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Lesson on obesity and anatomy of adipose tissue: new models of study in the era of clinical and translational research

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Abstract

Obesity is a serious global illness that is frequently associated with metabolic syndrome. Adipocytes are the typical cells of adipose organ, which is composed of at least two different tissues, white and brown adipose tissue. They functionally cooperate, interconverting each other under physiological conditions, but differ in their anatomy, physiology, and endocrine functions. Different cellular models have been proposed to study adipose tissue in vitro. They are also useful for elucidating the mechanisms that are responsible for a pathological condition, such as obesity, and for testing therapeutic strategies. Each cell model has its own characteristics, culture conditions, advantages and disadvantages. The choice of one model rather than another depends on the specific study the researcher is conducting. In recent decades, three-dimensional cultures, such as adipose spheroids, have become very attractive because they more closely resemble the phenotype of freshly isolated cells. The use of such models has developed in parallel with the evolution of translational research, an interdisciplinary branch of the biomedical field, which aims to learn a scientific translational approach to improve human health and longevity. The focus of the present review is on the growing body of data linking the use of new cell models and the spread of translational research. Also, we discuss the possibility, for the future, to employ new three-dimensional adipose tissue cell models to promote the transition from benchside to bedsite and vice versa, allowing translational research to become routine, with the final goal of obtaining clinical benefits in the prevention and treatment of obesity and related disorders.

Keywords Obesity, Adipose organ, Cell models, Translational research

Introduction

The obesity rate has been increasing worldwide in recent decades, and obesity is often associated with serious comorbidities [1]. A deep knowledge of the anatomy and pathophysiology of adipose organ is fundamental for choosing the best therapeutic strategies.

The goal of this review, after a concise overview of obesity, will be on describing adipose organ and discussing the many different cell models available for studying it. We will describe both two-dimensional and three-dimensional models, considering their respective benefits and constraints, with the ultimate goal of utilizing them for

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the evaluation of potential new drugs and the provision of information regarding adverse effects. Everything will be framed from the perspective of translational research, which aims to transform laboratory findings into clinical applications and convert clinical evidence into research questions, with the figure of the patient at the center. This means allowing the transition from the laboratory to the clinic and to industry.

Obesity

An overview

Obesity is a metabolic disease that is now considered a worldwide epidemic and represents a global public health problem [1]. It is associated with a reduction in quality of life and a shortened life span and can be described as a multifactorial chronic disease similar to the aging process [2, 3]. In a few simple words, it develops when the energy from the caloric intake exceeds energy expenditure, resulting in a state known as a positive energy balance. Under these conditions, progressive pathological remodeling of adipose tissues occurs because adipocytes accumulate energy in anticipation of a possible period of fasting. During the progression toward obesity, fat cells increase both in size (hypertrophy) and in number (hyperplasia) [4–7].

Overweight and obesity in adults are usually classified considering the weight-for-height index. It is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m^2) [8]. The result is commonly indicated as the body mass index (BMI). Considering the distribution of white adipose tissue, two kinds of obesity can be described: "apple-shaped obesity", also called visceral or android obesity, is typical of men and consists of an accumulation of fat in the face, neck, shoulders and, above all, abdominal cavity; "pear-shaped obesity", typically described as female or gynoid obesity, in which adipose tissue is mainly located beneath the skin (subcutaneous compartment) of hips, buttocks, thighs and belly [9].

Epidemiological evidence

Recent epidemiological studies indicate that the incidence of obesity has increased since 1975 in adults and children; in 2016, more than 1.9 billion adults were classified as overweight. In 2019, these numbers increased, and the overall prevalence of obesity was approximately 14%. Moreover, in the same year, approximately 38.2 million children under 5 years of age and more than 340 million children aged 5 to 19 years were overweight or obese [10, 11].

Overweight and obesity can develop because of unhealthy eating behaviors, such as preferring large amounts of food, which is often high in fat, sugar and refined carbs or is ultra-processed; drinking sugary beverages; and consuming too many alcohol drinks regularly. Moreover, high-calorie food has become less expensive and more convenient and is heavily advertised and promoted. Decreased or no physical activity and a sedentary lifestyle, as well as a shortened sleep duration but also genetic, endocrine, metabolic, and environmental factors, are currently considered the main common causes of the obesity epidemic worldwide [12, 13].

Associated comorbidities

Obesity is considered a risk factor for the onset of other chronic pathologies responsible for 86% of the deaths in Europe (60% worldwide) (https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight). These pathologies include metabolic disorders, such as type 2 diabetes [14], dyslipidemia, insulin resistance, metabolic syndrome, atherosclerosis, hypertension, cardiovascular diseases [15, 16], nonalcoholic fatty liver diseases [17], and other diseases that are not classified as metabolic, such as some forms of cancer [18, 19], osteoarthritis [20], obstructive sleep apnea [21] and neurodegenerative diseases [22, 23]. For all these complications, obesity curtails life expectancy and is associated with a reduction in quality of life and increased healthcare costs [14, 18]. In this regard, a loss of body weight through diet and exercise reduces all causes of mortality in obese individuals [24].

Insulin resistance and type 2 diabetes are complex multifactorial diseases and are the most well-known disorders related to obesity, but the prevalence of endocrine disorders in patients with obesity is considerable [25, 26]. In some cases, hormonal imbalances, including either peptide or steroid hormones, are related to type 2 diabetes. For example, it has been reported that the gastric ghrelin cells of obese patients who are in prediabetic conditions are hyperactive [27]. Considering the known inhibitory effect of ghrelin on insulin secretion [28, 29], its overproduction by the stomach of obese patients may be involved in the pathogenesis of type 2 diabetes.

Adipose organ

General features

The great diffusion of obesity in Western countries has attracted interest in adipose tissue, and a new anatomical and functional concept has been proposed: adipose organ. The interest in adipose organ is related to the discovery, in 1994, of the hormone leptin. It is secreted by a particular type of adipocyte (white adipocyte) and induces the search for food when its levels are low [30]. The discovery of leptin pushed many researchers to deepen their studies on adipose tissue, which was no longer considered a mere energy reserve organ but rather an important endocrine organ capable of interacting with brain centers [31]. In fact, adipose tissue represents a fundamental organ in the life of mammals because it

constitutes the main energy source for the approximately one hundred billion cells that require the organism to burn molecules for survival. The presence of adipose tissue allows us to have fasting intervals between one meal and another, and when the interval, due to lack of food, is prolonged, the adipose tissue becomes the tissue of survival.

Anatomy

The adipose organ of all mammals is composed of two main types of cells that can accumulate lipids (triglycerides) but exhibit notable anatomical and functional differences; these two cytotypes are defined as white adipocytes and brown adipocytes. The former are the best known because they constitute the parenchymal element of what is commonly defined as fat, which is abundant in humans and is pathologically increased in obese individuals. White adipocytes are unilocular, accumulate lipids for survival and secrete peptide hormones, cytokines known as adipokines [32], and exosomal miRNAs, which are involved in the control of metabolism [33, 34]. Adipokines produced by white adipocytes regulate energy expenditure, inflammation, and fibrosis. Imbalanced levels of adipokines in blood affect physiological homeostasis, are implicated in the pathogenesis of metabolic syndrome and play a role in metabolic dysfunction-associated steatotic liver disease (MASLD) [35].

Brown adipocytes are less known, and it was commonly believed that their presence in adult humans was negligible, at least from a functional point of view [36]. They are multilocular and rich of big mitochondria, which are typical of these cells. They produce UCP1 (uncoupling protein 1), a thermogenic protein exclusively expressed in these cells and are involved in the mechanism of thermogenesis because they dissipate lipids to produce heat [37, 38]. In recent years, brown adipocytes have also been recognized as endocrine cells that are able to secrete regulatory factors with local autocrine and paracrine effects but also act on distant tissues and organs [39].

Anatomical dissection has demonstrated that adipose cells are contained in subcutaneous (under the skin) and visceral (thoracic and abdominal regions) depots, indicating clear anatomical autonomy from the rest of the organism. A real organ with multiple depots is therefore configured [6]. Each depot is provided with its vascular-nervous peduncles and contains both white adipocytes and brown adipocytes. There are no anatomical barriers between regions containing white adipocytes (white adipose tissue or WAT) and those containing brown adipocytes (brown adipose tissue or BAT).

Plasticity of the adipose organ

WAT and BAT are often present simultaneously; they functionally cooperate and, under physiological conditions, they can interconvert into one another both in mice and humans [40–42]. In other words, adipose organ is endowed with remarkable physiological plasticity, which allows for the adaptation of organ function to specific needs [7, 43]. It is possible that under conditions of chronic cold exposure [44], physical exercise [45] and β-adrenergic stimulation [46], the white part of the organ becomes brown to increase thermogenesis, and this phenomenon is called "browning". On the other hand, the chronic positivity of the energy balance, which is present when the body is subjected to an obesogenic diet, requires an increase in lipid storage capacity, and the conversion of the brown component to the white component contributes to satisfying this need. This phenomenon is called "whitening" and is associated with hypertrophy and hyperplasia [47, 48].

According to different authors, in addition to brown fat cells, in mouse and human there are other fat cells. They are called "brown-in-white" ("brite") [49] or "beige" [50] adipocytes. As the brown ones, they are rich in mitochondria and express UCP1, but they are dispersed among white adipocytes. As brown adipocytes, they have also thermogenic properties, but these characteristics are UCP1-independent [51]. Beige adipocytes have different embryonic origins respect to brown ones, express a unique gene expression profile, and can derive from progenitors after de novo differentiation or from white adipocytes that can transform under specific conditions [52]. Considering the last option, under the conditions we have described (cold exposure, exercise, adrenergic stimulation), white adipose tissue undergoes morphological, molecular, metabolic and, consequentely, functional changes. These changes affect above all the mitochondria, whose number increases, and lead to obtain beige adipocytes, showing intermediate characteristics between those of white and those of brown adipocytes [53].

Direct transformation of brown/beige adipocytes into white adipocytes and vice versa, depending on the needs of the organism, is known as "transdifferentiation". It is a physiological phenomenon of cooperation between two adipose tissues in which differentiated mature adipose cells reversibly reprogram their genome without going through a dedifferentiation stage and therefore change phenotype and function in response to physiological stimuli [44, 54, 55]. Another example of transdifferentiation has been reported in breast WAT, which transdifferentiates into milk-secreting glands during pregnancy and lactation. Breast adipocytes change their anatomy, aggregate with each other, incorporating myoepithelial cells and developing a glandular alveolar anatomy. The color of the organ becomes pink because of the presence of pink cells, and they are considered adipocytes because they are rich in cytoplasmic lipids but lack the physiological characteristics of adipocytes. After lactation, the

adipoglandular (white–pink) conversion phenomenon reverses, and pink adipocytes transdifferentiate back into WAT [56]. Recently, gland-BAT (pink–brown) conversion in the postlactation period has been shown [57].

These data lead to two conclusions:

- Physiologic white–brown/beige adipose tissue transdifferentiation, given the ability of brown adipose tissue to disperse energy, could be a valid approach for treating obesity, type 2 diabetes and cardiovascular diseases;
- Mature cells can physiologically reprogram their genome to change their phenotype and, therefore, function. Perhaps, it could be useful to change, for example, tumor cells into normal cells [58].

The obese adipose organ: inflammation

The adipose organ of obese subjects undergoes pathological remodeling through a series of morphological and functional alterations. These events trigger proinflammatory immune cell responses. In particular, chronic low-grade inflammation, mainly supported by macrophages, occurs. Macrophage infiltration is strictly related to weight gain. In fact, under conditions of persistent positive energy balance, adipocytes expand, but their expansion capacity is limited, and the cells die [59, 60]. Because obese adipose tissue contains many dead adipocytes, inflammation becomes prolonged and persistent. Both in animals and in humans, a relationship exists between adiposity and adipose tissue inflammation; that is, the number of macrophages correlates with the number and size of adipocytes. It has been proposed that adipocyte death could occur through necrosis or pyroptosis [61, 62]. In this context, numerous cytokines are released into the microenvironment (TNF, IFNγ, IL-1β and IL-6), inducing innate immune responses [63]. Moreover, hypertrophic and stressed adipose cells secrete chemoattractants such as MCP-1 (monocyte chemoattractant protein-1) and HP (haptoglobin), which recruit macrophages to sequester and ingest adipocyte debris, particularly residual lipid droplets [64]. It has been shown that over 90% of macrophages are localized almost exclusively around dying fat cells to form typical structures called crown-like structures (CLSs), in which macrophages surround the cell residue in a crown-like manner and scavenge lipids and cellular debris. Sometimes, they evolve to form syncytia and multinucleated giant cells, which are much more common in obese animals and individuals than in lean animals [61]. Therefore, the presence of CLSs can be considered a biomarker of adipose tissue inflammation, and their formation is the principal inflammatory lesion. This is also evident for epicardial adipose tissue (EAT). A positive correlation between EAT thickness and cardiac diseases has been shown [65, 66]. Adipose tissue surrounding coronary arteries is considered EAT because no anatomical boundaries separate the two tissues and is a part of perivascular adipose tissue (PVAT) [67]. In general, PVAT is a special type of adipose tissue juxtaposing the outer adventitia of most blood vessels within the vascular tree of different districts of the human body [68]. It contains primarily preadipocytes, mature adipocytes, stem cells, fibroblasts, nerve cells, and various inflammatory cells [69].

From an anatomical point of view, PVAT is a heterogeneous tissue, exhibiting phenotypes from brown to white and beige adipose tissue, depending on the vascular bed where the PVAT is located [70].

As other fat depots, PVAT exerts important endocrine and paracrine functions, secreting adipokines and angiotensin, with a continuous bidirectional communication with the underlying vessels. These secreting properties are crucial for maintaining vascular homeostasis and health under normal physiological conditions [67, 71]. Under pathological conditions (i.e. patients with features of obesity, hyperlipidemia or metabolic syndrome), PVAT becomes dysfunctional and, together with endothelial dysfunction, can play a role in the development and progression of vascular diseases (hypertension or atherosclerotic plaques) [72, 73], predisposing the body to a prothrombotic or a proinflammatory profile.

Weight gain is also associated with cardiovascular diseases, such as myocardial infarction, and it is particularly true if weight gain is rapid. It has been reported that under these conditions, epicardial fat, which has anatomical continuity with the myocardium, is particularly rich in CLS. Therefore, CLS density could also be an important tool for predicting cardiovascular diseases [74]. A variety of other inflammatory changes also occurs, with activation and accumulation of different immune cell types in the white adipose tissue [75, 76].

It has been recently shown that the infiltration of adipose organ by macrophages represents an important early event in the onset of complications such as diabetes 2 and is chronically prolonged. This phenomenon correlates with BMI and with the systemic inflammatory state (measured by C-reactive protein, CRP, levels) and is reversible after weight loss. During this long process, macrophages produce cytokines that exert various side effects. Among these, insulin resistance, type 2 diabetes, and metabolic syndrome can be established because the function of the insulin receptor and the secretion of insulin itself are impaired [59, 60, 77].

Several lines of evidence suggest greater pathogenicity in visceral adipose tissue than in subcutaneous adipose tissue. Individuals with a high WHR ("waist hip-to-ratio"), which is typical of apple-shaped obesity, are more likely to develop comorbidities, such as diabetes, and,

in general, have a greater risk of developing metabolic syndrome. This is also true for individuals with a normal body weight and BMI if the body fat percentage is high. In contrast, a low WHR, which is typical of pear-shaped obesity, correlates to a low probability of comorbidity, despite a high BMI [78, 79]. These findings can be explained by the fact that the abdominal visceral fat, which is responsible for apple-shaped obesity in men, can expand less than the subcutaneous fat of the hips, which is typical of pear-shaped obesity in women. Because of this smaller critical death size, visceral adipocytes in men die earlier, visceral obesity is more pathogenic, inflammation is more severe, and comorbidities, such as insulin resistance and diabetes, can easily develop [80–82].

The obese adipose organ: remodeling of the extracellular matrix

CLS formation is due to general vascular dysfunction in the adipose tissue microenvironment, with areas of hypoxia and, as we have previously observed, adipocyte death. This vascular inflammation leads to changes in the matrix composition within tissues and in the extracellular matrix (ECM), which naturally embeds adipose cells, maintaining the structural integrity of these cells and therefore providing mechanical support [83, 84]. During the development of obesity, the ECM is subjected to structural remodeling, with an excessive accumulation of collagen fibrils and a change in its protein composition. Some proteins (especially various types of collagen) increase their expression and accumulate in the entire adipose tissue, inducing a state of local fibrosis, which increases the total rigidity of the tissue itself, reducing its expandability [85, 86]. Under physiological conditions, the matrix can remodel and stabilize the normal oscillations of adipocyte size. When fibrosis occurs, this dynamic remodeling is lost, and increased ECM stiffness counteracts the tendency of adipocytes to expand [84, 87]. The result of these two opposing actions could be, over time, an increase in mechanical stress, which, in turn, can determine adipocyte death and the consequent inflammation.

In conclusion, hypoxia and fibrosis are two important hallmarks of dysfunctional adipose tissue in obese individuals and are closely related to each other. A deeper knowledge of the morphology, histology and pathology of obese adipose tissue could help to develop effective strategies and treatment approaches to treat obesity and its related comorbidities.

Cell models for the study of adipose tissue

General features

As in industrialized countries, also in some low-middle-income developing countries the incidence and prevalence of obesity have increased, due to profound urbanization and changes in dietary lifestyle, with an adaptation to the standards of the Western diet. The onset of several obesity-associated diseases has important socioeconomic impacts. For these reasons and considering that adipocytes constitute almost 90% of the volume and up to 40% of the cells present in WAT [88], it is extremely crucial to have translational experimental systems (in vitro models) for functional studies of adipocytes. It is particularly interesting to understand the molecular mechanism of fat cell expansion and obesity, not only for better knowing the pathophysiology of adipose tissue but also because the uptake and release of lipids by adipocytes are likely involved in ectopic lipid deposition in different tissues, which can lead to lipotoxicity and other associated diseases [89, 90]. Moreover, we are still unable to maintain metabolic health in overweight patients by directly targeting their adipose tissue. The use of in vitro models could also allow the development of new treatments that could interfere with the progression of obesity and obesity-induced metabolic disorders [91].

Two-dimensional cell models

Mature white adipocytes are difficult to study because this model has some technical limitations. First, these cells are rich in lipids, and for this reason, they float in suspension [92]. Furthermore, they are difficult to isolate, do not proliferate [93], have a short life span ex vivo, and cannot be frozen without breaking. Finally, they are incompatible with many standard cell biology techniques, such as upright microscopy, cell counting, frozen sectioning, or flow cytometry [94]. To overcome the above-mentioned limitations regarding adipocyte buoyancy, 3D matrix cultures or ceiling cultures have been used. Although cell survival and metabolic activity increase, adipocytes undergo progressive dedifferentiation [95]. A variant of the ceiling culture is obtained using a small amount of freshly isolated human mature adipocytes, which aggregate under permeable small-pore transwell membranes (membrane-mature adipocyte aggregate cultures, MAAC). However, adipocytes cannot divide, and a constant supply of fresh samples, for example from surgery, is then required but is not always available [96]. Other primary mature adipocyte models are adipose tissue explant cultures, which are small pieces of WAT digested with collagenase to yield adipocytes [97, 98]. These explants remain metabolically active for several weeks and are used to investigate angiogenesis, inflammation and secretion after pharmacological stimuli [99, 100]. Altered adipocyte marker gene expression, with consequent changes in phenotype, has been described for this model [101, 102]. A modern variant of the above-described method is sandwiched WAT, in which a small piece of adipose tissue is enclosed between two

tissue-engineered layers of adipose-derived stromal cells [103] and collagenase allows the release of adipocytes. It is a model of a human three-dimensional organ construct, even though it is described among 2-dimensional (2D) models, maintaining metabolic activity for approximately four weeks without dedifferentiation, and it allows the study of WAT physiology, pathophysiology, personalized medicine, and pharmaceutical development.

Other 2D adipocyte culture models are cell lines that can be derived from mice or humans. Some of these models have been used to test the effects of nutraceuticals and compounds on obesity [104, 105] and are preadipocytes, often derived from fibroblast-like cell lines that undergo differentiation and adipogenesis after specific treatments to mimic beige, brown or white adipocytes [106–109].

Regarding preadipocyte differentiation, the change of preadipocyte morphology into the shape of mature adipocyte is influenced by epigenetic factors, miRNAs and paracrine factors, as well as by the composition and the microenvironments of the ECM [110]. Adipogenesis in mammals larger than rodents can be easily obtained stimulating transcription of specific factors that regulate the differentiation of preadipocytes into mature adipocytes. It is known that insulin is an adipogenic hormone and it has been reported that its effect can be amplified with a prolonged exposure of cells to a cocktail of specific differentiating agents. Among these, peroxisome proliferator-activated receptor γ (PPARγ) agonists (for example oleic acid, rosiglitazone, cycloglitazone) can be considered. They increase the expression of genes involved in the regulation of fatty acid synthesis and, consequently, of adipogenesis [111, 112]. Previously, MacDougald OA et al. had already shown that rodent 3T3-L1 cells become susceptible to complement of exogenous inducers, as insulin-like growth factor, glucocorticoid, fatty acids, which act as transcriptional activators promoting differentiation [113].

The reproducibility of experiments conducted using cell lines is an important characteristic and is undoubtedly an advantage.

A human 2D cell line model can be generated by the transformation and immortalization of human adipose-derived stem cells (hASCs) present in the adipose tissue stromal vascular fraction (SVF) [114, 115]. The SVF is a part of adipose tissue that includes progenitor cells such as preadipocytes, mesenchymal stem cells (including ASCs), pericytes, endothelial cells, and macrophages. The limitation of this model is that immortalization of the cells is accompanied by decreased adipogenic potential. Similar approaches can be used to isolate preadipocytes from the adipose tissue of patients with different pathologies [95].

When choosing to use the SVF fraction, some important aspects must be taken into account. In particular, adipose progenitor cells, including preadipocytes, are characterized by transcriptomic and proteomic heterogeneity, due to cell type, anatomical localization and sex [116]. Single-cell RNA sequencing (scRNA-seq) analysis has been used to analyze this molecular heterogeneity, allowing to identify, in adipose and progenitor stem cells, several subpopulations, according to different markers they express. This heterogeneity has implications in functional outcomes [117, 118]. Preadipocytes, for example, express a great number of markers, some are cell surface antigen markers and are related to adipogenesis; others are intracellular protein signaling and transcription factors which can influence the preadipocyte maturation process, promoting or repressing it [119].

A particular kind of hASC isolated from the SVF was described by Rodriguez in 2004 [120]. These cells are human multipotent adipose-derived stem (hMADS) cells that can differentiate into adipose cells in serum-free, chemically defined medium, resulting in lipid accumulation. Under other conditions, these cells can differentiate into osteoblasts or myoblasts [121]. They proliferate extensively without being transformed and have been shown to be a valid model for studies of fat tissue development and metabolism. Some of the preadipocyte cell lines that have been developed and used over the years are described in the scientific literature [109, 122–140] and reported in Table 1.

Preadipocytes, which can easily differentiate in vitro and are considered new, renewable and cryopreservable cell models [141], can be used to obtain primary cell cultures from both rodents [142] and humans [143]. Adipocyte-derived progenitors, or preadipocytes, isolated from SVFs can be expanded through a couple of passages and are compatible with most standard cell biology methods. After differentiation, these cells can accumulate small lipid droplets and express genes and adipogenic markers of mature adipocytes. A great limitation of primary but also cell line-derived preadipocytes is the lack of a unilocular morphology, that is, only a single large central lipid droplet. In contrast, they retain their multilocular morphology, which is typical of brown adipocytes, despite most cells not showing high thermogenic activity [95]. Small lipid droplets have a different membrane-to-volume ratio than large lipid droplets, and this feature influences the amount of membrane-associated proteins synthetized by adipocytes, as well as the accessibility of the lipolytic machinery to triglycerides and the lipolytic activity of cells. The final effect can be altered adipocyte functionality [144, 145]. The result is that preadipocytes are used mainly for studies of differentiation, such as the use of agents that favor adipose cell maturation. The failure of partial in vitro differentiation is probably due to

Table 1 Principal preadipocyte cell lines developed and used over the years

Cell line	Cell type	Source	Features	References
3T3-L1	Preadipose cells	Established starting from mouse fibroblast line 3T3	They accumulate triglycerides when opportunely stimulated. They are the most commonly used cellular model of murine preadipocyte	[122]
3T3-F442A	Preadipose cells	Subclone of 3T3-L1 cell line	Similar to 3T3-L1, but with a higher differentiation potential	[123]
Ob17	Preadipose cells	Dedifferentiated epididymal adipocytes of C57BL/6J ob/ob adult mice	They show morphological and biochemical characteristics of adipocytes upon confluence, with sensitivity to different adipogenic stimuli	[124]
ES cells	Stem cells	Deriving from the inner cell mass of mouse blastocyst	Used in studies on the conversion of stem cells toward adipocyte lineage	[125]
C3H10T1/2	Stem cells	Mouse embryonic mesenchymal stem cells	Used in studies on the conversion of stem cells toward adipocyte lineage	[126–129]
OP9	Stromal cells	Isolated from bone marrow mouse embryos	They can be converted into adipocytes and spontaneously express adipogenic markers. Useful model for adipocyte studies	[130, 131]
MEF	Embryonic fibroblasts	Deriving from disaggregation of mouse embryos	They can be differentiated to adipocytes using PPARγ agonists. Used to study genes and signaling pathways involved in adipogenesis	[132]
DFAT cells	Pluripotent cells	Mature adipocytes from WAT of various species	They can proliferate and differentiate into adipocytes and other cell types	[133–135]
Lisa-2	Adipose cells	Liposarcoma	Fibroblastoid cells with high capacity for acquiring adipocyte morphology and adipose differentiation	[109]
LS-14	Adipose cells	Liposarcoma	Long-lasting culture. They express adipocyte-specific genes and underdo terminal differentiation	[136]
PAZ6	Human Brown Preadipocytes	SVF	They can storage lipids and express several adipogenic markers	[137]
Chub-S7	Adipocyte precursors	Subcutaneous WAT	They express adipocyte markers and can differentiate and accumulate lipids	[138]
SGBS	Preadipocytes	Subcutaneous fat of a human infant with Simpson-Golabi-Behmel syndrome	Highly adipogenic, useful for studies of human adipocyte biology and adipogenesis	[139]
AML-1	Peripheral blood mononuclear cells	Acute myeloid leukemia	Ability to differentiate into adipocytes and to storage lipid droplets	[140]

the lack of an adequate cell microenvironment, that is, the lack of a vascular tree [146, 147] or of cell-cell and cell-matrix contacts [148].

Primary cultures of preadipocytes have also been obtained from pigs. They are useful for studying preadipocyte differentiation because they have adipogenic characteristics similar to those of human preadipocytes [149, 150].

Three-dimensional cell models

Cell lines often encounter genetic instability, and in general, 2D cell models fail to recapitulate the native microenvironment normally present in vivo. Furthermore, adipogenesis is a very complex process [151], and 2D models often cannot precisely reproduce it. On the other hand, 3-dimensional (3D) cell cultures have become a very attractive system for the scientific community [152] because they represent more realistic cell conditions in in vivo tissues.

In available 3D models of adipose tissue, adipocytes more closely resemble the phenotype of freshly isolated cells in terms of morphology, lipid droplet size (10–20 μm), expression pattern and lipid composition [153]. Some of them show obese characteristics such as hypertrophy, increased lipolysis, and insulin resistance, or can mimic an inflammatory microenvironment or fibrosis [154–157].

Furthermore, they are interesting models for studying gene expression and other biological activities because they closely mirror what occurs in living organisms. Interactions among cells and between cells and the matrix regulate proliferation [158], migration [159], and metabolism [160]. The first paper describing spheroidal self-aggregates, derived from carcinoma cell culture, was published approximately 50 years ago [161], but only in the last decade has the use of this kind of model largely been considered. Among 3D cell cultures, spheroids are likely the most studied and used. Cells of a specific type are seeded at a defined concentration and maintained in suspension. With time (hours or days), cells self-aggregate to form spheroidal structures.

Some of the described 2D adipocyte models can be used to establish 3D cultures. For example, spheroid cultures have been obtained from murine cell lines [154, 162] or from hASCs cultured on top of recombinant elastin-like polypeptide (ELP) conjugated to a charged polyelectrolyte, polyethyleneimine (PEI) [163]. hASCs have also been encapsulated in hydrogels to form spheroids

[164, 165]. In a modern approach, hASCs were forced to self-aggregate by a hanging-drop technique, and then spheroids were transferred to agar-coated cell culture dishes [166].

Recently, spheroids from the SVFs of human and murine adipose tissue have been derived [167]. It has also been reported that cells self-organize to form 3D vascularized structures with endogenous endothelial cells self-assembled to form highly organized endothelial networks among stromal cells [94, 168, 169], thus resembling miniature copies of adipose organ.

To conclude, different culture methods have been used to obtain 3D structures from hASCs or SVFs, and in some cases, they have allowed 3D microphysiological systems to be obtained. Either scaffold-based or scaffold-free culture systems have been described [170] (Fig. 1). In the first case, scaffolds are designed to reproduce the native extracellular matrix and can have a biological or a synthetic origin [165, 171]. Moreover, cultures can be set up under static conditions, with changes in the medium every few days, or in a perfusion bioreactor [172]. Unfortunately, the scaffold does not permit homogeneous delivery of nutrients to the cells, and the method has a high risk of contamination and does not accurately recapitulate interstitial flow through adipose tissue in vivo [171]. Culture in a perfusion bioreactor allows better cell viability, but adipogenesis is reduced compared with that in static culture [173].

With respect to scaffold-free culture systems, four main culture mechanisms have been described [173]: magnetic levitation [174], dynamic culture conditions [175], hanging drop, and low-attachment culture [176]. Scaffold-free culture mechanisms have increased adipogenic differentiation potential. Furthermore, they do not restrict adipocyte volume expansion, which could limit the use of scaffolds and could be useful for increasing the physiological properties of adipose tissue engineering applications. Truly, scaffold and scaffold-free 3D culture techniques have been employed to develop microphysiological models of adipose tissue [177] also in pathological states such as obesity [178]. The final goal is to recapitulate the same conditions that are present in a living organism but working with an organ system in vitro because it is now clear that the organotypic microenvironment is a fundamental

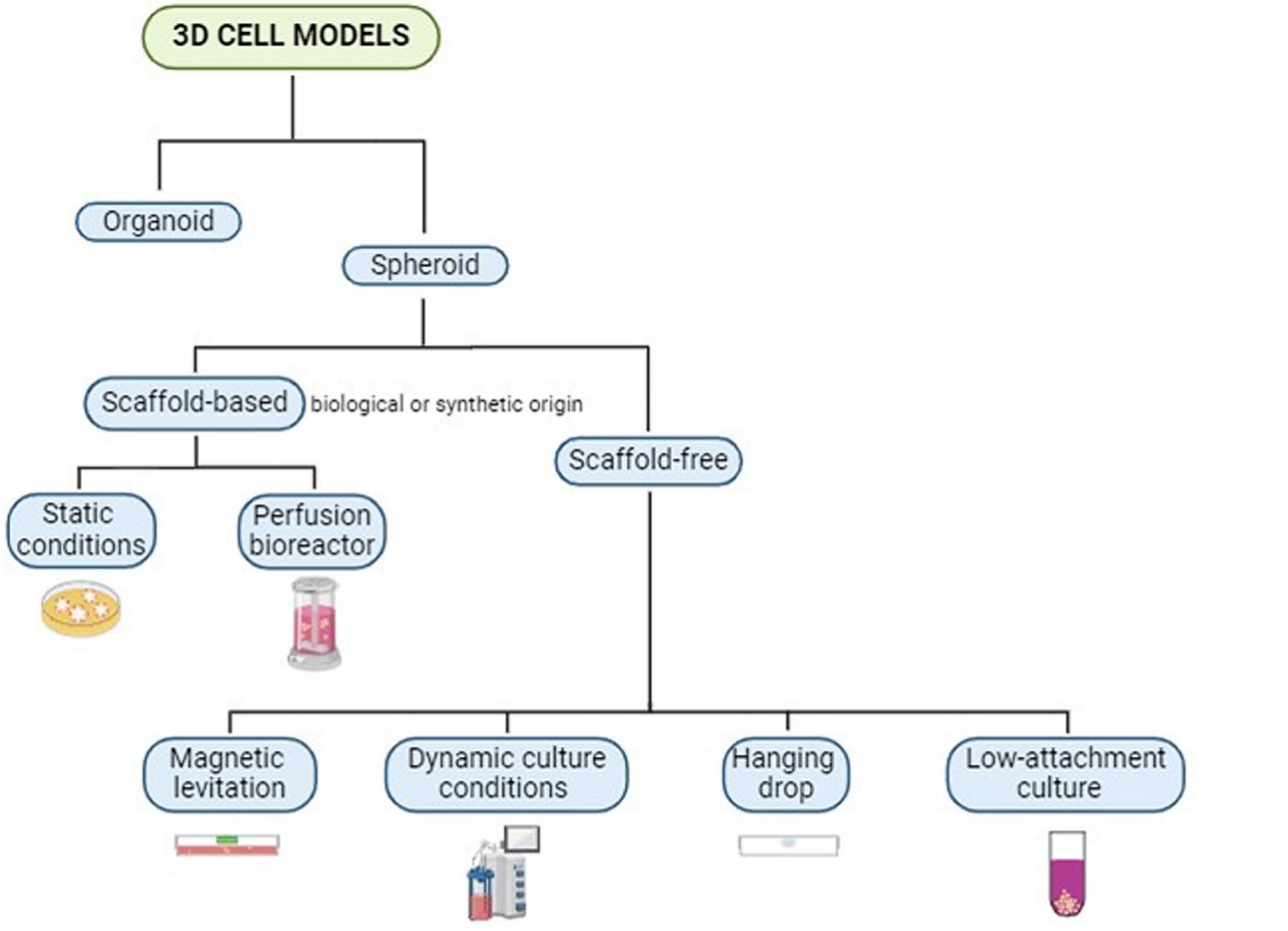

Fig. 1 Schematic overview of the different culture methods available for obtaining spheroid cultures. Created with https://BioRender.com

aspect of 3D models. Microphysiological models of adipose tissue can be regarded as adipose organoids, that is, advanced models for studying adipose tissue development and physiology.

3D models versus in vivo models

The most commonly used animal models are mice. They are easy to find and use, not very expensive, and their genetic manipulation follows well-standardized protocols [179]. However, their use has several limitations. First, the induction of obesity, which is linked to dietary modifications, is often accompanied by genetic and chemical modifications. The introduction of different variables does not always allow reproducibility of experiments in humans [180]. Second, substances and drugs active in mice might not be useful or may even be harmful to humans [181]. Rats have also been used, above all to obtain transgenic and knockout animals for the study of adipose tissue biology, but they have some limitations, and mice remain the preferred animal models for studying the expression of adipose tissue-specific genes [182].

Nonhuman primate models represent a good compromise for considering in vivo research because they are much more similar to humans. Moreover, obesity can be more easily induced in these animals than in mice, and the comorbidities that may occur are similar to those in humans [183, 184]. They can therefore represent a valid link between the simplest studies performed in mice and what is expected to be found in humans. However, their use is limited by their high costs and ethical issues.

In the future, sophisticated adipose organ-like structures could reduce, refine, and replace in vivo experiments and animal models because, more than other models, they can reproduce white and brown/beige adipose depots, avoiding interspecies variability and allowing more accuracy concerning possible outcomes in humans [185]. In general, the aggregation of multipotent mesenchymal stem cells in 3D multicellular spheroids results in improved anti-inflammatory and angiogenic properties beyond increased stemness and survival after experimental transplantation. Their therapeutic-associated properties open the possibility of translating the research achievements toward clinical applications [186]. In fact, adipose tissue is ubiquitous and can be easily obtained in large quantities using a minimally invasive procedure. Appropriate differentiation of autologous precursors of adipocytes could be employed for both regenerative medicine and organ damage caused by injury and disease [187, 188]. However, much work still needs to be done. Variables such as reagents (for example, growth medium or adipogenic factors that are used to routinely induce either beige/brown or white adipose differentiation), culture methods, and analysis methods must be standardized because different protocols are described in the scientific literature. To conclude, even if relevant microphysiological models of adipose tissue using hASCs and SVF cells have been developed [189], studies are still limited, especially regarding the influence of the culture environment on the development of adipose tissue constructs that physiologically mimic the same tissue in vivo.

Translational research: main topics of history, present and future

In recent decades, a new category of science, translational research (TR), has developed. Many definitions can be considered for TR. Briefly, it is "the application of findings from basic research to patient, community and population care and to the advancement of the delivery of health services", as reported by Cokkinos DV, in 2014 [190]. The term was introduced in the 1990s and referred to novel interventions directed to the treatment of lymphohematopoietic malignancies [191]. It was then extended to other fields and gained wide usage in the early 2000s [192]. TR has developed as a discipline from cross-talk among different scientific specialties, such as clinical pharmacology, cell biology, genetics, chemistry and physiology [193]. In other words, it can be considered an interdisciplinary branch of the biomedical field, supported by three main pillars: benchside, bedside and community, as stated in 2015 by the European Society for Translational Medicine [194]. In this context, patient participation is fundamental.

The principal goals of TR are not only to explore diagnostic and therapeutic interventions and facilitate their application in clinical practice but also to develop a scientific translational research approach to improve human health and longevity, considering the global increase in life expectancy. TR is a bidirectional concept, encompassing not only bench-to-bedside factors (testing new therapeutic strategies developed through research) but also bedside-to-bench factors, which provide feedback about the applications of new treatments and how they can be improved.

In 2003, TR was described as a two-phase process of research, which started from basic science to arriving at public health through clinical science [195–197]. Some years ago, a systematic analysis led to the identification of four different stages of TR: T1 is the phase of basic knowledge or basic scientific discovery, in which new methods of diagnosis, treatment and prevention are considered. It starts with basic research and ends with early testing in humans; the T2 phase consists of controlled studies to determine the effectiveness in humans and in establishing clinical guidelines; T3 is the phase of delivery, dissemination, and diffusion research to move evidence-based guidelines into health practice; the T4 phase regards outcomes and effectiveness research to determine the true benefit to the community. It ultimately

leads to clinical practices. After a systematic literature review, in 2017, an extra phase, T0, was proposed. It is the phase of basic research in which cell and animal models are used and mechanisms, targets and lead molecules are defined. It is also described as the phase of approaches to health problems [198]. All the phases are strictly connected to each other.

Starting from this description, it is useful to consider a translational science spectrum in which each TR stage is based on and strictly connected to the others, with patient involvement always as a key factor (Fig. 2).

Today, TR, also considered translational science or translational medicine, is an established concept in the medical field and is recognized by the scientific community, research councils and governments. The number of publications referring to "translational medicine" has noticeably increased in the last fifteen years [198, 199], indicative of a wider interest from the medical and scientific network.

The COVID-19 pandemic has presented itself as a case where the translational process has been fully exploited. Preclinical and clinical development, as well as production activities, were planned in parallel, and the global research community made great scientific progress in record time. Experts with necessary and complementary skills, knowledge and experience worked together and created a very collaborative network. Many randomized controlled trials have been proposed and used for confirmed or suspected COVID-19 patients, revealing the possibility of a new integrated future of healthcare and clinical research.

Another aspect to consider during the emergence of the COVID-19 pandemic was the complexity and rising cost of conducting cancer clinical trials. It was necessary to reevaluate all the procedures and to adapt them to the emergent situation. A great challenge was to allow patients continued access to their experimental cancer therapies, ensuring both efficacy and safety.

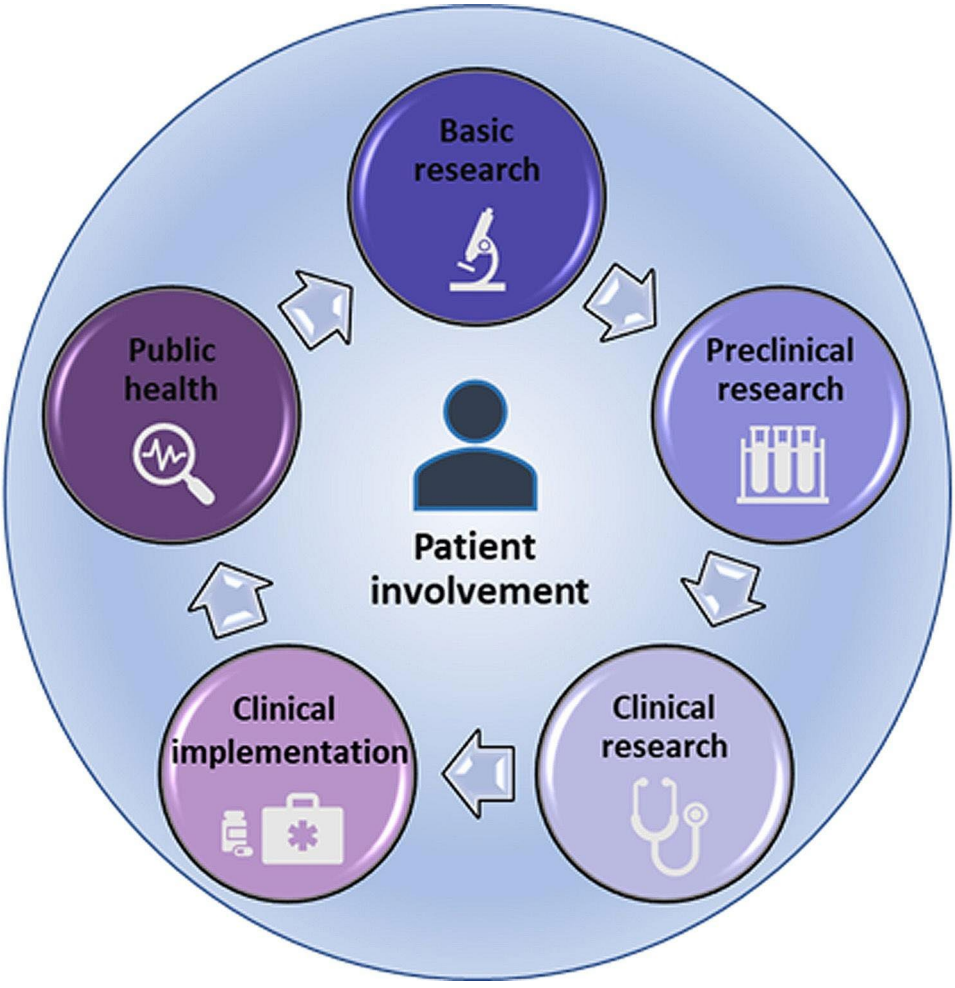

Fig. 2 Spectrum showing the interconnections among the stages of translational research, with patient involvement in all the stages. Created with https://BioRender.com

The adaptation to the new situation paved the way for incorporating changes and novel flexible workflows within clinical trials and for setting more patient-centered clinical trials to easily collect information regarding the effects of new anticancer therapies. The medical and scientific communities now have the opportunity to use these necessitated novel strategies to design a more patient-focused approach to improve impact and efficiency [200, 201].

However, much work is still needed before TR can be incorporated it into everyday health practice and used to achieve optimal patient care and treatment. In this way personalized therapies will provide efficient and cost-effective healthcare. In the future, understanding science and utilizing scientific knowledge will be even more important than they are today [202]. Artificial intelligence, genetically based patient care, use of novel molecular biomarkers and drug testing on organoids/spheroids grown from patient cells will likely be an integral part of healthcare. For example, several institutes and laboratories for metabolism and diabetes already use metabolomics, genomics, and proteomics technologies to combine basic and clinical research [203]. Such a similar approach allows the introduction of new useful biomarkers for the subclassification and management of diabetes in clinical practice [204].

Clinical results can be implemented for the benefit of the patient, even in other fields. The incorporation of new three-dimensional (3D) simulation techniques is highly recommended in orthopedics for articular cartilage repair and regeneration [205] and represents an example of a translational approach in which basic science has a profound effect on clinical practice.

3D simulation, and computer science in general, allows us to obtain image data of anatomical structures of the body, also from the most complex, and helps surgeons to address these problems in depth. This approach translates current discoveries into clinical benefits. Imaging, together with new technologies, such as proteomic, transcriptomic, and metabolomic analyses, can also be used for translational research in bone tumors [206]. In recent years, regenerative and translational dentistry has also been developing to evaluate different new approaches for the implementation of protocols and strategies [207]. TR and precision medicine are also facilitated by the generation of tissue biobanking. Today, biobanking in health care is organized in dynamic units and is extremely useful for modern medical research, for example, in the field of cardiovascular diseases. The use of human biobanks allows us to overcome the limitations related to the intrinsic anatomical, functional and molecular differences in tissue samples that must be considered when animal species are employed [208].

For the examples we have reported and for several other similar reasons, continuous collaborations among universities, hospitals and academies as well as renewed and reviewed healthcare strategies must always be arranged. Continual communication between researchers in shared and different disciplines is fundamental to accelerating scientific progress. It is particularly beneficial when scientific exchanges are multidisciplinary.

3D culture systems and translational research

In vitro 2D cell cultures are still used in several laboratories throughout the world to study, for example, proliferation and/or differentiation. Their use presents the important advantage of being a standardized procedure. However, they also have some disadvantages. For example, the use of fetal bovine serum as an adjuvant can lead to cellular senescence and aging. Furthermore, this kind of model does not always represent the original tissue. For these reasons, 2D cultures are often replaced by 3D culture systems, which more closely resemble in vivo conditions.

Starting from these considerations, the new culture models represent robust and translational experimental systems that could have, in the near future, clinical application, favoring the creation of *ad personam* therapy.

Spheroids and organoids can be used for bench-to-bedside translation in several research fields, such as tissue engineering and regeneration, cancer cell biology, stem cell research, regenerative therapies, drug testing, and preclinical/clinical models for the treatment of different disorders and pathologies [188, 209–232]. Some examples of the possible applications of 3D multipotent mesenchymal stem cell spheroids are reported in Fig. 3.

The translation of these findings to obesity and spheroid models is related to the fact that adipose tissue is considered an endocrine organ and obesity is considered an inflammatory chronic disease.

Interestingly, the production of adipose spheroids from the SVF of mouse interscapular adipose tissue has already been reported. After the adipogenic protocol, spheroids had a complex internal structure with fully differentiated adipocytes and are viable, leptin-secreting, and lipolysis-responsive. They expressed the same adipocyte markers as adult mouse adipose tissue, and without an adipogenic stimulus, they maintained leptin secretion and were able to respond to adrenergic lipolytic stimulation by secreting glycerol. It has been reported that after implantation in mouse models, they could sustain leptin production [167].

The biological differences among WAT and SAT depots, leading to functional differences and to a more frequent onset of obesity-induced metabolic complications and comorbidities in visceral adipose tissue, are due not only to differences in their SVF composition but also

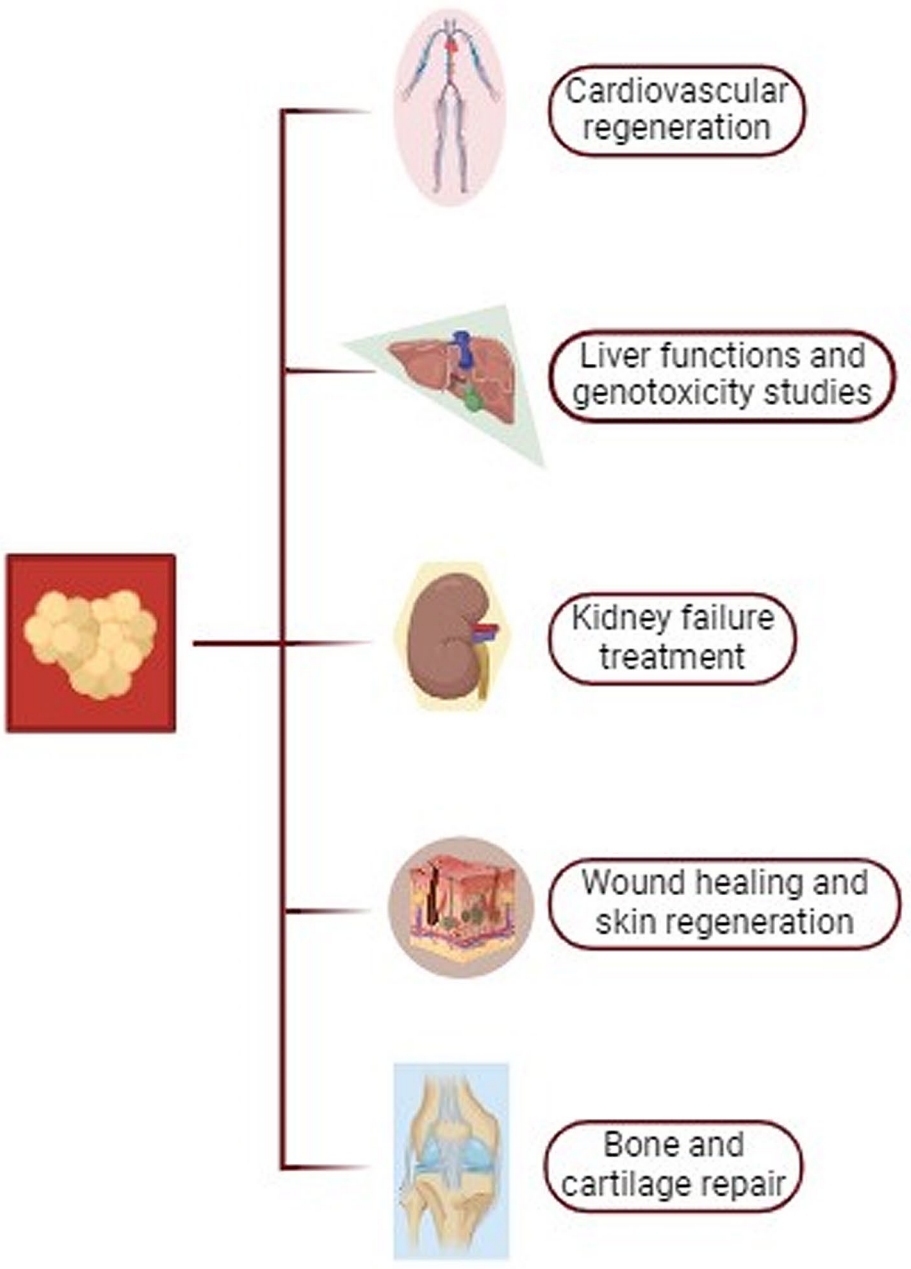

Fig. 3 Therapeutic potential of 3D multipotent mesenchymal stem cell spheroids and their application in translational medicine. Created with https://BioRender.com

to the presence of specific adipocyte stem cells and progenitor cells. Specialized adipocyte subtypes are derived from these cells [233–236] and must be selected for different purposes.

Another aspect to be considered is that some differences may exist not only because of different depot-targeted human tissue samples, as we have described above but also because hASCs can be derived from different sexes or different species. The first aspect may be useful for studying and evaluating sex-related biological differences in WAT expansion and obesity, and the second could be a limitation in establishing obesity cell models derived from species other than humans, limiting the translation of obesity-related findings to humans.

Therefore, experimental systems engineered with human-derived cells with depot-specific profiles are extremely useful for studying every aspect of the physiology and pathology of adipose tissue [166] and developing novel therapies.

However, some limitations must be considered. First, costs, absence of automation, diverse laboratory protocols, poor reproducibility, and limited scale of production are often associated with these procedures. Moreover, new therapies and technologies, chosen according to specific requirements, can be developed and employed only if regulated by approved guidelines to avoid harming the intended beneficiaries, that is, the patients and the public in general.

In terms of modern medicine, recent advances in adipose tissue 3D models (spheroids or organoids, more complex structures) may contribute to a deep understanding of the mechanisms involved in the prevention and treatment of obesity and related disorders (diabetes, metabolic syndrome, nonalcoholic fatty liver disease, cancer, etc.) in humans, reducing the preclinical evaluation process and improving predictive accuracy. Such an approach might also contribute to drug discovery for safe and effective personalized therapy, helping with translation to the pharmaceutical industry and providing efficient and cost-effective healthcare.

Conclusions

The prevalence of obesity is increasing at an alarming rate worldwide, leading to a major increase in the incidence of severe obesity-related conditions such as type 2 diabetes, ischemic heart disease, stroke, and many forms of cancer. The use of different types of study models is crucial for comprehending the pathophysiology of adipose organ and effectively implementing therapeutic strategies to combat obesity, which is now acknowledged as a serious illness. Constant sharing of opinions, information, skills, data and ideas among various professional figures through effective communication and facilitating the flow of knowledge from the laboratory to the bedside, and vice versa, becomes essential to ensure a seamless transition from laboratory settings to clinics and industries. This continuum, with the patient as the focal point, will enable the integration of translational research into routine operations.

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