Tissue Eng Regen Med (2020) 17(2):123–140
https://doi.org/10.1007/s13770-019-00230-x

Online ISSN 2212-5469 TERM

REVIEW ARTICLE

Human Adipose Tissue Derivatives as a Potent Native Biomaterial for Tissue Regenerative Therapies

Siva Sankari Sharath[1] · Janarthanan Ramu[2] · Shantikumar Vasudevan Nair[1] · Subramaniya Iyer[2] · Ullas Mony[1] · Jayakumar Rangasamy[1]

Received: 30 September 2019 / Revised: 7 November 2019 / Accepted: 15 November 2019 / Published online: 17 January 2020

Abstract

BACKGROUND: Human adipose tissue is a great source of translatable biomaterials owing to its ease of availability and simple processing. Reusing discardable adipose tissue for tissue regeneration helps in mimicking the exact native microenvironment of tissue. Over the past 10 years, extraction, processing, tuning and fabrication of adipose tissue have grabbed the attention owing to their native therapeutic and regenerative potential. The present work gives the overview of next generation biomaterials derived from human adipose tissue and their development with clinical relevance.

METHODS: Around 300 articles have been reviewed to widen the knowledge on the isolation, characterization techniques and medical applications of human adipose tissue and its derivatives from bench to bedside. The prospective applications of adipose tissue derivatives like autologous fat graft, stromal vascular fraction, stem cells, preadipocyte, adipokines and extracellular matrix, their behavioural mechanism, rational property of providing native bioenvironment, circumventing their translational abilities, recent advances in featuring them clinically have been reviewed extensively to reveal the dormant side of human adipose tissue.

RESULTS: Basic understanding about the molecular and structural aspect of human adipose tissue is necessary to employ it constructively. This review has nailed the productive usage of human adipose tissue, in a stepwise manner from exploring the methods of extracting derivatives, concerns during processing and its formulations to turning them into functional biomaterials. Their performance as functional biomaterials for skin regeneration, wound healing, soft tissue defects, stem cell and other regenerative therapies under *in vitro* and *in vivo* conditions emphasizes the translational efficiency of adipose tissue derivatives.

CONCLUSION: In the recent years, research interest has inclination towards constructive tissue engineering and regenerative therapies. Unravelling the maximum utilization of human adipose tissue derivatives paves a way for improving existing tissue regeneration and cellular based therapies and other biomedical applications.

Keywords Human adipose tissue · Adipose tissue derivatives · Adipose derived stem cell · Preadipocyte · Extracellular matrix · Biomaterial

1 Introduction

Integration of biologists, reconstruction practitioners and tissue engineers is useful to develop a functional biomaterial to replace the regenerative loss in humans [1]. Most widely occurring regenerative repair includes engineering of soft tissues like flap reconstruction [2], dermal fillers [3], implants for soft tissue augmentation [4]. Soft tissue regeneration is the challenging field of interest attracting both researchers and clinicians around the globe to address

✉ Ullas Mony
ullas19351@aims.amrita.edu

✉ Jayakumar Rangasamy
rjayakumar@aims.amrita.edu

[1] Centre for Nanosciences and Molecular Medicine, Amrita Institute of Medical Sciences and Research Centre, Amrita Vishwa Vidyapeetham, Kochi 682041, India

[2] Department of Plastic and Reconstructive Surgery, Amrita Institute of Medical Sciences and Research Centre, Amrita Vishwa Vidyapeetham, Kochi 682041, India

the necessity of appropriate substitutes to retain the complex architecture of fat depots. Major proportion of patients undergoes soft tissue reconstruction for traumatic injuries [5], cutaneous scars [6], cosmetic surgeries [7], congenital defects [8], tumor resection [9] and contouring [10]. Therefore, a universal method of regenerative treatment is not suitable for the different types of soft tissue defects. Medical care for soft tissue defects has evolved from tissue autotransplantation to contemporary reconstruction [11, 12]. These kinds of procedure need biomimetic materials which can take the shape of defects, restore the volume, decrease resorption and enhance regeneration [13]. So, a multifaceted biomaterial is required to quench the demands of defective adipose tissue sites.

Currently prevailing trend for adipose tissue restoration includes incorporation of biological additives and factors along with biomaterials to accelerate adipose regeneration. This comprehensive review is to apprehend the rationale for the need of human source of adipose tissue for adipose tissue engineering applications. Human adipose tissue derivatives hold a great promise in soft tissue regeneration owing to the less invasive technique of liposuction [14]. Unlike other human tissue source, liposuctioned adipose tissue can be obtained in adequate quantities with minimal complications and does not need laborious techniques before implantation. It would serve as a better alternative source for deriving multipotent cells and extracellular matrix (ECM). Source of material plays a major role in properties of the extracted biomaterials. Successful regeneration of adipose tissue depends on the composition, rheological behaviour, tunability, formulation and functionalities of adipose tissue derivatives or other biomaterials used for regeneration. Having an intrinsically appropriate biomaterial is crucial for grafts to function properly post-implantation [15].

In this review, we discuss the roadmap on materialistic approach and tailoring of human adipose tissue, challenges involved, current strategies employed to overcome those hurdles towards a promising future in clinics. Here, we reviewed wide range of studies dealing with the efficient conversion of adipose into a functional biomaterial, followed by the critical analysis of adipose tissue derivatives and their role in clinics. Figure 1 illustrates the human adipose tissues derivatives and its application in autologous therapy to humans for soft tissue defects.

2 Structural and molecular aspect of human adipose tissue

Adipose tissue is the metabolic endocrinal organ, also known as a universal regulator of energy homeostasis [16]. It serves as a caloric reservoir by storing the excess nutrients in lipid formulation and provides nutrients to tissues by lipolysis during nutrient deficient conditions. Adipose tissue remodelling greatly contributes to the maintenance of endocrinal function by controlling thermogenesis and adipogenesis, removing and/or repairing damaged tissue to cope up with the external environmental modifications [17]. Obesity is due to dynamic remodelling of adipose tissue, inducing aberrant changes in the hormonal and signalling pathways leading to disease. Adipose tissue depots act as miniorgan to native environment and helps in sustaining the homeostatic balance by releasing paracrine factors [18].

In order to understand the therapeutic approach of human adipose tissue, deeper mechanistic understanding of human adipose tissue architecture is necessary. There are two prominent categories of adipose tissues, brown adipose tissue (BAT) and white adipose tissue (WAT). WAT can be further categorised into two forms, subcutaneous WAT (SWAT) and visceral WAT (VWAT), enclosing internal organs. Prime function of WAT is to serve as thermal insulator and energy reservoir whereas, BAT has significant contribution to lipid oxidation and heat generation. But, BAT gradually decreases with aging and is replaced by WAT [19]. Adipose tissue is a smart connective tissue and its omnipresence in human body serves specialized functions such as cushioning, energy storage, insulation and vasculature [20, 21]. Lipid-filled adipocytes are held together by collagen fibers, reticular fibers, nerve fibers, elastin fibers, lymph nodes, vascular stroma and adipokines. Stromal-vascular fraction of adipose tissue includes smooth muscle cells, fibroblasts, endothelial cells, and adipose-derived stem cells (ADSCs) [22]. Therefore, treatment of soft tissue defect involves replacement of WAT supported by vascular network.

Adipose tissue undergoes continuous remodelling due to its increased metabolic rate. Thus, adipogenesis is governed by several autocrine and paracrine inducers, regulators and inhibitors involving a series of complex process from transcriptional, gene and protein expression finally leading towards phenotypical lipogenesis [23]. Choi et al. [24] has demonstrated the mechanism of signalling pathways towards adipogenesis as mentioned in Fig. 2. WNT and hedgehog signalling pathways regulate adipogenesis through transcriptional regulators, peroxisome proliferator activated receptor gamma (PPARγ) and CCAAT/enhancer binding protein alpha (C/EBPα) to induce adipogenic genes like glycerol-3-phosphate dehydrogenase (GAPDH), fatty acid binding protein (FABP4) and acyl-CoA synthetase (ACS) ensuing triglyceride (TG) synthesis, ECM production and adipokine secretion [25–29]. Detailed discussion on biochemical pathways is beyond the scope of this article.

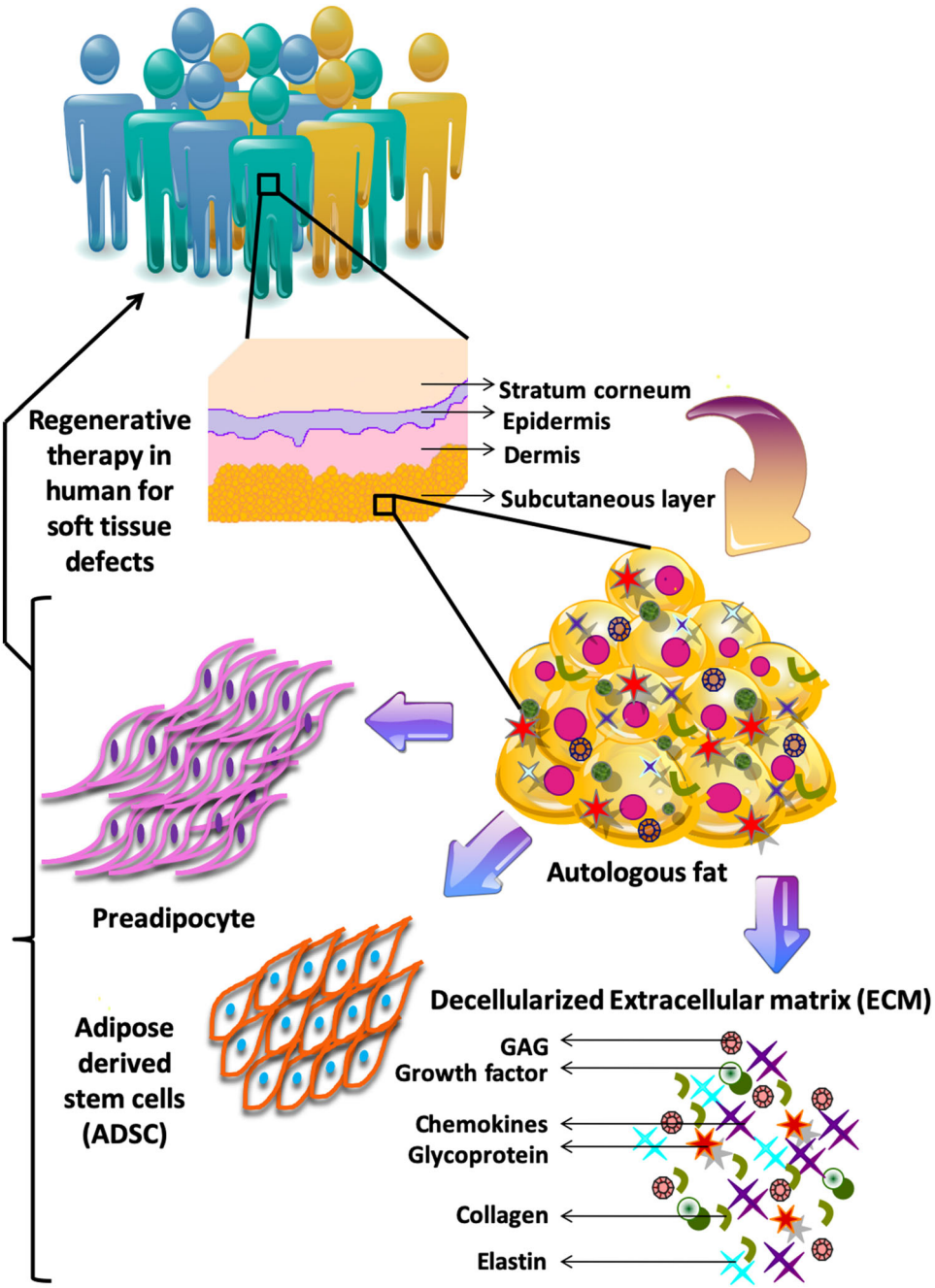

Fig. 1 Schematic representation of human adipose tissue derivatives, and its application in regenerative therapy

3 Engineering human adipose tissue derivatives

Fat graft, adipose derived cells like preadipocytes, ADSCs and ECM are the major derivatives of human liposuctioned adipose tissue. These derivatives can be engineered in an entailed fashion and utilized in combination with or without polymers for regenerative medicine, reconstructive therapies and tissue engineering. The subsequent section will be addressing the biology, processing and characterization techniques of adipose tissue derivatives as potential biomaterials.

3.1 Fat grafting: adipose tissue analogue

Autologous fat graft consists of mature adipocytes, pre adipocytes, stem cells and other growth factors and less likely to induce immunogenic response. Owing to the advantages and ease of processing, autologous fat grafting (AFG) has been considered as the widely used treatment for soft tissue defects for a long time. [30, 31]. AFG method includes donor site selection, optimal method of fat harvesting, processing and fat transfer [32]. Surgeons have freedom in choosing the donor site depending on the

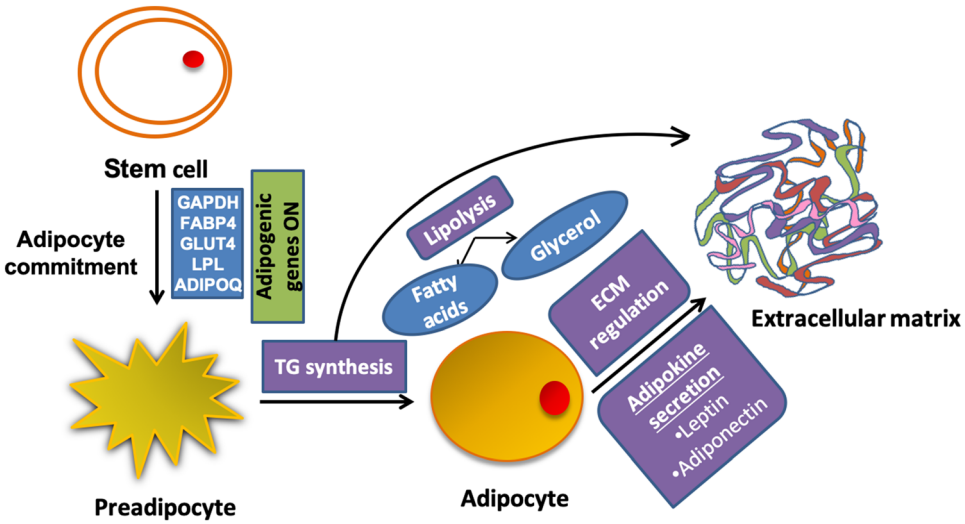

Fig. 2 Cartoon showing adipogenic differentiation; *TG* triglyceride

requirement and patient's health condition. In general, inner thighs and lower abdomen are considered to have more viable adipocytes with increased volume of fat than other sites [33]. Choice of donor site has direct impact on the viable cell concentration during fat transfer. But, the composition of fat varies with age, sex, size and metabolism of the patient. There is no clear evidence suggesting an ideal donor site to get more number of viable cells. Considering these factors, clinician can take charge in choosing the site, most preferably thigh and abdomen. Following the site selection, fat harvesting method has to be optimized to have minimal detrimental effect on cell viability. Pu et al. [34] concluded their study stating conventional method of fat harvest maintains the morphology and viability but survival rate of cells after transplantation is likely to be less. Syringe aspiration is considered to be better than conventional method since latter involves suction assisted liposuction above 700 mm Hg vacuum which is likely to disrupt cells as confirmed by histological examination. Anesthesia drugs like lidocaine reduces cell viability and affects the morphology but these effects can be eliminated by proper washing of harvested fat during processing [35–37]. In general, tumescent solution contains very less concentration of lidocaine and by reducing the time of exposure, its negative impact on cells can be reduced. Optimal method of fat harvest has to be balanced in such a way that cell morphology and viability has to be retained with greater yield. Coleman et al. refined the harvesting method to such an extent where minimal procedure is needed without exploiting the nativity of cells [38, 46]. They showed that selection of cannula with blunt end and broad diameter would help in acquiring more cells without rupturing them. Overall, syringe aspiration with 10 mm diameter is preferred for small defects whereas Coleman technique of lipoaspiration with 3 mm cannula is preferred for large area defects or for repeated injections during fat transfer.

3.1.1 Processing, adipo-transfer and concerns

Fat processing followed by adipo-aspiration is still ambiguous due to lack of study and evaluation of cellular functionality using quantitative analysis. Filtration, centrifugation and decantation are widely used refinery methods to segregate fat from blood, lipid and other components, so that entire fat can be utilized for fat transfer. Decantation is the simplest method that can be used for fat graft processing where the fat graft obtained through liposuction or syringe is allowed to settle on its own by the influence of gravity. Conde-Green et al. [39] studied the difference in retaining ADSCs and viable adipocytes after processing using decantation and centrifugation. This study revealed that decantation is not proficient enough to separate contaminating blood cells whereas centrifugation being a defiant step to adipocytes retains maximum viability and more number of ADSCs when optimal force is used. Closed technique was a traditional method of fat processing due to the risk of contamination when fat is exposed to air. Another study demonstrated the difference in cell viability by comparing open filtration method using cotton cloth with closed centrifugation technique using histological examination [40]. No remarkable difference was found between the two methods in cell survivability and minimal fibrosis was observed in nude mice. A similar study was conducted by Minn and his co-workers concluded stating either of the methods can be used for fat processing [41]. Efficiency of centrifugation method depends on the time and speed of centrifugation of fat for separation. It is evaluated by several researchers and have optimized based on cell viability, proliferation,

glucose uptake by cells and histology [42]. Xie et al. [43] found linear decrease in cell viability with increase of centrifugal force above 4000 rpm. Increase in centrifugal force may rupture or fuse cells and cause cell lysis and morphological changes affecting its function. Optimization of centrifugal force was also studied by Kim et al. [44] and recommended to use 3000 rpm for 3 min by evaluating viability through trypan blue staining. But, other study recommends 1200 g to separate oil and blood from fat as well as to retain more adipose derived stem cells (ADSCs) [45]. Coleman technique also suggests using 1286 g for an efficient fat graft processing with increased tolerance of tissue to ischemia [46]. It has been proved that centrifugation method around 1200*g* for 3 min is most efficient in retaining growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) as well as ADSCs [47, 48].

Common concerns of fat transfer include site, volume and method of injection to prevent necrosis, quick resorption, blindness, insidious lesions and stroke with minimal inflammatory response [49–52]. There is no standard method of injection for fat grafting although bolus injection is prevalently used technique of fat transfer. Karacaoglu et al. [53] has studied the role of recipient site in fat grafting using rabbit. Supramuscular injection was found to have profound effect on cell survival rate and volume preservation with reduced resorption rate when compared with subcutaneous and submuscular injections on rabbit face. The survival rate also depends on early vascularisation and injected volume of fat graft. Nishimura et al. [54] confirms the presence of growth factor VEGF and stem cells that assisted in vascularization within 7 days of fat transfer. Though Autologous Fat Grafting (AFG) is currently used as a gold standard method in clinics (Fig. 3), it is associated with unpredicted host response depending on intensity of tissue damage. This might be due to insufficient penetration of oxygen to cells leading to ischemia, apoptosis, necrosis, dedifferentiation affecting revascularization leading to the formation of cyst as given in Table 1. It has been found that survival rate increases when the fat graft is injected at proximity of 2 mm from arterial blood supply [55–57].

3.2 Adipose tissue-derived cells (stromal vascular fraction (SVF), adipose-derived stem cells (ADSCs), preadipocyte)

Adipose depots consist of heterogeneous population of stromal vascular fraction (SVF) cells, mature adipocytes, lymphocytes and macrophages, vascular and stromal cells. Adipose-derived stromal/stem cells (ADSC) possess multilineage (adipo-, chondro-, osteo-, hepato-, teno-, neuro-, cardiomyo-) differentiation potential [58]. Kim and his coworker have explored the cellular based therapeutic potential of ADSCs based on several *in vitro* and *in vivo* studies accomplished around the globe [59]. Specifically, Jeon and his colleagues carried out a study where ADSCs were differentiated osteogenically and aided bone formation under *in vivo* conditions [60]. Similarly, a study was conducted to expose the chondrogenic ability of ADSCs by culturing and differentiating it into chondrocytes and evaluated its potential through mice transplantation [61]. Another study revealing its hepatogenic potential, where injured hepatic cell line was cocultured along with ADSCs. Cocultured ADSCs expressed significantly higher regenerative factors and promoted recovery of injured cell line and also showed early hepatogenic differentiation [62]. They also have the potential to trans-differentiate into non-mesenchymal cells such as endocrinal cells, neurons and hepatocytes [63–65]. Zhu et al. [66] compared bone marrow and adipose tissues for which is a more suitable for isolation of mesenchymal stem cells (MSCs) for soft tissue regeneration. They observed that adipose tissue serves as better source for MSCs than bone marrow due to minimal invasiveness, higher yield of cells with enhanced proliferation rate and greater frequency of stemness maintenance. On the other hand, Gimble et al. [67] divides source of adipose tissue into at least five categories based on the functionality of tissue like bone marrow, brown, white, mechanical (abdomen, hand and thighs) and mammary. Depending on the required function, site and source of adipose tissue has to be chosen. White, mechanical adipose tissue is widely used source for acquiring ASCs.

3.2.1 Isolation and differentiation

The isolation and characterization methods for adipose tissue-derived stem cells were well established and standard protocols were followed [68–71]. Briefly, the isolation of stem cells includes washing, enzymatic digestion and cellular extraction (Fig. 4). Enzymatic digestion yields SVF composed of stromal and vascular cells, which were then centrifuged followed by adherent culturing. The expanded cells can then be differentiated into various lineages according to the application [58–65] (Fig. 5).

Adipogenic differentiation of adipose-derived stem cells can be distinguished by the gene expression levels and classified into early, middle and late phases. Early phase involves the suppression of pref-1 (preadipocyte factor-1, negative regulator), with the coordinated stimulation of several genes by PPARγ and C/EBPα. Intermediate phase consists of triglyceride (TG) synthesis which is influenced and regulated by genes such as fatty acid binding protein (FABP4) and acetyl-coenzyme A synthetase (ACS). Metabolism of TG constitute the terminal phase of adipogenesis where adipokines like adiponectin and leptin are

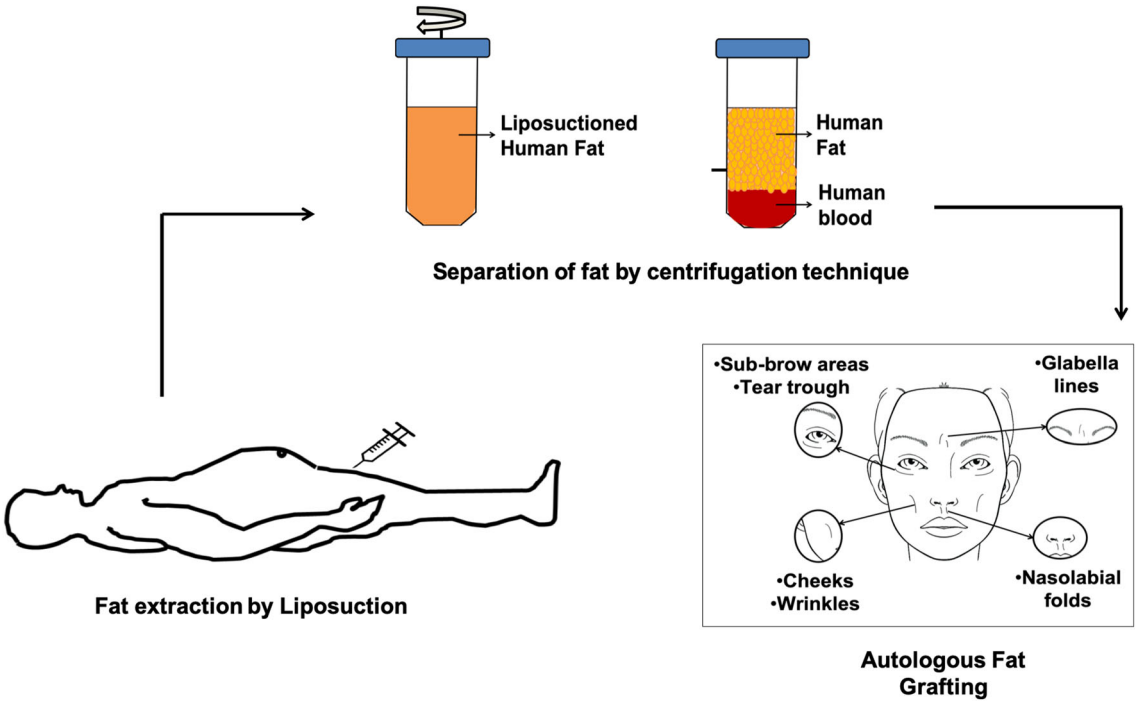

Fig. 3 Frequently used technique of autologous fat grafting

Table 1 Limitations of fat grafting

No.	Limitations of fat grafting	References
1.	Increased volume resorption	[57]
2.	Necrosis due to lack of cell viability	[49–52]
3.	Inadequate mass transport—exchange of gases and nutrients	[46]
4.	Vascularization deficit	[45, 54]
5.	Multiple injections required for collection, processing and adipo-transfer	[57]
6.	Injection site influences volume retention of fat	[48, 53]
7.	Unpredictable results	[55, 57]
8.	Less shelf life—immediate adipo transfer is better	[47]

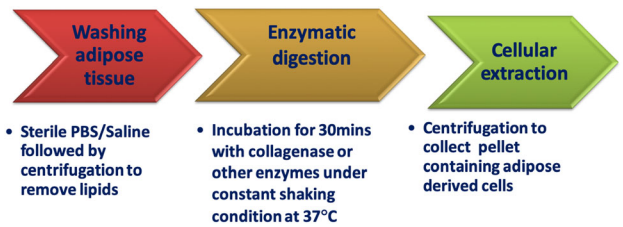

Fig. 4 Isolation of ADSCs from human adipose tissue

secreted, lipoprotein lipase (LPL) catabolises TG resulting in glycerol and fatty acids. The synthesized TG anabolizes giving rise to ECM production. Insulin, dexamethazone, indomethacin, isobutyl methylxanthine (IBMX) and rosiglitazone are the commonly used adipogenic supplements under *in vitro* conditions to induce adipogenesis for 14–21 days of ADSCs with slight modifications in concentrations [72, 73]. Insulin tends to cross-activate insulin growth factor (IGF) which then mediates the downstream stimulation of adipogenesis by accelerating lipid accumulation. Dexamethasone acts through glucocorticoid receptor, inducing C/EBPδ whereas IBMX is believed to enhance the expression of C/EBPβ, thereby differentiating adipocytes [74–76]. Various adipogenic differentiation strategies have been developed where adipocytes respond differently depending on the type and concentration of inducers used [77]. However, to obtain successful adipogenic differentiation of ADSCs, factors such as source, site, age, sex, metabolism, cell plating density and surgery protocol of patient etc., need to be considered during isolation for better reproducibility [78].

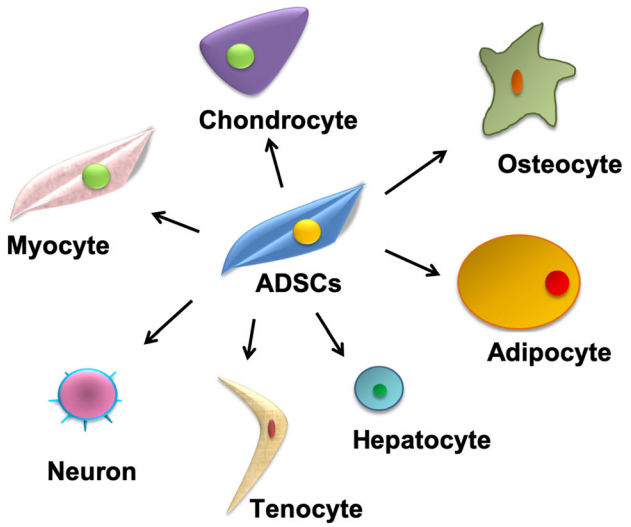

Fig. 5 Differentiation potential of ADSCs

3.2.2 Characterization of ADSCs

In addition to inducing adipogenesis, ADSCs have shown to be vested in perivascular site facilitating the neovasculogenesis by formation of new blood vessels with endothelial and smooth muscle cells. They are also known to partake in tissue repair of systemic circulation [79]. SVF or ADSCs could be characterized using positive and negative expression of surface markers on the cells. Bourin et al. [80] explains the difference in the expression of markers specific to SVF and ADSCs using flow cytometry technique. ADSCs are shown to be positive for CD29, CD54, CD90, CD73, CD105, CD10, CD166, CD9 and CD146 whereas Negative for hematopoietic markers such as CD31, CD45. Greater percentage viability (around 90%) of cells was observed in case of ADSCs than SVF. This might be due to the increased presence of mature adipocytes in SVF. Bourin and his coworkers classified the immunophenotypic markers as primary and secondary based on the intensity of their expression. Colony forming units (CFU) studies have shown a higher proliferation capacity in SVF. Immunohistochemistry or immunocytochemistry, RT-PCR, western blotting techniques can also use to evaluate the expression of specific biomarkers such as adiponectin, LPL, leptin, FABP4, C/EBPα and PPARγ. Alternatively, Oil red O staining is most prevalently used method to analyse adipogenic differentiation qualitatively based on cellular lipid synthesis. Varma et al. [81] did not found any significant phenotypic and functional behaviour differences between freshly isolated and cultured ADSCs. Immunomodulatory effect of ADSCs is less due to the decreased expression of human leukocyte antigen (HLA) and does not incite allogenic reactions [82, 83].

3.3 Decellularized adipose tissue: extracellular matrix

Extracellular matrix (ECM) is a heterogenous and highly dynamic, non-cellular constituent existing within all the living tissues. Fundamentally, ECM is composed of proteins, polysaccharides, proteoglycans, growth factors, receptors and some molecular cues necessary for biochemical, biomechanical signalling and remodelling of tissue microenvironment. Its unique three-dimensional ultrastructure has profound significance in cell adhesion, migration, morphological organization of a tissue, physiological support and maintenance of tissue architecture [84]. Multifunctionality of ECM is markedly illustrated by wide range of responsibilities that includes, serving as a pool of growth factors for signalling, providing binding sites for cell surface receptors, modulating the structural properties of tissue, immunological response, thereby meeting all the requirements of a tissue to maintain homeostasis. ECM is primarily composed of basement membrane and interstitial matrix. Basement membrane forms a layer between epithelial and stromal cells whereas interstitial matrix is composed of surrounding cells (adipocytes, preadipocytes, stromal cells). Basement membrane can be further differentiated into two layers, basal lamina and reticular lamina.

The adipose derived ECM is a complex entity of functional and structural proteins, arranged in a distinct fashion, rendering tissue specific three-dimensional architecture (Fig. 6). Altogether, these proteins with other mechanical cues sets out diverse cellular functions including attractive ligand binding site, maintenance of cellular coherence and integrity and signalling reservoir for host processes like cell adhesion and migration, cell proliferation, orientation and differentiation, inflammation, vasculogenesis and immunomodulatory response, etc. [24, 85].

Copious presence of collagen in mammalian source accounts to approximately 70% of the dry weight of the ECM from most tissues and organs [86]. Collagen types other than type I exist in naturally occurring ECM, albeit in much lower quantities and contribute predominantly to tissue structure and function. Different collagen types offer unique mechanical properties to the ECM thereby, contributing to the abundant utility of the intact collagen in mammalian ECM. Each type of collagen has its own distinct functional property, contributing to the mechanical integrity of intact ECM. For instance, type IV collagen has significant role in vascularization by acting as ligand for vascular and endothelial cells of plasma membrane [87]. ECM, a rich source of collagen and other proteins makes it suitable environment for cells to adapt and grow under *in vitro* and *in vivo* conditions. Collagen along with elastin forms fibrous networks to provide mechanical strength to organs by cementing cells, help withstand stress and

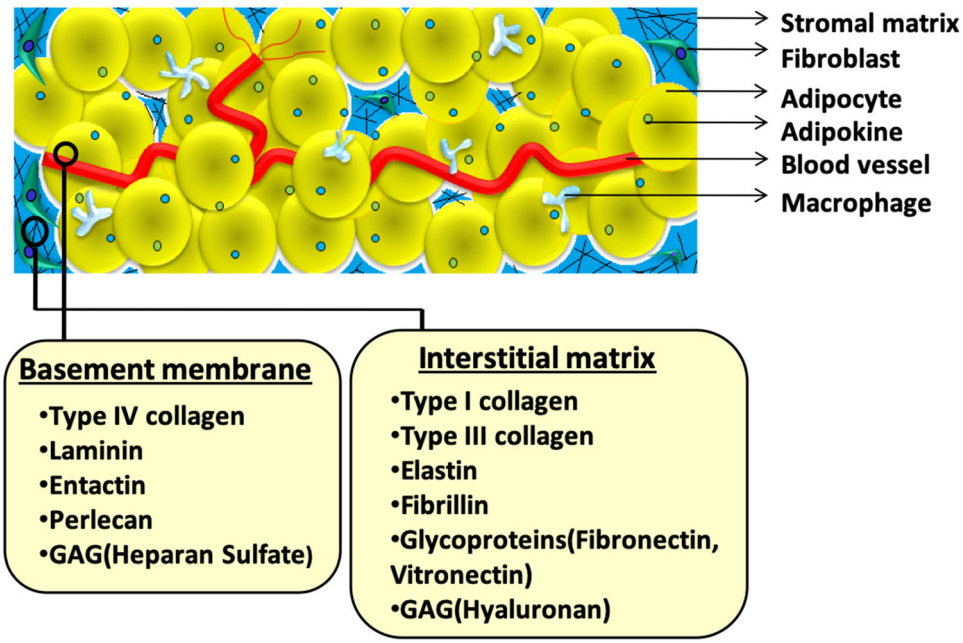

Fig. 6 Schematic representation of extracellular matrix (ECM) components

provide tensile strength [88]. Collagen derived from human can be used in regenerative medicine which has reduced the immunogenicity due to pathogenic transmission from xenogenic sources [88, 89].

Glycosaminoglycans (GAGs) are negatively charged polymers having adequate affinity for water and sequester the prime functions of cell. The heparin binding properties of GAGs prevents the blood coagulation, binds with numerous growth factors and ligands necessary for tissue repair and function [90]. Laminins are the major additional integral proteins present in the basal lamina of ECM, contributing to the integration of scaffold. They promote cellular polarization and organization of tissues to maintain the mature tissue functionality [91]. Fibronectin favours the adhesion of cells by acting as ligand binding sites facilitating the tissue repair [92]. Fibronectin coating *in vitro* enhances the immobilization of cell by interacting with the cell binding sites having greater impact on cell behaviour [93]. In light of these properties a variety of Heparin and GAG functionalized biomaterials are available for regenerative medicine and drug delivery applications [94]. Elastin fibers are influenced by the spatio-temporal elastogenesis from precursor tropoelastin molecules to form a stable microfibril sheet. These fibrous bundles present in ECM contribute to dynamic stretch and elasticity of cells, tissues and organs and to maintain vascular homeostasis [95]. Human adipose tissue consists of elastin molecules which can be used as potential biomaterial for muscle and tendon development or arterial replacement [96, 97] and other tissues requiring high range of elasticity [98].

Negligible presence of growth factors and cytokines in ECM makes them rich potent modulators of cell behaviour. vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF) transforming growth factor-β (TGF-β) and keratinocyte growth factor (KGF) exist in multiple isoforms of adipose derived ECM, exerts specific biological function [99–106]. Although purified forms of certain growth factors are commercially available and well known for their functional activity of neovascularisation and angiogenesis, studies are restricted to various parameters like optimal dose, release profile, site of release and regulation, which is a prerequisite for accelerating the wound repair and regeneration [107]. An alternate strategy might be utilizing ECM in its native state which will be advantageous due to the presence of all of the attendant cues in the relative amounts and concentration required for the microenvironment to functionalize the necrotic niche.

In human body, cells are supported by ECM for their growth since it provides interconnected framework of proteins, proteoglycans and growth regulators essential for cell attachment, migration, polarity, differentiation and development. ECM is an ideal, biological scaffold like framework having unique composition contributing to the crucial biochemical and mechanical factors responsible for tissue morphogenesis [108, 109]. It is primarily composed of both soluble and insoluble proteins, growth factors and polysaccharides thereby, providing structural framework for efficient cellular interactions. There might be supplementary cues, yet to be identified as the component of

ECM. Owing to the complexity of native ECM, mimicking ideal niche of cells becomes tricky during the development of synthetic polymeric scaffolds. The adipose tissue-derived ECM helps in maintenance of multiple cell types, differentiation of ADSCs into most cell lineages etc., which was studied by Kim et al. by proliferating human dermal fibroblasts, chondrocytes, aortic smooth muscle cells, human umbilical vein endothelial cells (HUVECs) and ADSCs on adipose derived ECM [110].

Unlike ADSC/SVF or fat graft, processing of ECM does not have a universal standard protocol. Hence, we have discussed the processing and characterization techniques of ECM elaborately in this review. Adipose-derived ECM from human fat is the best accessible source due to easy processing and wide availability. ECM is an active ingredient of the specific tissue, determining function and fate of surrounding cell (Table 2). These attributes of ECM makes it unique, naturally obtained outstanding three dimensional complex scaffolds.

3.3.1 *Decellularization approach*

In contrast to other human adipose tissue derivatives, methodologies for processing and characterization of adipose tissue have not been well defined. In this section, techniques for decellularization (Fig. 7) of ECM followed by its characterization to evaluate the efficiency of the decellularization process has been critically analysed and discussed a summary of which is listed in Table 3.

3.3.1.1 Physical methods of decellularization Freeze–thaw cycle is the cell membrane disrupting technique eventually, releasing the factors present inside cell. Flynn et al, Francis et al. and Brown et al. used this technique as the first step of decellularization in their protocols [110–112, 121]. Cells when subjected to stress while adapting temperature fluctuations, leads to disruption. This method has minimal impact on the deviation from native architecture of adipose ECM. Alternatively, salt buffer could be used along with freeze–thaw cycle to promote cell lysis. In order to prevent the adverse effects of this technique, 5% trehalose was used as cryoprotectant during the process [113].

Homogenization is mechanical method of cell disruption to minimise the heterogeneity of tissue digestion. This is the widely used method for adipose tissue, due to less time consumption and its effectiveness in cell lysis [114]. At times, this method is used in combination with other physical treatment such as ultrasonication or chemical treatment such as hypertonic buffer [115]. Decellularization protocol of Choi et al. [116] is extensively based on homogenization method followed by several times of washing. Effectiveness of this protocol is unknown since they used the decellularized powders and scaffolds derived from human adipose tissue in immunecompromised nude mice. This lacks proper immunological evidence when ECM is used as xenograft.

These physical treatments are more advantageous over chemical methods since washing out of unreacted chemicals after reaction is not needed. Less time is needed for the physical treatment to cause cell lysis. On the other hand, it is generally accompanied by chemical treatment for effective decellularization and for specific removal of cellular components, likely to cause immunogenicity. Physical treatment alone is sufficient enough to cause cell lysis but not effective in removal of cellular remnants. Therefore, sensible combination of physical and chemical treatment is required to retain the native architecture of ECM by eliminating antigens.

3.3.1.2 Chemical methods of decellularization Osmolysis is the process where cells are subjected to stress by altering the concentration of salt. This in turn, causes cell

Table 2 Properties of human adipose derived ECM [108–125, 127–134]

No.	Edging properties of human adipose derived ECM
1.	Native niche preservation
2.	Tunable mechanical and functional properties
3.	Biocompatible
4.	Biodegradable
5.	Customizable forms of bioscaffold
6.	Longer shelf life
7.	Ease handling and maintenance

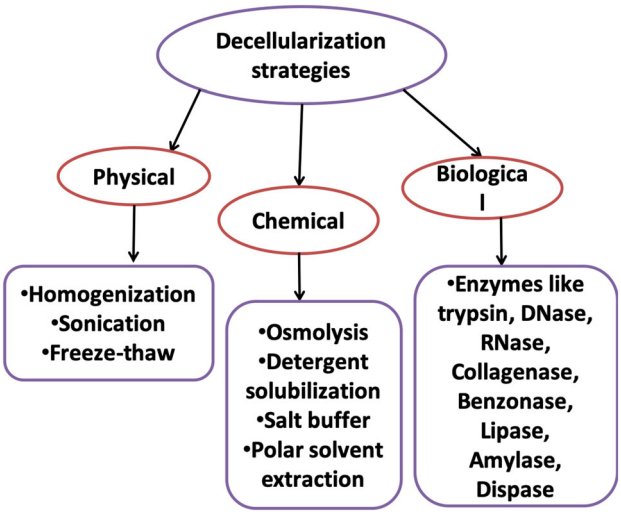

Fig. 7 Strategies used for decellularization of adipose tissues

Table 3 Overview of decellularization of human adipose tissue

No.	Author and year	Decellularization strategies	Formulation of processed ECM	References
1.	Uriel *et al*, (2008)	Dispase High salt buffer + homogenization Urea buffer	Physically crosslinked hydrogel	[119]
2.	Choi *et al*, (2009)	Homogenization	Powder, Scaffold, Hydrogel	[114–117]
3.	Flynn *et al*, (2010)	Freeze–thaw cycle Enzyme—Trypsin, RNase, DNase Polar solvent extraction—Isopropanol Salt buffer	Scaffold	[110, 121]
4.	Young *et al*, (2011)	Detergent—SDS/SDC Enzyme—Lipase/Colipase	Physically crosslinked hydrogel	[120, 124]
5.	Yu *et al*, (2011)	Salt buffer Enzyme—α-Amylase Peiodic homogenization	Microcarrier foams	[110, 125]
6.	Kochhar *et al*, (2011)	Peracetic acid Detergent—Triton X-100 DNase	Chemically crosslinked hydrogel	[132]
7.	Francis *et al*, (2012)	Freeze–thaw Acetic acid	Electrospun scaffold	[111]
8.	Wang *et al*, (2013)	Ultrasonic homogenization Enzyme: Pancreatin	Microparticle	[130]
9.	Sano *et al*, (2013)	Enzyme: DNase Detergent: SDC Sodium azide	Scaffold	[129]
10.	Poon *et al*, (2013)	Enzyme: Dispase II Salt buffer and Urea buffer Guanidine-HCl buffer	Physically crosslinked hydrogel	[118]
11.	Pati *et al*, (2014)	Detergent: SDS Isopropanol Peracetic acid, Ethanol	Physically crosslinked hydrogel	[131]
12.	Song *et al*, (2014)	Detergent: SDS, SDC Salt buffer	Scaffold	[134]

rupture, thereby releasing cellular components. This method can be coupled with physical method like freeze–thaw cycle to increase the efficiency of cell membrane disruption [117]. However, osmotic treatment to cells is not sufficient enough to remove the nucleic acids and other cellular remnants of ECM. Choi et al. has chosen this technique as an initial step of decellularization process to rupture the cells.

In general, extracellular matrix is comprised of proteins with varying degree of solubility. Variation in pH is necessary to dissolve the desired components of ECM by treating the tissue in both alkaline and acidic environments. Poon et al. [118] uses salt buffer and urea buffer in alkaline pH to solubilise hydrophobic proteins followed by treating the remaining tissue under acidic pH. This protocol is efficient to completely solubilise components of ECM. Uriel et al. [119] uses urea buffer to dissolve the extracellular proteins, formulating into temperature induced hydrogel. These buffers tend to destabilize the hydrophobic protein interaction allowing them to solubilise in the solution. Yu et al. [125] used salt buffer containing as the initial step of decellularization for membrane lysis followed by periodic homogenization along with acetic acid to remove the carbohydrate and glycoproteins by breaking the bond between adjacent carbon atoms. This can also be used as a disinfectant and to remove nucleic acids with minimal effect on the native proteins. However, optimal balance in the usage of highly acidic or alkaline solvents is necessary to prevent detrimental effects to ECM constituents.

Solubilisation with detergents is also commonly used chemical treatment for decellularization. Detergents such as sodium dodecyl sulphate (SDS), sodium deoxycholate (SDC) and triton X-100 are used for protein solubilisation. Kim et al. [117] used osmolysis by using increased concentration of salt along with physical treatment, followed by detergent solubilisation using SDS. Sodium dodecyl sulphate (SDS) tends to disrupt the disulphide bonds present in the proteins causing solubilisation. Young et al. [120] optimized the protocol using detergents such as SDS or SDC. Care should be taken to ensure preservation of native ECM architecture by limiting factors such as over-exposure to high concentration, since the greater time of exposure causes denaturation of proteins. Adipose tissue consists of lipids that need to be removed to minimize immunogenicity. Polar solvent extraction is used wherein isopropanol and ethanol react with lipids present in the adipose tissue and can be removed by washing [134]. Flynn et al. [121] employed polar solvent extraction method, specifically to remove lipids present in the adipose tissue, followed by immersing the tissue in various salt buffers to increase solubility without causing precipitation of proteins.

3.3.1.3 Biological methods of decellularization Decellularization process efficiency is evaluated by the presence of nucleic acids, which are normally secured from degradation by matrix bound nanovesicles (MBV). Nuclease secured nucleic acids are packed into these MBVs making their removal difficult [122]. In *in vitro* the removal of nucleic acids is achieved by enzymes such as RNase and DNase which are specific and well used for removing the nucleic acids. Another critical step in decellularization process is usage of proteolytic enzymes like pepsin, papain, dispase [118] to dissolve proteins in the tissue by cleaving specific bonds between amino acids [123]. Dispase is used as initial step of decellularization to disrupt the integrity of proteins in the adipose tissue, thereby increasing the solubility of desirable factors of ECM. Uriel et al. and Poon et al. used dispase enzyme to increase the efficiency of decellularization process [118, 119]. Lipase in combination with colipase was used by Young et al. [124] to replace the usage of organic solvents to remove lipids present in adipose tissue. α-Amylase was used by Claire Yu et al. and Benzonase was used by Zhang et al. to disintegrate the clustered proteins of ECM [125–127]. Since, collagen is the most prominent protein present in ECM, most of these biological agents aim in cleaving the collagen peptides.

3.3.2 Evaluation of decellularization process

Analogous to the increasing demand for standardized protocol, we need to have reliable set of characterization assays to evaluate the efficiency of decellularization process. These characterization assays (Fig. 6) will allow a comparison of tissue contents before and after processing and also facilitate large scale production of ECM. Although studies from different research groups adopted different processing steps, same evaluation assays were used which includes histological staining, immunohistochemistry and electron microscopy to analyse the constituents of native and decellularized tissue. Here, we have summarized the detailed study of those techniques and their implications.

Histological staining is the simplest technique widely employed for the identification of lipids, nucleic acids, proteins and other cellular components of ECM after processing. H&E staining reveals the whole essence of processing by staining cellular membrane, proteins and cytoplasmic granules and nucleic acids. In case of *in vivo* studies of ECM scaffold transplantation, Hematoxylin shows the infiltration of host cells into the ECM scaffold. Oil Red O staining reveals the efficient removal of lipids whereas Masson trichrome staining shows retained collagen fibres post decellularization. Proteins are visualized using specific antibodies like laminin, types of collagen, elastin by immunohistochemistry technique [127–130]. Proficiency of ECM processing can be evaluated by quantifying the proteins, proteoglycans and growth factors present in the acellular ECM. Nucleic acids present in the ECM such as DNA can be quantified using picogreen assay and gel electrophoresis to prove the reduced level of dsDNA remnants after decellularization. It has been reported that < 200 bp and < 50 ng of DNA is not capable of eliciting immune response in the host [116]. Similarly, SDS-PAGE can be used to quantify proteins by comparing with commercially available protein ladder [117]. To analyze and confirm the percentage of individual proteins and growth factors, corresponding ELISA and biochemical assays are used. For example, hydroxyproline, DMMB and Fastin assays are used for quantifying collagen, GAG and elastin respectively [131, 132]. In case of growth factors, corresponding ELISA kits are used [127]. LC–MS technique is the most accurate technique to quantify all the known and unknown components present in processed ECM [128]. Though it is a quite complicated and time consuming process, accuracy of quantification makes it unique from other methods. Although several other methods can be combined and utilized to evaluate the efficiency of decellularization process, researchers are still to simplify these methods of evaluation with higher accuracy. But, so far, there is no universal standard method of either processing the tissue or quantification methods to estimate its efficiency analysis. The overall fibrous architecture of ECM can be studied using electron microscopy. Mechano-stress testing can be conducted to estimate the ability of ECM to withstand force by measuring its tensile and

compressive strength [133]. Mechanical strength of scaffolds plays an evident role in favouring the specific lineage of cellular differentiation. Adipogenic scaffolds are prescribed to have approximately 2 kPa similar to primordial adipose tissue to ease the differentiation regime. Being derived from natural source, immunological rejections in the host could be associated with ECM hindering its translational potential (Table 4).

4 Translatable biomaterials of adipose tissue derivatives

Applications of adipose tissue derivatives have been categorised on the basis of their translational potential for soft tissue regeneration and have been briefly discussed here. Autologous fat graft (AFG) is the currently existing treatment for soft tissue defects since it has wide range of advantages like minimal invasiveness, no immune response, cell based therapy, greater regenerative potential and safe to use [135, 136]. But, AFG is restricted to certain limitations such as unavailability of fat in patients, patient specific treatment, necrosis due to matured cells, volume resorption, repeated injection, poor vascularization. Improved AFG treatment by use of platelet rich plasma (PRP) and/or ADSCs along with native fat graft has been observed to improve the vascularisation and survival of graft [137]. PRP contains the notable growth factors and blood products to interact with the surrounding cells and enhance the adipogenesis. PRP and ADSCs based therapies are also used for hair follicle treatment, soft tissue facial defects [138–140]. Apart from AFG, most preferred form of scaffold for soft tissue defects is injectable hydrogel. Ease of handling, hydrophilicity, fluid absorption capacity, viscoelasticity, biocompatibility, and minimal invasive procedure are substantial properties adequate to induce adipogenesis by closely resembling the loose mass of native adipose depots. Semi-autologous therapies are plethora of interest in clinics where patient specific ADSCs or PRP or adipose derived growth factors are used along with polymeric hydrogels as therapeutic agents [141, 142]. Adipose derived growth factors like IGF, TGF, VEGF, bFGF, EGF can also be used as therapeutic vehicles alone or in combination with polymeric or biologic scaffolds [143–146].

The biomaterial or biomolecule formulations having translational potential should exert certain criteria to be applied in clinics. It should be able to minimise the complexities of transplantation with simple processing techniques, ease of handling and economic. Mandatory product quality standards like safety, reproducibility, purity, potency and efficacy has to be maintained before using

Table 4 Evaluation techniques for decellularized ECM

No.	Evaluation methods of decellularized ECM	References
1.	Nucleic acid	[110, 115, 116, 120, 121, 130]
	Picogreen assay—dsDNA quantification	
	Gel electrophoresis—DNA and RNA	
	Immunohistochemistry/histology—DAPI, H&E, Hoescht	
2.	Proteins	[111, 115, 117–120, 127–132]
	SDS-PAGE	
	Western blot	
	Mass spectrometry	
	Immunohistochemistry/Histology—Masson trichrome/Picrosirius red (Collagen), Laminin, Fibronectin	
	Biochemical assays- Collagen (Hydroxyproline assay)	
	Proteoglycan (DMMB assay), Elastin (Fastin assay)	
3.	Mechanical—Tensile and compressive strength	[114, 124, 132]
4.	Lipids	[120, 127–130]
	Oil Red O	
5.	Viscoelastic properties	[132]
	Rheometer, Viscometer	
6.	Morphological observation	[110, 111, 115, 119, 120, 128]
	Electron microscopy	

these adipose tissue derivatives in a clinical setting. Designing a biomaterial through an engineer's perspective is an essential feature to construct a functional scaffold by balancing both medical and biological requirements along with materialistic parameters [147]. Next generation biomaterials like ECM needs extra attention during the development process since decellularization is a property changing complex technique [148]. Around 80 commercially available ECM based products are in clinical use for treating hernia mesh [149], topical wounds [150], skin defects [151], breast and pediatric heart reconstruction [152, 153]. Apart from these, there are substantial preclinical studies where ECM is used for musculotendon repair, temperomandibular joints, limb and digit regeneration [154]. Different forms of ECM can be used along with patient- specific cells to cure soft tissue defects as mentioned in Fig. 8. Unlike AFG, ECM possess outrageous properties like tunability, retainment of injected volume, enhanced cell viability, longer shelf life and mechanical cues necessary for soft tissue reconstruction [155, 156]. In the light of evidence, a number of *in vitro* and *in vivo* studies have been reported unravelling the mysterious mechanism of adipose derived ECM action along with the neighbouring cells [115–120, 122, 124, 125, 127]. Flynn et al. [121] was the first research team to develop detergent free method of decellularization process to extract ECM out of human adipose tissue followed by development of ECM-alginate microbeads by electrospraying technique to treat soft tissue defects [123, 157]. They further fabricated adipose ECM foams and microcarriers by electrospraying technique and used them to develop 3D cell culture platform to simulate the ECM microenvironment under *in vitro* conditions [158]. The microcarrier was then evaluated for trilineage differentiation (adipogenic, chondrogenic and osteogenic) wherein fold increase was observed. But, major limitation of this study is that they failed to evaluate its differentiation potential at different time points [125]. Further, *in vivo* studies and pre clinical trials would help the ECM microcarriers to reach the next level to be used in clinical setup. Shridhar and her colleagues developed adipose ECM-methacrylated chondroitin sulphate composite hydrogel for cartilage regeneration [159]. Both UV crosslinked and thermal crosslinked composite hydrogels exhibited increased cell viability. But, gene and protein expression studies will fetch us knowledge of hydrogel interaction with cells and its commitment towards specific cell lineage. Falguni pati and his coworkers formulated the bioprinting ability of adipose derived ECM along with alginate and evaluated their biological interaction with cells towards native lineage [131]. Francis and his colleagues elucidated the adipogenic ability of electrospun ECM [111]. These *in vitro* studies serve as a primitive work for the development of next generation biomaterial, ECM with various formulations. Pre-clinical studies involving adipose derived ECM upgrades it to a level ahead, bringing them closer in proximity to the clinical setup. Choi and his co-workers used adipose ECM powders as an injectable cell delivery for adipogenesis and established 3D culture system to demonstrate trilineage property of ECM powder [114, 160]. They also used nude mice to assess the adipogenic potential of developed ECM powder and is the first *in vivo* study carried out using adipose derived ECM. But the exact mechanism by which cells were recruited, migrated and interacted with ECM remains unclear. Another study involved differentiation of ADSCs towards adipogenic and endothelial lineage using adipose ECM-fibroin hydrogel signifies the angiogenic and adipogenic property of ECM in rats for 2 weeks [161]. Electrospun TiO_2-chitosan/adipose ECM bilayer composite was used to treat rat wound for 2 weeks by Woo et al. [162]. This shows that adipose derived ECM is not mechanically stable and needs the aid of polymer to be used as a wound dressing. Similarly, an injectable, thermo-sensitive adipose ECM-methylcellulose hydrogel was developed as a cellular delivery vehicle for treating dermal wounds [117]. This study demonstrated the wound closure ability of ECM hydrogel with and without ADSCs in rats for 21 days. Recently, polymeric, adhesive adipose derived ECM has been used as an implant for voice recovery [163]. This kind of research plays an eminent role in revealing the translational ability of adipose ECM which can be further expanded using large animal studies. These adipose tissue derivatives tend to have prominent properties similar to the source adipose tissue. It is likely to use these derivatives as biomaterials for homologous tissue regeneration for effective usage.

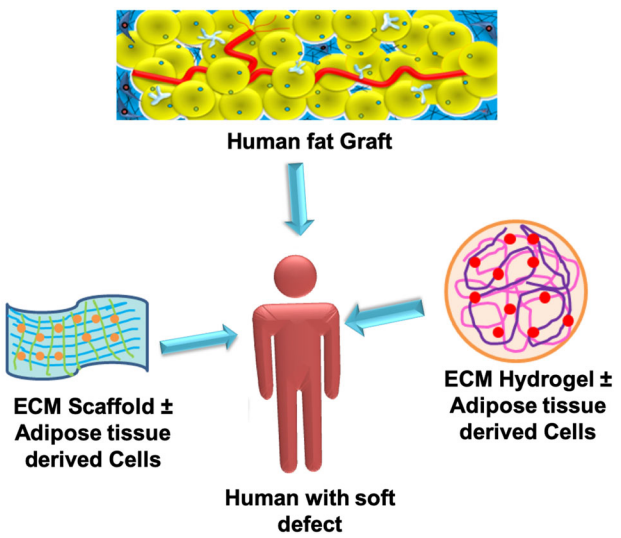

Fig. 8 Application potential of human adipose tissue derivatives

5 Concluding remarks and future prospective

Adipose tissue has the unique ability to undergo continuous remodelling and should be considered during regeneration of scaffolds. Though large scale soft tissue defects are very difficult to treat, semi-autologous and hybrid biomaterials meeting the demands could to be designed. Emphasis has to be made in investigating and deciphering the immunomodulatory effects of adipose derived ECM. Since soft tissue engineering is an interdisciplinary field, recycling of liposuctioned adipose tissue for regenerative therapies is possible with the expertise from biomaterialists, cell biologists, biomedical engineers and plastic surgeons. Active research going on in this direction to unfold the science and mechanism of soft tissue engineering would fetch researchers closer to the reality of soft tissue regenerative therapies. It can be contemplated that innovative efforts of researchers and suitable platforms will enable in constructing straightforward scaffolds for human soft tissue regeneration.

Acknowledgements The authors are thankful to Nanomission, Department of Science and Technology (DST), Government of India for funding under the "Thematic Projects on Frontiers of NanoScience and Technology (TPF-Nano)" program.

Compliance with ethical standards

Conflict of interest All authors declare that there is no conflict of interest.

Ethical statement There were no animal experiments carried out for this article.

References

1. Poss KD. Advances in understanding tissue regenerative capacity and mechanisms in animals. Nat Rev Genet. 2010;11:710–22.
2. Haughey BH, Wilson E, Kluwe L, Piccirillo J, Fredrickson J, Sessions D, et al. Free flap reconstruction of the head and neck: analysis of 241 cases. Otolaryngol Head Neck Surg. 2001;125:10–7.
3. Funt D, Pavicic T. Dermal fillers in aesthetics: an overview of adverse events and treatment approaches. Clin Cosmet Investig Dermatol. 2013;6:295–316.
4. Lemperle G, Morhenn V, Charrier U. Human histology and persistence of various injectable filler substances for soft tissue augmentation. Aesthetic Plast Surg. 2003;27:354–66.
5. Patrick CW Jr. Adipose tissue engineering: the future of breast and soft tissue reconstruction following tumor resection. Semin Surg Oncol. 2000;19:302–11.
6. Aarabi S, Bhatt KA, Shi Y, Paterno J, Chang EI, Loh SA, et al. Mechanical load initiates hypertrophic scar formation through decreased cellular apoptosis. FASEB J. 2007;21:3250–61.
7. Alster TS, West TB. Human-derived and new synthetic injectable materials for soft-tissue augmentation: current status and role in cosmetic surgery. Plast Reconstr Surg. 2000;105:2515–25.
8. Pinsolle V, Chichery A, Grolleau JL, Chavoin JP. Autologous fat injection in Poland's syndrome. J Plast Reconstr Aesthet Surg. 2008;61:784–91.
9. Lindberg RD, Martin RG, Romsdahl MM, Barkley HT Jr. Conservative surgery and postoperative radiotherapy in 300 adults with soft-tissue sarcomas. Cancer. 1981;47:2391–7.
10. Paul M, Mulholland RS. A new approach for adipose tissue treatment and body contouring using radiofrequency-assisted liposuction. Aesthetic Plast Surg. 2009;33:687–94.
11. de Bree R, Rinaldo A, Genden EM, Suárez C, Rodrigo JP, Fagan JJ, et al. Modern reconstruction techniques for oral and pharyngeal defects after tumor resection. Eur Arch Otorhinolaryngol. 2008;265:1–9.
12. Tintle SM, Gwinn DE, Andersen RC, Kumar AR. Soft tissue coverage of combat wounds. J Surg Orthop Adv. 2010;19:29–34.
13. Shandalov Y, Egozi D, Koffler J, Dado-Rosenfeld D, Ben-Shimol D, Freiman A, et al. An engineered muscle flap for reconstruction of large soft tissue defects. Proc Natl Acad Sci U S A. 2014;111:6010–5.
14. Matsumoto D, Sato K, Gonda K, Takaki Y, Shigeura T, Sato T, et al. Cell-assisted lipotransfer: supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. Tissue Eng. 2006;12:3375–82.
15. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001;7:211–28.
16. Rosen ED, Spiegelman BM. Molecular regulation of adipogenesis. Annu Rev Cell Dev Biol. 2000;16:145–71.
17. Katz AJ, Llull R, Hedrick MH, Futrell JW. Emerging approaches to the tissue engineering of fat. Clin Plast Surg. 1999;26:587–603.
18. Serrero G, Lepak N. Endocrine and paracrine negative regulators of adipose differentiation. Int J Obes Relat Metab Disord. 1996;20:S58–64.
19. Gesta S, Tseng YH, Kahn CR. Developmental origin of fat: tracking obesity to its source. Cell. 2007;131:242–56.
20. Eto H, Suga H, Matsumoto D, Inoue K, Aoi N, Kato H, et al. Characterization of structure and cellular components of aspirated and excised adipose tissue. Plast Reconstr Surg. 2009;124:1087–97.
21. Fraser JK, Wulur I, Alfonso Z, Hedrick MH. Fat tissue: an underappreciated source of stem cells for biotechnology. Trends Biotechnol. 2006;24:150–4.
22. Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, Bovenkerk JE, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. Circulation. 2004;109:1292–8.
23. Casadei A, Epis R, Ferroni L, Tocco I, Gardin C, Bressan E, et al. Adipose tissue regeneration: a state of the art. J Biomed Biotechnol. 2012;2012:462543.
24. Choi JH, Gimble JM, Lee K, Marra KG, Rubin JP, Yoo JJ, et al. Adipose tissue engineering for soft tissue regeneration. Tissue Eng Part B Rev. 2010;16:413–26.
25. Christy RJ, Yang VW, Ntambi JM, Geiman DE, Landschulz WH, Friedman AD, et al. Differentiation-induced gene expression in 3T3-L1 preadipocytes: CCAAT/enhancer binding protein interacts with and activates the promoters of two adipocyte-specific genes. Genes Dev. 1989;3:1323–35.
26. Juge-Aubry CE, Gorla-Bajszczak A, Pernin A, Lemberger T, Wahli W, Burger AG, et al. Peroxisome proliferator-activated receptor mediates cross-talk with thyroid hormone receptor by competition for retinoid X receptor possible role of a leucine zipper-like heptad repeat. J Biol Chem. 1995;270:18117–22.

27. Prestwich TC, Macdougald OA. Wnt/β-catenin signaling in adipogenesis and metabolism. Curr Opin Cell Biol. 2007;19:612–7.
28. Barker N. The canonical Wnt/β-catenin signalling pathway. In: Vincan E. editor. Wnt signaling. Methods in molecular biology. Humana Press; 2008. p. 5–15.
29. Fontaine C, Cousin W, Plaisant M, Dani C, Peraldi P. Hedgehog signaling alters adipocyte maturation of human mesenchymal stem cells. Stem Cells. 2008;26:1037–46.
30. Billings E Jr, May JW Jr. Historical review and present status of free fat graft autotransplantation. Plast Reconstr Surg. 1989;83:368–81.
31. Locke MB, de Chalain TM. Current practice in autologous fat transplantation: suggested clinical guidelines based on a review of recent literature. Ann Plast Surg. 2008;60:98–102.
32. Sajjadian A, Tandav Magge K. Treating facial soft tissue deficiency: fat grafting and adipose-derived stem cell tissue engineering. Aesthet Surg J. 2007;27:100–4.
33. Padoin AV, Braga-Silva J, Martins P, Rezende K, Rezende AR, Grechi B, et al. Sources of processed lipoaspirate cells: influence of donor site on cell concentration. Plast Reconstr Surg. 2008;122:614–8.
34. Pu LL. Towards more rationalized approach to autologous fat grafting. J Plast Reconstr Aesthet Surg. 2012;65:413–9.
35. Moore JH, Kolaczynski JW, Morales LM, Considine RV, Pietrzkowski Z, Noto PF, et al. Viability of fat obtained by syringe suction lipectomy: effects of local anesthesia with lidocaine. Aesthetic Plast Surg. 1995;19:335–9.
36. Keck M, Zeyda M, Gollinger K, Burjak S, Kamolz LP, Frey M, et al. Local anesthetics have a major impact on viability of preadipocytes and their differentiation into adipocytes. Plast Reconstr Surg. 2010;126:1500–5.
37. Shoshani O, Berger J, Fodor L, Ramon Y, Shupak A, Kehat I, et al. The effect of lidocaine and adrenaline on the viability of injected adipose tissue-an experimental study in nude mice. J Drugs Dermatol. 2005;4:311–6.
38. Erdim M, Tezel E, Numanoglu A, Sav A. The effects of the size of liposuction cannula on adipocyte survival and the optimum temperature for fat graft storage: an experimental study. J Plast Reconstr Aesthet Surg. 2009;62:1210–4.
39. Condé-Green A, de Amorim NF, Pitanguy I. Influence of decantation, washing and centrifugation on adipocyte and mesenchymal stem cell content of aspirated adipose tissue: a comparative study. J Plast Reconstr Aesthet Surg. 2010;63:1375–81.
40. Ramon Y, Shoshani O, Peled IJ, Gilhar A, Carmi N, Fodor L, et al. Enhancing the take of injected adipose tissue by a simple method for concentrating fat cells. Plast Reconstr Surg. 2005;115:197–201.
41. Minn KW, Min KH, Chang H, Kim S, Heo EJ. Effects of fat preparation methods on the viabilities of autologous fat grafts. Aesthetic Plast Surg. 2010;34:626–31.
42. Boschert MT, Beckert BW, Puckett CL, Concannon MJ. Analysis of lipocyte viability after liposuction. Plast Reconstr Surg. 2002;109:761–5.
43. Xie Y, Zheng D, Li Q, Chen Y, Lei H, Pu LL. The effect of centrifugation on viability of fat grafts: an evaluation with the glucose transport test. J Plast Reconstr Aesthet Surg. 2010;63:482–7.
44. Kim IH, Yang JD, Lee DG, Chung HY, Cho BC. Evaluation of centrifugation technique and effect of epinephrine on fat cell viability in autologous fat injection. Aesthet Surg J. 2009;29:35–9.
45. Kurita M, Matsumoto D, Shigeura T, Sato K, Gonda K, Harii K, et al. Influences of centrifugation on cells and tissues in liposuction aspirates: optimized centrifugation for lipotransfer and cell isolation. Plast Reconstr Surg. 2008;121:1033–41.
46. Coleman SR. Avoidance of arterial occlusion from injection of soft tissue fillers. Aesthet Surg J. 2002;22:555–7.
47. Pallua N, Pulsfort AK, Suschek C, Wolter TP. Content of the growth factors bFGF, IGF-1, VEGF, and PDGF-BB in freshly harvested lipoaspirate after centrifugation and incubation. Plast Reconstr Surg. 2009;123:826–33.
48. Canizares O, Scharff CL, Nguyen PD. Centrifugation creates unique fractions of lipoaspirate: implications for fat graft survival. Plast Reconstr Surg. 2009;126:S75.
49. Sherman JE, Fanzio PM, White H, Leifer D. Blindness and necrotizing fasciitis after liposuction and fat transfer. Plast Reconstr Surg. 2010;126:1358–63.
50. Anwar UM, Ahmad M, Sharpe DT. Necrotizing fasciitis after liposculpture. Aesthet Surg J. 2004;28:426–7.
51. Gibbons MD, Lim RB, Carter PL. Necrotizing fasciitis after tumescent liposuction. Am Surg. 1998;64:458–60.
52. Heitmann C, Czermak C, Germann G. Rapidly fatal necrotizing fasciitis after aesthetic liposuction. Aesthetic Plast Surg. 2000;24:344–7.
53. Karacaoglu E, Kizilkaya E, Cermik H, Zienowicz R. The role of recipient sites in fat-graft survival: experimental study. Ann Plast Surg. 2005;55:63–8.
54. Nishimura T, Hashimoto H, Nakanishi I, Furukawa M. Microvascular angiogenesis and apoptosis in the survival of free fat grafts. Laryngoscope. 2000;110:1333–8.
55. Bourne DA, James IB, Wang SS, Marra KG, Rubin JP. The architecture of fat grafting: what lies beneath the surface? Plast Reconstr Surg. 2016;137:1072–9.
56. Mahoney CM, Imbarlina C, Yates CC, Marra KG. Current therapeutic strategies for adipose tissue defects/repair using engineered biomaterials and biomolecule formulations. Front Pharmacol. 2018;9:507.
57. Strong AL, Cederna PS, Rubin JP, Coleman SR, Levi B. The current state of fat grafting: a review of harvesting, processing, and injection techniques. Plast Reconstr Surg. 2015;136:897–912.
58. Schäffler A, Büchler C. Concise review: adipose tissue-derived stromal cells-basic and clinical implications for novel cell-based therapies. Stem Cells. 2007;25:818–27.
59. Kim EH, Heo CY. Current applications of adipose-derived stem cells and their future perspectives. World J Stem Cells. 2014;6:65–8.
60. Jeon O, Rhie JW, Kwon IK, Kim JH, Kim BS, Lee SH. In vivo bone formation following transplantation of human adipose–derived stromal cells that are not differentiated osteogenically. Tissue Eng Part A. 2008;14:1285–94.
61. Yoon HH, Bhang SH, Shin JY, Shin J, Kim BS. Enhanced cartilage formation via three-dimensional cell engineering of human adipose-derived stem cells. Tissue Eng Part A. 2012;18:1949–56.
62. Liau LL, Makpol S, Azurah AGN, Chua KH. Human adipose-derived mesenchymal stem cells promote recovery of injured HepG2 cell line and show sign of early hepatogenic differentiation. Cytotechnology. 2018;70:1221–33.
63. Timper K, Seboek D, Eberhardt M, Linscheid P, Christ-Crain M, Keller U, et al. Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. Biochem Biophys Res Commun. 2006;341:1135–40.
64. Kang SK, Lee DH, Bae YC, Kim HK, Baik SY, Jung JS. Improvement of neurological deficits by intracerebral transplantation of human adipose tissue-derived stromal cells after cerebral ischemia in rats. Exp Neurol. 2003;183:355–66.
65. Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. Biochem Biophys Res Commun. 2005;328:258–64.

66. Zhu Y, Liu T, Song K, Fan X, Ma X, Cui Z. Adipose-derived stem cell: a better stem cell than BMSC. Cell Biochem Funct. 2008;26:664–75.
67. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. Circ Res. 2007;100:1249–60.
68. Rodbell M. Metabolism of isolated fat cells II. The similar effects of phospholipase C (Clostridium perfringens α toxin) and of insulin on glucose and amino acid metabolism. J Biol Chem. 1966;241:130–9.
69. Hauner H, Entenmann G, Wabitsch M, Gaillard D, Ailhaud G, Negrel R, et al. Promoting effect of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in a chemically defined medium. J Clin Invest. 1989;84:1663–70.
70. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, et al. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell. 2002;13:4279–95.
71. Lee RH, Kim B, Choi I, Kim H, Choi HS, Suh K, et al. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. Cell Physiol Biochem. 2004;14:311–24.
72. Van Harmelen V, Röhrig K, Hauner H. Comparison of proliferation and differentiation capacity of human adipocyte precursor cells from the omental and subcutaneous adipose tissue depot of obese subjects. Metabolism. 2004;53:632–7.
73. Liu L, Liu H, Chen M, Ren S, Cheng P, Zhang H. miR-301b ~ miR-130b-PPARγ axis underlies the adipogenic capacity of mesenchymal stem cells with different tissue origins. Sci Rep. 2017;7:1160.
74. Moustaid N, Lasnier F, Hainque B, Quignard-Boulange A, Pairault J. Analysis of gene expression during adipogenesis in 3T3-F442A preadipocytes: insulin and dexamethasone control. J Cell Biochem. 1990;42:243–54.
75. Smas CM, Chen L, Zhao L, Latasa MJ, Sul HS. Transcriptional repression of pref-1 by glucocorticoids promotes 3T3-L1 adipocyte differentiation. J Biol Chem. 1999;274:12632–41.
76. Wu Z, Bucher NL, Farmer SR. Induction of peroxisome proliferator-activated receptor gamma during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPbeta, C/EBP-delta, and glucocorticoids. Mol Cell Biol. 1996;16:4128–36.
77. Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. Physiol Rev. 1998;78:783–809.
78. Mauney JR, Volloch V, Kaplan DL. Matrix-mediated retention of adipogenic differentiation potential by human adult bone marrow-derived mesenchymal stem cells during ex vivo expansion. Biomaterials. 2005;26:6167–75.
79. Kim WS, Park BS, Sung JH, Yang JM, Park SB, Kwak SJ, et al. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. J Dermatol Sci. 2007;48:15–24.
80. Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, March KL, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy. 2013;15:641–8.
81. Varma MJ, Breuls RG, Schouten TE, Jurgens WJ, Bontkes HJ, Schuurhuis GJ, et al. Phenotypical and functional characterization of freshly isolated adipose tissue-derived stem cells. Stem Cells Dev. 2007;16:91–104.
82. Calderon D, Planat-Benard V, Bellamy V, Vanneaux V, Kuhn C, Peyrard S, et al. Immune response to human embryonic stem cell-derived cardiac progenitors and adipose-derived stromal cells. J Cell Mol Med. 2012;16:1544–52.
83. Frese L, Dijkman PE, Hoerstrup SP. Adipose tissue-derived stem cells in regenerative medicine. Transfus Med Hemother. 2016;43:268–74.
84. Villena JA, Cousin B, Pénicaud L, Casteilla L. Adipose tissues display differential phagocytic and microbicidal activities depending on their localization. Int J Obes Relat Metab Disord. 2001;25:1275–80.
85. Choi JS, Choi YC, Kim JD, Kim EJ, Lee HY, Kwon IC, et al. Adipose tissue: a valuable resource of biomaterials for soft tissue engineering. Macromol Res. 2014;22:932–47.
86. Cen L, Liu W, Cui L, Zhang W, Cao Y. Collagen tissue engineering: development of novel biomaterials and applications. Pediatr Res. 2008;63:492–6.
87. Muiznieks LD, Keeley FW. Molecular assembly and mechanical properties of the extracellular matrix: a fibrous protein perspective. Biochim Biophys Acta. 2013;1832:866–75.
88. Bayrak A, Prüger P, Stock UA, Seifert M. Absence of immune responses with xenogeneic collagen and elastin. Tissue Eng Part A. 2013;19:1592–600.
89. Willard JJ, Drexler JW, Das A, Roy S, Shilo S, Shoseyov O, et al. Plant-derived human collagen scaffolds for skin tissue engineering. Tissue Eng Part A. 2013;19:1507–18.
90. Bernfield M, Götte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, et al. Functions of cell surface heparan sulfate proteoglycans. Annu Rev Biochem. 1999;68:729–77.
91. Yurchenco PD, Amenta PS, Patton BL. Basement membrane assembly, stability and activities observed through a developmental lens. Matrix Biol. 2004;22:521–38.
92. Migliorini E, Thakar D, Sadir R, Pleiner T, Baleux F, Lortat-Jacob H, et al. Well-defined biomimetic surfaces to characterize glycosaminoglycan-mediated interactions on the molecular, supramolecular and cellular levels. Biomaterials. 2014;35:8903–15.
93. Calonder C, Matthew HW, Van Tassel PR. Adsorbed layers of oriented fibronectin: a strategy to control cell–surface interactions. J Biomed Mater Res A. 2005;75:316–23.
94. Liang Y, Kiick KL. Heparin-functionalized polymeric biomaterials in tissue engineering and drug delivery applications. Acta Biomater. 2014;10:1588–600.
95. Rosenbloom J, Abrams WR, Mecham R. Extracellular matrix 4: the elastic fiber. FASEB J. 1993;7:1208–18.
96. Brooke BS, Bayes-Genis A, Li DY. New insights into elastin and vascular disease. Trends Cardiovasc Med. 2003;13:176–81.
97. Waterhouse A, Wise SG, Ng MK, Weiss AS. Elastin as a nonthrombogenic biomaterial. Tissue Eng Part B Rev. 2011;17:93–9.
98. Simionescu DT, Lu Q, Song Y, Lee JS, Rosenbalm TN, Kelley C, et al. Biocompatibility and remodeling potential of pure arterial elastin and collagen scaffolds. Biomaterials. 2006;27:702–13.
99. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr. 2004;92:347–55.
100. Wang B, Jenkins JR, Trayhurn P. Expression and secretion of inflammation-related adipokines by human adipocytes differentiated in culture: integrated response to TNF-α. Am J Physiol Endocrinol Metab. 2005;288:E731–40.
101. Trayhurn P, Beattie JH. Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. Proc Nutr Soc. 2001;60:329–39.
102. Yudkin JS. Adipose tissue, insulin action and vascular disease: inflammatory signals. Int J Obes Relat Metab Disord. 2003;27:S25–8.
103. Fonseca-Alaniz MH, Takada J, Alonso-Vale MI, Lima FB. Adipose tissue as an endocrine organ: from theory to practice. J Pediatr (Rio J). 2007;83:S192–203.

104. Cao Y. Adipose tissue angiogenesis as a therapeutic target for obesity and metabolic diseases. Nat Rev Drug Discov. 2010;9:107–15.
105. Peinado JR, Pardo M, de la Rosa O, Malagón MM. Proteomic characterization of adipose tissue constituents, a necessary step for understanding adipose tissue complexity. Proteomics. 2012;12:607–20.
106. Trujillo ME, Scherer PE. Adipose tissue-derived factors: impact on health and disease. Endocr Rev. 2006;27:762–78.
107. Cheung HK, Han TT, Marecak DM, Watkins JF, Amsden BG, Flynn LE. Composite hydrogel scaffolds incorporating decellularized adipose tissue for soft tissue engineering with adipose-derived stem cells. Biomaterials. 2014;35:1914–23.
108. Badylak SF. The extracellular matrix as a scaffold for tissue reconstruction. Semin Cell Dev Biol. 2002;13:377–83.
109. Kim BS, Choi JS, Kim JD, Choi YC, Cho YW. Recellularization of decellularized human adipose-tissue-derived extracellular matrix sheets with other human cell types. Cell Tissue Res. 2012;348:559–67.
110. Yu C, Bianco J, Brown C, Fuetterer L, Watkins JF, Samani A, et al. Porous decellularized adipose tissue foams for soft tissue regeneration. Biomaterials. 2013;34:3290–302.
111. Francis MP, Sachs PC, Madurantakam PA, Sell SA, Elmore LW, Bowlin GL, et al. Electrospinning adipose tissue-derived extracellular matrix for adipose stem cell culture. J Biomed Mater Res A. 2012;100:1716–24.
112. Brown BN, Freund JM, Han L, Rubin JP, Reing JE, Jeffries EM, et al. Comparison of three methods for the derivation of a biologic scaffold composed of adipose tissue extracellular matrix. Tissue Eng Part C Methods. 2011;17:411–21.
113. Keane TJ, Swinehart IT, Badylak SF. Methods of tissue decellularization used for preparation of biologic scaffolds and in vivo relevance. Methods. 2015;84:25–34.
114. Choi JS, Kim BS, Kim JD, Choi YC, Lee EK, Park K, et al. In vitro expansion of human adipose-derived stem cells in a spinner culture system using human extracellular matrix powders. Cell Tissue Res. 2011;345:415–23.
115. Choi JS, Yang HJ, Kim BS, Kim JD, Lee SH, Lee EK, et al. Fabrication of porous extracellular matrix scaffolds from human adipose tissue. Tissue Eng Part C Methods. 2010;16:387–96.
116. Choi JS, Kim BS, Kim JD, Choi YC, Lee HY, Cho YW. In vitro cartilage tissue engineering using adipose-derived extracellular matrix scaffolds seeded with adipose-derived stem cells. Tissue Eng Part A. 2012;18:80–92.
117. Kim EJ, Choi JS, Kim JS, Choi YC, Cho YW. Injectable and thermosensitive soluble extracellular matrix and methylcellulose hydrogels for stem cell delivery in skin wounds. Biomacromolecules. 2016;17:4–11.
118. Poon CJ, Pereira E Cotta MV, Sinha S, Palmer JA, Woods AA, Morrison WA, et al. Preparation of an adipogenic hydrogel from subcutaneous adipose tissue. Acta Biomater. 2013;9:5609–20.
119. Uriel S, Labay E, Francis-Sedlak M, Moya ML, Weichselbaum RR, Ervin N, et al. Extraction and assembly of tissue-derived gels for cell culture and tissue engineering. Tissue Eng Part C Methods. 2009;15:309–21.
120. Young DA, Ibrahim DO, Hu D, Christman KL. Injectable hydrogel scaffold from decellularized human lipoaspirate. Acta Biomater. 2011;7:1040–9.
121. Flynn LE. The use of decellularized adipose tissue to provide an inductive microenvironment for the adipogenic differentiation of human adipose-derived stem cells. Biomaterials. 2010;31:4715–24.
122. Huleihel L, Hussey GS, Naranjo JD, Zhang L, Dziki JL, Turner NJ, et al. Matrix-bound nanovesicles within ECM bioscaffolds. Sci Adv. 2016;2:e1600502.
123. Turner AE, Yu C, Bianco J, Watkins JF, Flynn LE. The performance of decellularized adipose tissue microcarriers as an inductive substrate for human adipose-derived stem cells. Biomaterials. 2012;33:4490–9.
124. Adam Young D, Bajaj V, Christman KL. Decellularized adipose matrix hydrogels stimulate in vivo neovascularization and adipose formation. J Biomed Mater Res A. 2014;102:1641–51.
125. Yu C, Kornmuller A, Brown C, Hoare T, Flynn LE. Decellularized adipose tissue microcarriers as a dynamic culture platform for human adipose-derived stem/stromal cell expansion. Biomaterials. 2017;120:66–80.
126. Steven FS. Nishihara technique for the solubilization of collagen: application to the preparation of soluble collagens from normal and rheumatoid connective tissue. Ann Rheum Dis. 1964;23:300–1.
127. Zhang S, Lu Q, Cao T, Toh WS. Adipose tissue and extracellular matrix development by injectable decellularized adipose matrix loaded with basic fibroblast growth factor. Plast Reconstr Surg. 2016;137:1171–80.
128. Thomas-Porch C, Li J, Zanata F, Martin EC, Pashos N, Genemaras K, et al. Comparative proteomic analyses of human adipose extracellular matrices decellularized using iternative procedures. J Biomed Mater Res A. 2018;106:2481–93.
129. Sano H, Orbay H, Terashi H, Hyakusoku H, Ogawa R. Acellular adipose matrix as a natural scaffold for tissue engineering. J Plast Reconstr Aesthet Surg. 2014;67:99–106.
130. Wang JQ, Fan J, Gao JH, Zhang C, Bai SL. Comparison of in vivo adipogenic capabilities of two different extracellular matrix microparticle scaffolds. Plast Reconstr Surg. 2013;131:174e–87.
131. Pati F, Jang J, Ha DH, Kim SW, Rhie JW, Shim JH, et al. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun. 2014;5:3935.
132. Kochhar A, Wu I, Mohan R, Condé-Green A, Hillel AT, Byrne PJ, et al. A comparison of the rheologic properties of an adipose-derived extracellular matrix biomaterial, lipoaspirate, calcium hydroxylapatite, and cross-linked hyaluronic acid. JAMA Facial Plast Surg. 2014;16:405–9.
133. Lee K, Kuo CK. Extracellular matrix remodeling and mechanical stresses as modulators of adipose tissue metabolism and inflammation. In: Benayahu D, Gefen A, editors. The mechanobiology of obesity and related diseases. Studies in mechanobiology, tissue engineering and biomaterials. Cham: Springer; 2013. p. 105–22.
134. Song M, Liu Y, Hui L. Preparation and characterization of acellular adipose tissue matrix using a combination of physical and chemical treatments. Mol Med Rep. 2018;17:138–46.
135. Khater R, Atanassova P. Autologous fat grafting—factors of influence on the therapeutic results. In: Agullo F editor. Current concepts in plastic surgery. InTech; 2012. p. 183–210.
136. Marcus BC. The use of autologous fat for facial rejuvenation. Obstet Gynecol Clin North Am. 2010;37:521–31.
137. Piccinno MS, Veronesi E, Loschi P, Pignatti M, Murgia A, Grisendi G, et al. Adipose stromal/stem cells assist fat transplantation reducing necrosis and increasing graft performance. Apoptosis. 2013;18:1274–89.
138. Liao HT, Marra KG, Rubin JP. Application of platelet-rich plasma and platelet-rich fibrin in fat grafting: basic science and literature review. Tissue Eng Part B Rev. 2014;20:267–76.
139. Gentile P, De Angelis B, Pasin M, Cervelli G, Curcio CB, Floris M, et al. Adipose-derived stromal vascular fraction cells and platelet-rich plasma: basic and clinical evaluation for cell-based therapies in patients with scars on the face. J Craniofac Surg. 2014;25:267–72.
140. Tanikawa DY, Aguena M, Bueno DF, Passos-Bueno MR, Alonso N. Fat grafts supplemented with adipose-derived stromal

cells in the rehabilitation of patients with craniofacial microsomia. Plast Reconstr Surg. 2013;132:141–52.
141. Van Nieuwenhove I, Tytgat L, Ryx M, Blondeel P, Stillaert F, Thienpont H, et al. Soft tissue fillers for adipose tissue regeneration: from hydrogel development toward clinical applications. Acta Biomater. 2017;63:37–49.
142. Sivashanmugam A, Kumar RA, Priya MV, Nair SV, Jayakumar R. An overview of injectable polymeric hydrogels for tissue engineering. Eur Polym J. 2015;72:543–65.
143. Chun SY, Lim JO, Lee EH, Han MH, Ha YS, Lee JN, et al. Preparation and characterization of human adipose tissue-derived extracellular matrix, growth factors, and stem cells: a concise review. Tissue Eng Regen Med. 2019;16:385–93.
144. Morissette Martin P, Shridhar A, Yu C, Brown C, Flynn LE. Decellularized adipose tissue scaffolds for soft tissue regeneration and adipose-derived stem/stromal cell delivery. In: Bunnell BA, Gimble JM, editors. Adipose-derived stem cells: Methods in Molecular Biology. New York: Humana Press; 2018. p. 53–71.
145. Huang YB, Lin MW, Liu MY, Chen CL. Composite of decellular adipose tissue with chitosan-based scaffold for tissue engineering with adipose-derived stem cells. J Biomater Tissue Eng. 2015;5:56–63.
146. Niemelä S, Miettinen S, Sarkanen JR, Ashammakhi N. Adipose tissue and adipocyte differentiation: molecular and cellular aspects and tissue engineering applications. In: Ashammakhi N, Reis R, Chiellini F. editor. Topics in tissue engineering. 2008. p. 1–26.
147. Webber MJ, Khan OF, Sydlik SA, Tang BC, Langer R. A perspective on the clinical translation of scaffolds for tissue engineering. Ann Biomed Eng. 2015;43:641–56.
148. Brown BN, Badylak SF. Extracellular matrix as an inductive scaffold for functional tissue reconstruction. Transl Res. 2014;163:268–85.
149. Cevasco M, Itani KM. Ventral hernia repair with synthetic, composite, and biologic mesh: characteristics, indications, and infection profile. Surg Infect (Larchmt). 2012;13:209–15.
150. Romanelli M, Dini V, Bertone MS. Randomized comparison of OASIS wound matrix versus moist wound dressing in the treatment of difficult-to-heal wounds of mixed arterial/venous etiology. Adv Skin Wound Care. 2010;23:34–8.
151. Naranjo JD, Scarritt ME, Huleihel L, Ravindra A, Torres CM, Badylak SF. Regenerative medicine: lessons from mother nature. Regen Med. 2016;11:767–75.
152. Spear SL, Sinkin JC, Al-Attar A. Porcine acellular dermal matrix (strattice) in primary and revision cosmetic breast surgery. Plast Reconstr Surg. 2013;131:1140–8.
153. Woo JS, Fishbein MC, Reemtsen B. Histologic examination of decellularized porcine intestinal submucosa extracellular matrix (CorMatrix) in pediatric congenital heart surgery. Cardiovasc Pathol. 2016;25:12–7.
154. Swinehart IT, Badylak SF. Extracellular matrix bioscaffolds in tissue remodeling and morphogenesis. Dev Dyn. 2016;245:351–60.
155. Flynn L, Woodhouse KA. Adipose tissue engineering with cells in engineered matrices. Organogenesis. 2008;4:228–35.
156. Mojallal A, Lequeux C, Shipkov C, Rifkin L, Rohrich R, Duclos A, et al. Stem cells, mature adipocytes, and extracellular scaffold: what does each contribute to fat graft survival? Aesthet Plast Surg. 2011;35:1061–72.
157. Turner AE, Flynn LE. Design and characterization of tissue-specific extracellular matrix-derived microcarriers. Tissue Eng Part C Methods. 2012;18:186–97.
158. Kornmuller A, Brown CF, Yu C, Flynn LE. Fabrication of extracellular matrix-derived foams and microcarriers as tissue-specific cell culture and delivery platforms. J Vis Exp. 2017;122:e55436.
159. Shridhar A, Gillies E, Amsden BG, Flynn LE. Composite bioscaffolds incorporating decellularized ECM as a cell-instructive component within hydrogels as in vitro models and cell delivery systems. In: Turksen K. editor. Decellularized scaffolds and organogenesis. Methods in molecular biology. Humana Press, New York. 2018. p. 183–208.
160. Choi JS, Yang HJ, Kim BS, Kim JD, Kim JY, Yoo B, et al. Human extracellular matrix (ECM) powders for injectable cell delivery and adipose tissue engineering. J Control Release. 2009;139:2–7.
161. Kayabolen A, Keskin D, Aykan A, Karslıoglu Y, Zor F, Tezcaner A. Native extracellular matrix/fibroin hydrogels for adipose tissue engineering with enhanced vascularization. Biomed Mater. 2017;12:035007.
162. Woo CH, Choi YC, Choi JS, Lee HY, Cho YW. A bilayer composite composed of TiO_2-incorporated electrospun chitosan membrane and human extracellular matrix sheet as a wound dressing. J Biomater Sci Polym Ed. 2015;26:841–54.
163. Lee SS, Kim HD, Kim SH, Kim I, Kim IG, Choi JS, et al. Self-healing and adhesive artificial tissue implant for voice recovery. ACS Appl Bio Mater. 2018;1:1134–46.