mimicking AT mechanical properties relies on mimicking the anatomical site-specific features of AT [53,54] and the dynamic variation of properties between healthy and dysfunctional states [55,56]. To date, the impact of the mechanical properties of AT models has been generally overlooked, with only a few studies reporting the mechanical characterisation of AT models designed to mainly mimic the mechanical properties of generic healthy AT, such as collagen hydrogels with elastic modulus (E = 1.6 kPa) reported to be comparable with that of AT *in vivo* (E = 2.2 kPa) [30]. Even fewer models considered the importance of the architectural and mechanical changes of ECM in obesogenic conditions, where the development of fibrotic tissue is a crucial element contributing to AT inflammation [57]. In fact, modulating the mechanical properties and architecture of 3D collagen hydrogels by varying their crosslinking led to an increased actin cytoskeleton fibre formation and profibrotic gene expression of embedded adipocytes (Figure 3B), confirming that ECM structure and mechanical properties regulate adipocyte (dys)functions [49].

While the biomaterial mimics AT ECM, the cell component is fundamental to mimic its biological functions and dysfunctions. In obesogenic conditions, hyperplasia and hypertrophy trigger inflammation and these pathological changes contribute to dysregulated adipokine secretion and impaired glucose metabolism (Figure 2C) [58]. Adipocyte hypertrophy contributes to metabolic dysfunction and should be incorporated into a biomimetic model of dysfunctional AT. Adipocyte hypertrophy has been achieved in 2D *in vitro* cultures by adding fatty acids such as oleic and palmitic acid to the cell culture medium [59]. Adipocyte hypertrophy has also been achieved for adipocytes cultured on 3D scaffolds. In a recent work, mesenchymal stem cells were differentiated into mature adipocytes on porous cellulose scaffolds. After adipogenic maturation, the authors optimised cell culture medium formulations by adding fatty acids and promoting adipocyte hypertrophy. This resulted in increased lipid droplet size, impaired insulin sensitivity, and a rise in macrophage proinflammatory markers when cultured in a medium conditioned by hypertrophic adipocytes (Figure 3C) [51]. In another study, preadipocyte-laden bioinks were bioprinted into densely packed adipocyte-laden hydrogels. After adipogenic maturation, culture in a glucose-enriched medium promoted adipocyte hypertrophy that resulted in increased proinflammatory cytokine secretion, monocyte recruitment and M1 activation, mimicking the progression of chronic inflammation in obesogenic AT [47].

While adipocytes are a critical cellular component of AT, various cell types constitute AT, with their ratios and distributions varying significantly between healthy and pathological states [60]. Macrophages play a crucial role in determining AT chronic inflammation and its related metabolic disorders. In lean individuals, macrophages make up around 5% of cells in AT and are distributed throughout the tissue in an alternative activation state (M2-like). In individuals with obesity, the macrophage component increases to up to 50% and macrophages cluster around apoptotic adipocytes in crown-like structures, assuming a proinflammatory state (M1-like) [61,62]. Mimicking the dynamic composition, distribution, and phenotype of macrophages is essential for developing a model of AT capable of replicating AT inflammation in obesity (Figure 2D). In a bioprinted scaffold made of alginate and decellularised ECM, human preadipocytes were differentiated and cultured to obtain mature adipocytes mimicking lean and obese features. When monocytes were co-cultured with these lean and obese AT-mimicking scaffolds, they displayed predominant M2 and M1 activation, respectively. Additionally, macrophages in the 3D models mimicking obese AT were distributed in crown-like structures, replicating the pathophysiological activation of macrophages observed *in vivo* (Figure 3D) [47].

Finally, the introduction of vasculature in the 3D construct is fundamental to guarantee the perfusion of nutrients and oxygen to support cell survival and to mimic AT vascularisation and its

changes in pathophysiological conditions (Figure 2E). Several strategies have been proposed to vascularise AT scaffolds for AT engineering, including sacrificial networks [63] and co-culture of endothelial cells [64]. For AT modelling, microfluidic chips have been the most explored choice for modelling and replicating AT vascularisation. 3D chambers have been designed and fabricated to be perfused for the culture and maintenance of mature adipocytes [50]. To improve the biomimetics of the microfluidic systems, decellularised ECM from lean and obese mice were used to 3D culture adipocytes in the chips. Mature adipocytes were maintained in culture, and the different ECMs (lean versus obese) guided adipocyte response, confirming the capability of adipocytes to sense and respond to their microenvironment. Moreover, microfluidic channels were included to replicate vascular microvessels (Figure 3E) [52]. In another example, a microfluidic chip was engineered to replicate the heterogeneity of AT. This chip, built as a modular tool to increase complexity on demand, allowed the culture of mature adipocytes in a hydrogel matrix, incorporating an endothelial barrier to mimic microvasculature and SVF cells to mimic the AT physiological environment. After the inclusion of macrophages, the vascularised model was shown to mimic immunomodulatory cytokine release and response to proinflammatory stimulation [24].

Concluding remarks and future perspectives

The World Health Organisation (WHO) declared obesity a global epidemic and its rapid increase in prevalence is posing a severe threat to overall human health [65]. AT plays a crucial role in determining obesity-related severe comorbidities, such as cardiovascular disease and insulin resistance, that heavily impact the lives of obese subjects. Tackling these complications requires the development of human-relevant, biomimetic, and ethical models to study mechanisms related to AT dysfunctions and test new treatments. Among currently available 2D *in vitro* models and animal models, 3D scaffold-based AT models represent a potential breakthrough to overcome current barriers and obtain translational AT models and constructs helpful in metabolic research and beyond (Figure 4).

Scaffold-based 3D AT models can be used to study AT functions and dysfunctions (Figure 4 top), both in the short term and as a long-term alternative to 2D *in vitro* models and animal models. These human-relevant models could unveil new insights into the vital role of AT in overall physiology and the pathogenesis of obesity. The development of healthy AT models as baselines and dysfunctional AT models as pathological replicas will enable the use of these platforms to screen new therapeutics and drugs. These models can be used to prevent or mitigate the metabolic consequences of AT dysfunction and restore healthy AT functions. These platforms are particularly promising on account of the ability to control their physical, chemical, and mechanical properties to replicate AT functions/dysfunctions on demand, allowing them to test drugs and inform clinical trials and therapeutic interventions for metabolic diseases. Moreover, the biomaterial component, if appropriately designed, can be used to fabricate high-throughput platforms to further facilitate and accelerate drug screening by allowing simultaneous and cost-effective evaluation of drug libraries [16]. The 3D scaffold-based *in vitro* models also enable a 'bottom-up' approach to introducing biological complexity based on the demands of the assay in question by including the appropriate cellular components. This strategy adds versatility and complexity to the models, which can be engineered using the desired various cell populations, such as adipocytes and vascular stromal cells, as well as induced pluripotent stem cells (iPSCs) [66], to create patient-specific body fat replicas. The generation of patient-specific AT models enables the investigation of specific individuals' pathophysiological pathways and the formulation of personalised therapeutic approaches [67]. Combining 3D scaffold-based models with microfluidic platforms allows for the integration of AT vasculature and the simulation of oxygenation changes during obesity. This approach enables the development of multi-organ chips connecting AT models with other tissue/organ models, facilitating the study of the effect of AT inflammation on other tissues and the development of obesity-related comorbidities. This is a step towards the achievement of a

Outstanding questions

How can the complexity of biomaterial-based 3D *in vitro* models be increased while maintaining tunability, control, and reproducibility, to better mimic the *in vivo* microenvironment and adipose tissue pathophysiology? To what extent are 3D models capable of mimicking the complex pathophysiology and drug response of inflammatory and dysfunctional adipose tissue in obesity?

Can 3D models be used to mimic depot-specific adipose tissue properties and dysfunctions? Which design parameters should be considered and how will biomaterial selection influence the biomimetics of different adipose tissue depots and their contribution to metabolic disease?

Can 3D adipose tissue models take a further step in enabling drug high-throughput screening to facilitate and accelerate the discovery of obesity-linked treatments? How can their scalability and reproducibility be maximised to obtain reliable replicas and readouts in high-throughput platforms?

Can adipose tissue 3D *in vitro* models be integrated in multi-organ platforms to investigate the severe comorbidities of obesity, understand their link with adipose tissue inflammation, and be used to formulate advanced treatments? Can these models be engineered as patient-specific body fat replicas to advance towards personalised medicine?

How will biomaterial-based 3D *in vitro* models advance the 3Rs principles in obesity and metabolic research? Will these platforms enable the reduction and eventually replacement of animal testing? Which ethical and regulatory challenges need to be overcome to enable their use for drug testing and screening?

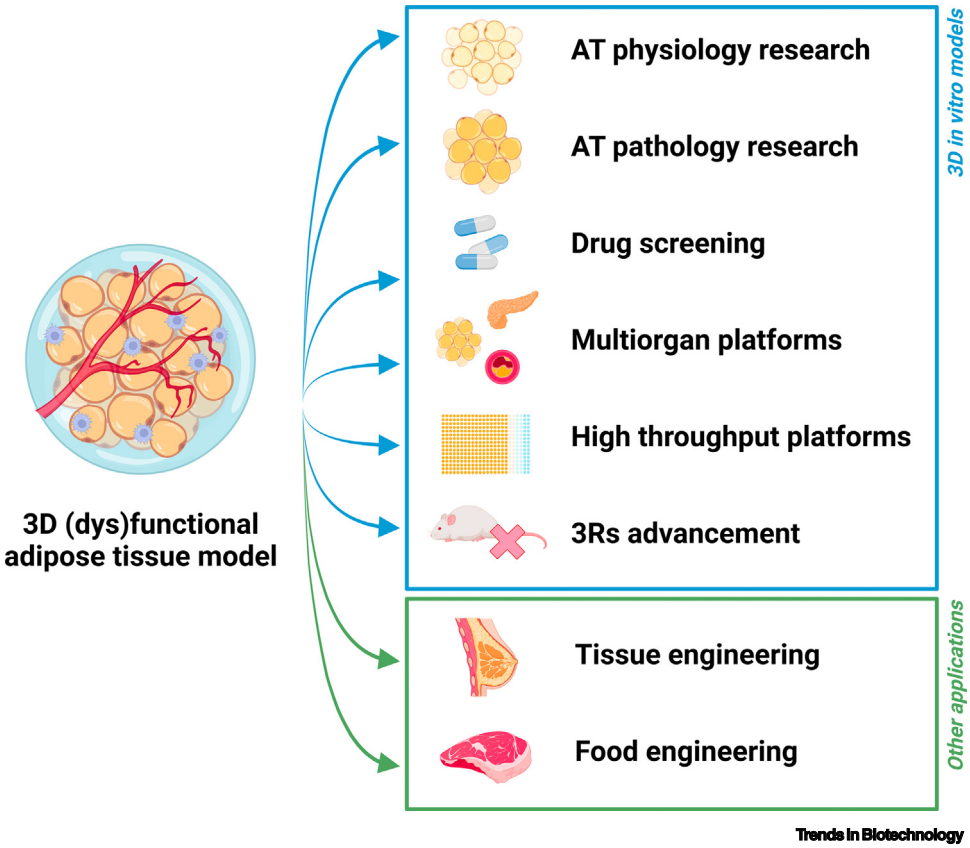

Figure 4. Future perspective and potential applications of scaffold-based 3D *in vitro* constructs of (dys) functional adipose tissue as 3D models of pathophysiological adipose tissue and beyond. Created with BioRender.com. Abbreviations: AT, adipose tissue; 3Rs, reduction, refinement, and replacement.

metabolically dysfunctional human-on-chip [68]. Finally, the development of non-animal 3D *in vitro* models will facilitate the pre-screening of pathophysiological pathways of interest and treatments, scaling down the number of conditions to be tested and reducing the reliance on animal models to the most promising candidates. In line with ongoing legislation trends in promoting alternatives to animal models [69], non-animal 3D models will eventually and ideally substitute animal testing. Moreover, engineering human models will increase the human relevance of AT models and their translation to metabolic research and accurate drug screening [70].

Scaffold-based AT models also have the potential for socio-economic impact beyond AT modelling (Figure 4 bottom). The developed 3D models will provide new insights on biomaterial and scaffold design and contribute to understanding how biomaterials drive adipocyte response, advancing the field of AT engineering and regeneration, contributing to the progress of technologies to replace dysfunctional AT or for the substitution/augmentation of AT in case of loss or removal, such as in the case of breast AT engineering [71]. Moreover, the cultivated meat sector will benefit from the development of (animal) AT scaffolds, as the development of more ethical meat/fat food alternatives is a steeply growing business [72], and a deeper understanding and capability of tailoring biomaterial properties are required to mimic fat texture and taste towards the fabrication of new cultivated fat/meat products [73].

3D scaffold-based *in vitro* models of AT are an outstanding alternative to model, understand, and treat pathological AT. However, the complexity of these models requires a joint effort to tackle the multifaceted challenges from a materials science, tissue engineering, biology, metabolic, and clinical perspective, as only this interdisciplinary effort would allow the development of a genuinely biomimetic, human-relevant, and translational model (see Outstanding questions).

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Declaration of interests

The authors declare no conflicts of interest.

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