


Preparation and Characterization of Human Adipose Tissue-Derived Extracellular Matrix, Growth Factors, and Stem Cells: A Concise Review

So Young Chun¹ · Jeong Ok Lim¹ · Eun Hye Lee²  · Man-Hoon Han² · Yun-Sok Ha³ · Jun Nyung Lee³ · Bum Soo Kim³ · Min Jeong Park⁴ · MyungGu Yeo⁴ · Bongsu Jung⁴ · Tae Gyun Kwon^{3,5}

Received: 20 May 2019 / Accepted: 6 June 2019 / Published online: 5 July 2019
© The Korean Tissue Engineering and Regenerative Medicine Society 2019

Abstract

BACKGROUND: Human adipose tissue is routinely discarded as medical waste. However, this tissue may have valuable clinical applications since methods have been devised to effectively isolate adipose-derived extracellular matrix (ECM), growth factors (GFs), and stem cells. In this review, we analyze the literature that devised these methods and then suggest an optimal method based on their characterization results.

METHODS: Methods that we analyze in this article include: extraction of adipose tissue, decellularization, confirmation of decellularization, identification of residual active ingredients (ECM, GFs, and cells), removal of immunogens, and comparing structural/physiological/biochemical characteristics of active ingredients.

RESULTS: Human adipose ECMs are composed of collagen type I–VII, laminin, fibronectin, elastin, and glycosaminoglycan (GAG). GFs immobilized in GAG include basic fibroblast growth factor (bFGF), transforming growth factor beta 1 (TGF- β 1), insulin like growth factor 1 (IGF-1), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), BMP4 (bone morphogenetic protein 4), nerve growth factor (NGF), hepatocyte growth factor (HGF), and epidermal growth factor (EGF). Stem cells in the stromal-vascular fraction display mesenchymal markers, self-renewal gene expression, and multi-differentiation potential.

CONCLUSION: Depending on the preparation method, the volume, biological activity, and physical properties of ECM, GFs, and adipose tissue-derived cells can vary. Thus, the optimal preparation method is dependent on the intended application of the adipose tissue-derived products.

Keywords Human adipose tissue · Extracellular matrix · Growth factors · Adipose-derived stem cell · Optimum method

So Young Chun and Jeong Ok Lim have contributed equally to this work.

✉ Bongsu Jung
bsjung@dgmif.re.kr

✉ Tae Gyun Kwon
tgkwon@knu.ac.kr

¹ BioMedical Research Institute, Joint Institute for Regenerative Medicine, Kyungpook National University Hospital, Daegu 41940, Republic of Korea

² Department of Pathology, School of Medicine, Kyungpook National University, Daegu 41944, Republic of Korea

³ Department of Urology, School of Medicine, Kyungpook National University, Daegu 41944, Republic of Korea

⁴ Medical Device Development Center, Daegu-Gyeongbuk Medical Innovation Foundation (DGMIF), Cheombok-ro 80, Dong-gu, Daegu 41061, Republic of Korea

⁵ Department of Urology, Kyungpook National University Chilgok Hospital, 807 Hoguk-ro, Buk-gu, Daegu 41404, Republic of Korea

1 Introduction

Human adipose tissue is removed safely and easily with local anesthesia using simple techniques, and the amount of material removed is sufficient for many clinical applications without further manipulation [1, 2]. Once removed via clinical liposuction, body contouring, breast reduction, or abdominoplasty procedures, the tissue is typically discarded as medical waste [3]. However, human adipose tissue is an ideal biomaterial for implantation because it contains large amounts of bioactive constituents such as extracellular matrix (ECM) components, growth factors (GFs), and stem/progenitor cells [4, 5].

ECM components that can be found in human adipose tissue include collagen, fibronectin, elastin, laminin, and glycosaminoglycan (GAG). These components have minimal immunogenicity (i.e. no xenogeneic antigens, such as α -Gal epitopes), induce angio-/adipogenesis, and promote long-term retention of soft-tissue [6]. GFs that can be found in human adipose tissue include bFGF, TGF- β 1, IGF-1, VEGF, PDGF, BMP4, NGF, HGF, and EGF. They are persistent even after the decellularization process and can promote cell differentiation into several target lineages [7]. The fractionated cell types include smooth muscle, endothelial, fibroblasts, lipogenic, hematopoietic, and stem cells. Adipose-derived stem cells display mesenchymal stem cell characteristics [8], and cell quality is not dependent on donor age [9].

However, the type, components, volume, biological activity, and physical properties of adipose-derived ECM, GFs, and cells vary depending on how they are extracted. This can result in a variety of regeneration effects during *in vivo* application [10, 11]. Thus, this review surveys the characteristics that ECM, GFs, and cells can acquire based on the method used to extract the original adipose tissue (Fig. 1). Such information is critical to maximizing target tissue regeneration capabilities.

2 Human adipose-derived ECM

To collect ECM, human adipose tissue is obtained via liposuction using a commercially available device. The extracted tissue is washed with distilled water to remove blood, and then the tissue/water mixture is homogenized and centrifuged. The oil contained upper layer is discarded, and the remaining gel-like suspension is washed with distilled water and decellularized to obtain ECM [12]. During the decellularization step, the cellular and nuclear materials are removed because they can cause an *in vivo* inflammatory response and immune rejection [13].

2.1 Decellularization methods

There are three common decellularization methods: physical (e.g. freezing, pressure, agitation, sonication, and radiation) [14], chemical (e.g. detergent, acid, and alkaline), and biological treatment with enzymes [15]. To increase the efficiency of decellularization, these treatments are often combined. For example, freeze–thaw treatments loosen connective tissue, and chemical and enzymatic agents facilitate the removal of cellular components [15, 16].

When choosing a decellularization protocol, several factors are considered such as origin, thickness, density, cellularity, and lipid content [17]. This is also true for human adipose tissue, which is composed of loose connective tissue; therefore, a mild method is recommended over a harsher method. For example, extensive freezing ($-80\text{ }^{\circ}\text{C}$) and thawing ($37\text{ }^{\circ}\text{C}$) to remove lipid tissue causes severe alterations in the matrix architecture. Therefore, this physical method has been replaced by chemical methods that use polar solvents such as acetone, ethanol, or isopropanol (with isopropanol being the most effective) [18, 19]. Some chemical detergents such as (sodium dodecyl sulfate, SDS) have a strong decellularization effect resulting in the disruption of the matrix architecture. Usually, SDS is applied to dense, compact organs such as the heart or porcine adipose tissue decellularization [13]. But human adipose tissue is a gel-like substance [19], and SDS treatment causes dramatic swelling of the ECM and irreversible macroscopic degradation of its structure [19]. Furthermore, SDS strongly interacts with ECM components and renders them difficult to remove at the end of processing. SDS is also cytotoxic when reseeding cells on a matrix [20]. For these reasons, SDS has been replaced with the milder detergent EDTA when decellularizing human adipose tissue [19]. The ECM extraction method was visualized on Fig. 1.

2.2 Adipose ECM composition

After the decellularization process, the volume of ECM extracted accounts for approximately 5% of the original adipose tissue [16]. The types of ECM in human adipose tissue include collagen type I–VII, laminin, fibronectin, elastin, and GAGs [21, 22]. Collagens are the most abundant proteins after decellularization [23], and there are four different types: the densely-packed type, the lumen type with vascular structure, the void space type with interwoven fiber, and the network type [19]. Type I collagen surrounds adipocytes with thin fibers and thick bundles composed of heterotrimers in a triple helix and provides the major ECM framework necessary to sustain the structure [24]. Collagen type III also surrounds adipocytes [10].



Fig. 1 Scheme for preparation of human adipose tissue-derived extracellular matrix (ECM), growth factors (GFs), and stem cells. Human adipose-derived ECM is collected from liposuction, decellularized, and then manufactured to the required form. The GFs are

prepared from decellularized ECM using optimized extraction buffer, then collected through dialysis and concentration. The stem cells are isolated from stromal-vascular fraction (SVF) through physical and enzymatic process

Collagen type IV serves as a basement membrane for the adipocyte area and is located under the vascular endothelial cell layer [10]. Fibrillar collagen type IV contains various binding sites for bioactive molecules that modulate cellular behavior; thus, it acts as a functional and structural protein [25]. Collagen type V and VI form micro-fibers composed of heterotrimers and are located between type I collagen fibers [26]. Collagen type VII is located within the matrix between adipocytes and near blood vessels in small amounts [10].

Non-collagen ECM proteins found in adipose tissue include laminin, fibronectin, elastin, and GAGs. Laminin composes trimeric α -, β -, and γ -chain structures [27]; promotes epithelial cell attachment to the basal lamina; and is involved in cell migration and growth [28]. Laminin also forms a network with type IV collagen α -chain [27]. Fibronectin dictates cytoskeletal and ECM organization, which controls cell shape and contractility [29]. Also, fibronectin maintains the fibrillary organization of collagen type I by cross-linking to an $\alpha 1(I)$ chain [30]. Elastin is related to collagen type VI [31], and its network density increases with obesity [32]. In visceral fat, elastin fiber forms a mesh-like net, and in subcutaneous fat, it is linear [33] and involved in the elasticity of adipose tissue

[34, 35]. Sulfated GAG sequesters growth factors and presents them to cells creating a path for bioactive molecule delivery [36, 37].

Extracted adipose ECMs are quantified with commercial kits. For collagen quantification, the Sircol Soluble Collagen Assay Kit is used [38]. Collagen types that are rich in adipose tissue are I, II, and IV [15]. Masson's trichrome and Picrosirius (Sirius) red are used for collagen fiber staining. For laminin and fibronectin quantification and identification, commercial ELISA kits and immunohistochemical staining are available, respectively. For elastin quantification, the Fastin Elastin Assay Kit is used [13], and elastic fiber is stained with Verhoeff's-Van Gieson elastic stain. For GAG quantification, the Blyscan Sulfated GAG Assay Kit is used [38]. Reported GAG content is approximately 40 μg per 1 g of adipose tissue [15]. Sulfated GAG is stained with Alcian blue.

2.3 Confirmation of decellularization

The inefficient removal of cellular contents such as cell membranes, nucleic acids, lipids, and cytosolic components can cause inflammatory responses and low regeneration of target tissue *in vivo* [39, 40]. DNase, RNase, and

lipase are added during the decellularization process to remove these immunogens. Quantification of residual DNA is performed with a commercial kit, and DNA content is normalized to the initial wet weight of the sample. The elimination of DNA is also validated by polymerase chain reaction (PCR) and histological staining [16]. For PCR, primers designed to amplify the glyceraldehyde-3-phosphate dehydrogenase (GAPDH, NM002046) gene are used with total DNA serving as the template. For end-point PCR, the amplified product is analyzed with agarose gel electrophoresis. When real-time PCR is used, Ct values are observed. Standard criteria state that residual DNA fragments should be < 200 bp in length, and the total DNA content should be < 50 ng of double-stranded DNA/mg of ECM (dry weight) [10]. For histological staining, ECM is fixed in paraformaldehyde, embedded in paraffin or frozen, and stained with 4,6-diamidino-2-phenylindole (DAPI) or acridine orange (AO). Routine hematoxylin and eosin staining (H&E) can also show residual cytosolic and nuclear components [14]. The residual lipids within the ECM are observed with Oil red O staining; the red color indicates the presence of lipids [10].

2.4 Mechanical properties

Hydrated human adipose ECM exhibits a whitish gel-like appearance, and the final product volume is 30–40% of the original tissue mass [38]. Freeze-dried ECM resembles a porous sponge where the pore sizes can range between 20 and 200 μm , and the average porosity is approximately 90% [15]. The porosity can be controlled by temperature; pore size is relatively decreased as the temperature [22]. The collagen structure is interconnected with much finer, network-type collagen [19]. The extensive surface area and median pore size achieved by freeze-drying are favorable for cell adhesion, penetration, proliferation, oxygen/nutrient transport, and waste exchange [15]. With respect to mechanical properties, initial tensile strength is approximately 0.2 MPa, Young's modulus is about 65 MPa, and the maximum elongation at the breakage point is approximately 65%. ECM swells in a time-dependent manner, and the absorption ratio is approximately six times [15]. Gel-type ECM stiffness is approximately 1.8 MPa, and Young's modulus is between 1 and 2.5 kPa [41].

2.5 Clinical application

When compared to xenogeneic ECM-based scaffolds, human adipose-derived ECM is superior in terms of biocompatibility, biodegradability, and/or bioinductivity because it has lower immunogenicity and is less likely to transmit pathogens during clinical application [12, 42]. In a comparison of naïve autologous adipose tissue

transplantation, an adipose ECM graft could maintain volume, structure, strength, and/or stiffness, which promoted mechanical support, cell shape/function stability, the transport of chemical signals, angiogenesis, and vascularization of the graft [12, 43, 44]. Adipose ECM can form 3D scaffolds with a variety of shapes such as sheet, microsphere, square, film, hollow tube, bead [16], or injectable gel type [12, 45]. The above contents were summarized in Table 1.

3 Human adipose-derived growth factors

Adipose tissue is a major organ of the endocrine system, and adipocytes secrete various paracrine factors to induce cell adhesion, proliferation, migration, and differentiation [19, 46]. These bioactive molecules, including GFs, exist in ECM and retain their activity even after the decellularization process [7]. This phenomenon is associated with the binding with GAG. The heparan sulfate in GAG immobilizes GFs rendering them resistant to degradation by protein hydrolytic enzymes. Decorin and biglycan also help bind bioactive molecules to ECM [47].

3.1 Extraction steps

Extraction of GFs from decellularized adipose ECM consists of 6 steps: (1) place the ECM in an extraction buffer containing a protease inhibitor. Commonly used extraction buffers are acetic acid, urea, NaCl, or Triton X100. (2) Stir the solution for 1–3 days at 4 °C. (3) Centrifuge the solution at 3000–12,000 g for 30 min at 4 °C. (4) Dialyze the supernatant using an MWCO 3–15 k membrane and 80–100 volumes of ddH₂O at 4 °C over 2 days. (5) Centrifuge the dialyzed product at 3000–12,000 g for 30 min at 4 °C. (6) Collect the supernatant and lyophilize [38]. The quantified GFs (per g of dry human adipose ECM) are transforming growth factor-beta1 (TGF- β 1, approximately 8 ng), insulin growth factor-1 (IGF1, approximately 13 ng), basic fibroblast growth factor (bFGF, approximately 80 ng), and vascular endothelial growth factor (VEGF, approximately 25 ng) [38]. The GF extraction method was summarized on Fig. 1.

3.2 Importance of solvent selection

The type, concentration, and activity of extracted GF's can vary depending on the solvent used in the extraction. TGF- β 1, IGF-1, VEGF, PDGF, BMP4, HGF and EGF are extracted in large amounts with acetic acid [38]. For extraction of bFGF, NaCl is preferred. NGF is only extracted with urea, but BMP4 cannot be detected in urea (Table 2). The assumed reason for these differences are:

Table 1 Human adipose-derived ECM

Category	Contents	References
Decellularization methods	Physical	Freezing, pressure, agitation, sonication and radiation
	Chemical	Detergent, acid, and alkaline
	Biological	Enzymes
Adipose ECM composition	Collagens	Type I–VII
	Non-collagen	Laminin, fibronectin, elastin and GAGs
Confirmation of decellularization	DNA quantification with PCR, IHC	< 50 ng ds DNA/mg < 200 bp in length
	H&E stain	DAPI, acridine orange
Mechanical property (lyophilized)	Pore size	20 and 200 μ m
	Porosity	\approx 90%
	Tensile strength	\approx 0.2 MPa
	Young's modulus	\approx 65 MPa
	Maximum elongation	\approx 65%
Possible 3D scaffolds types	Sheet, microsphere, square, film, hollow tube, bead, injectable gel	[12, 16, 45]

(1) depending on the decellularization detergent used on ECM, the remaining GF's type, concentration, and activity can vary [7]; and (2) the solubility and stabilization of each GF can vary depending on the solvent's pH, composition, and excipients [48]. For example, each GF is stabilized at a different pH. bFGF is stabilized at pH 7 [48], EGF is stabilized at pH 5.3 [49], and VEGF is stabilized at pH 5.5–6 [50]. NaCl electrostatically bonds with proteins in aqueous solution, thus the binding force increases according to the degree of ionization of the GF [51]. Triton X100 readily reacts with hydrophobic proteins and NGF [51]. In addition, GF stability is affected by the ECM structure. Acetic acid damages collagen and weakens the extracellular matrix but does not affect GAG [52]. Urea damages collagen but does not affect elastin [53]. Triton X100 effectively conserves the structure and concentration of GAG [54]. Therefore, a GF extraction solution should be

carefully selected with the end-purpose in mind because each target tissue requires different growth factors. For example, chondrogenesis requires TGF- β 1, IGF-1, bFGF, and VEGF; adipogenesis requires insulin and insulin-like growth factor (IGF) [55]; myogenesis requires IGF [56]; osteogenesis and neurogenesis requires growth hormone (GH) and IGF-I [57, 58]; and endothelial differentiation requires VEGF and hepatocyte growth factor (HGF).

4 Human adipose-derived stem/progenitor cells

To increase the yield of adipose-derived cells, collection of fat en bloc (mass) is more efficient than liposuction. Liposuction yields 1 L of adipose tissue per donor while collection en bloc yields 2 L of adipose tissue per donor. Thus, the en bloc adipose collection method effectively increases the yield by increasing the total volume of adipose tissue per donor [14].

4.1 Extraction method

To fractionate cells from adipose tissue, the tissue is washed with PBS (containing penicillin/streptomycin) to remove blood. It is then treated with collagenase to digest connective fibers and filtered through 100- μ m filter to suspend into single cell. The collagenase activity is stopped with media containing 10% FBS. The mixture is then centrifuged at 200 g for 10 min to obtain the stromal-vascular fraction (SVF) [12]. The stem cells are contained in the SVF [8]. If a milder fractionation is necessary,

Table 2 Human adipose-derived growth factors

Extractable growth factors	Recommended extraction buffer	References
TGF- β 1	Acetic acid	[38, 48–51]
IGF-1		
VEGF		
PDGF		
BMP4		
EGF		
HGF		
bFGF	NaCl	
NGF	Urea	

Table 3 Human adipose-derived stem/progenitor cells

Category	Contents	References
Morphology	Fibroblastic-like	[59, 60]
	Diameter	Up to 120 μm
	Adherent period	15 h–1 week
Stem cell characters	Self-renewal	OCT4, SOX2, NANOG expression [8]
	High proliferation rate	[60–64]
	Multi-differentiation potential	Adipocyte, fibroblasts, smooth muscle cells, endothelial cells, immune cells, pancreatic cells, hepatocytes, neurons, cardiomyocytes, osteocytes, chondrocyte [63, 65–69]
	Secreted cytokines	HGF, granulocyte and macrophage colony stimulating factors, IL-6, -7, -8 and -11, TNF- α , VEGF, BDNF, NGF, adipokines, MMP-1, CXCL8, Ang-1, Ang-2, FGF-1, TIMP-1, CCL2, TGF, VCAM, EGF, FGF [41, 64, 78]
Recommended passage limitation	\approx 5–6 [60]	
Cell surface phenotype	Mesenchymal markers	CD44+, CD73+, CD90+, CD105+, CD166+ [61, 62]
	Endothelial markers	CD31–, CD34–, CD144–, CD146–
	Hematopoietic markers	CD45–, CD133–

mechanical force can be used instead of collagenase treatment. Fractionation with mechanical force reduces processing and possibly increases cell survival rate and stem cell yield [8, 44]. The SVF extraction method using automated machine was visualized on Fig. 1.

4.2 Characterization of fractionated cells

4.2.1 Morphology and growth features

Adhered cells display fibroblastic-like features when cultured (diameter up to 120 μm) [59], and hematopoietic and lipogenic cells are removed through washing and culture expansion. The late adherent cells (15 h–1 week) to plastic surface exhibits higher self-renewal, proliferation rate, differentiation potency, and cytokine secretion than the early adherent cells (before 3 h) [60]. The cells are used within passages 5–6 because of the accumulation of cytotoxic effects by the digestive enzymes used in passaging and cellular senescence induced by *in vitro* culture conditions.

4.2.2 Cell surface phenotype

Expression of classical mesenchymal markers (CD44, CD73, CD90, CD105, and CD166) is high while expression of endothelial (CD31, CD34, CD144, CD146) and hematopoietic (CD45, CD133) markers is low [61–63].

4.2.3 Self-renewal

OCT4, SOX2, and NANOG expression are identified [8].

4.2.4 Secreted cytokines

HGF, granulocyte and macrophage colony stimulating factors, IL-6, -7, -8 and -11, TNF- α , VEGF, BDNF, NGF, adipokines, MMP-1, CXCL8, Ang-1, Ang-2, FGF-1, TIMP-1, CCL2 [41, 64].

4.2.5 Multi-differentiation potential

Stem cells from adipose tissue can differentiate into adipocytes, fibroblasts, smooth muscle cells, endothelial cells, immune cells [65], pancreatic cells, hepatocytes, neurons, cardiomyocytes [66, 67], osteocyte [68], or chondrocyte [69].

The above contents were summarized in Table 3.

4.3 Clinical application

For clinical application, cells are combined with SVF to mimic the cells' native microenvironment [70]. This increases graft survival and vessel formation while decreasing inflammatory cell migration and fibrosis. The positive effects of SVF are demonstrated by histological changes in the introduced fat mass into the body. If an

in vivo immune response is stimulated, inflammatory cells are penetrated and form cysts, fat cells' size became larger than normal, and the transplanted fat volume decreases. As the inflammatory reaction progresses, granulomatous syncytial-like structures form and lobular panniculitis occurs. As necrosis progresses, connective bundles are formed, and the adipose tissue becomes an amorphous and disorganized mass. The occurrence of these histologic transformations is significantly reduced by SVF [8]. Thus, combining SVF with adipose tissue-derived cells is useful in cell therapy and fat enrichment [8].

5 Further considerations

5.1 Differences between subcutaneous and visceral adipose tissue

Depending on the deposition site, adipose tissue can be divided into two types: subcutaneous and visceral adipose tissue [71]. These adipose tissues display different physiological functions because they have different ECM compositions and secrete different bioactive molecules [72]. Visceral adipose tissue displays excess collagen deposition [71], which is indicative of severe inflammation and enhanced infiltration by neutrophils, lymphocytes, and macrophages when compared to subcutaneous adipose tissue [73]. Visceral adipose tissue displays a mesh-like, dense elastin fiber while subcutaneous adipose tissue displays a linear form of elastin, which is related to macrophage activity [33].

5.2 Loss of fat tissue and health

Excessive adipose tissue harvesting increases the risk of diabetes and cardiovascular disease because fat loss stimulates macrophage infiltration, inflammation, and fibrotic responses [74]. Body mass index (BMI) and the risk of these diseases have a U- or J-curve relationship [75, 76]. Thus, the importance of balanced adiposity needs to be considered for people with metabolic diseases.

5.3 Guidelines for ECM, GFs, and adipose tissue-generated cell as therapeutic agents

In order to use ECM, GFs, and adipose-generated cells in a clinical setting, it is necessary to follow the laws and regulations established by the appropriate regulatory body. For example, products must be manufactured according to mandated quality standards and be safe and effective when applied to a patient.

The required considerations are: (1) an analysis that explains the final form, function, and safety of the material

being used; (2) a quality evaluation that examines the sampling method, the identification method, purity, potency, and stability of the material being used as well as cell viability within the scaffold; (3) a non-clinical evaluation that examines the morphological and functional characteristics of the material being used as well as their transplant duration, tumorigenicity, and safety; and (4) a clinical trial that includes a functional, structural, pharmacodynamic (functional), and pharmacokinetic (persistence) evaluation [77].

Acknowledgements This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) and funded by the Korean government (MSIT) (2014M3A9D3034164), (2016R1C1B1011180), (2018R1C1B5040264) (2019R1A2C1004046), and the Ministry of Trade, Industry and Energy (MOTIE), Korea, under the “Regional Industry Infrastructure and R&D Support Program” (R0005886) supervised by the Korea Institute for Advancement of Technology (KIAT), and 2017–2019 Medical Cluster R&D Support Project through Daegu Gyeongbuk Medical Innovation Foundation funded by the Ministry of Health & Welfare, the Republic of Korea (HI16C2505).

Compliance with ethical standards

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

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

References

- Gomillion CT, Burg KJ. Stem cells and adipose tissue engineering. *Biomaterials*. 2006;27:6052–63.
- Patrick CW Jr. Tissue engineering strategies for adipose tissue repair. *Anat Rec*. 2001;263:361–6.
- Banyard DA, Borad V, Amezcua E, Wirth GA, Evans GR, Widgeow AD. Preparation, characterization, and clinical implications of human decellularized adipose tissue extracellular matrix (hDAM): a comprehensive review. *Aesthet Surg J*. 2016;36:349–57.
- Zhou Q, Desta T, Fenton M, Graves DT, Amar S. Cytokine profiling of macrophages exposed to *Porphyromonas gingivalis*, its lipopolysaccharide, or its FimA protein. *Infect Immun*. 2005;73:935–43.
- Trayhurn P, Wood IS. Signalling role of adipose tissue: adipokines and inflammation in obesity. *Biochem Soc Trans*. 2005;33:1078–81.
- Young DA, Christman KL. Injectable biomaterials for adipose tissue engineering. *Biomed Mater*. 2012;7:024104.
- Chun SY, Oh SH, Yoo JJ, Kwon TG. Fabrication and characterization techniques for decellularized organ scaffolds. *Tissue Eng Regen Med*. 2014;11:S1–10.
- Gentile P, Piccinno MS, Calabrese C. Characteristics and potentiality of human adipose-derived stem cells (hASCs) obtained from enzymatic digestion of fat graft. *Cells*. 2019;8:E282.
- Dufrane D. Impact of age on human adipose stem cells for bone tissue engineering. *Cell Transplant*. 2017;26:1496–504.

10. 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.
11. Wu I, Nahas Z, Kimmerling KA, Rosson GD, Elisseff JH. An injectable adipose matrix for soft-tissue reconstruction. *Plast Reconstr Surg*. 2012;129:1247–57.
12. 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.
13. Choi YC, Choi JS, Kim BS, Kim JD, Yoon HI, Cho YW. Decellularized extracellular matrix derived from porcine adipose tissue as a xenogeneic biomaterial for tissue engineering. *Tissue Eng Part C Methods*. 2012;18:866–76.
14. Shi Y, Dittman B, Atkinson B, Stilwell RL. Cell repopulated collagen matrix for soft tissue repair and regeneration. US Patent 20140286911A1, 9 Sept 2014 (2014).
15. 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.
16. Choi JS, Kim BS, Kim JY, Kim JD, Choi YC, Yang HJ, et al. Decellularized extracellular matrix derived from human adipose tissue as a potential scaffold for allograft tissue engineering. *J Biomed Mater Res A*. 2011;97:292–9.
17. Gilbert TW, Sellaro TL, Badylak SF. Decellularization of tissues and organs. *Biomaterials*. 2006;27:3675–83.
18. Gilbert TW, Freund JM, Badylak SF. Quantification of DNA in biologic scaffold materials. *J Surg Res*. 2009;152:135–9.
19. 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.
20. Rieder E, Kasimir MT, Silberhumer G, Seebacher G, Wolner E, Simon P, et al. Decellularization protocols of porcine heart valves differ importantly in efficiency of cell removal and susceptibility of the matrix to recellularization with human vascular cells. *J Thorac Cardiovasc Surg*. 2004;127:399–405.
21. Chung YC, Su YP, Chen CC, Jia G, Wang HL, Wu JC, et al. Relationship between antibacterial activity of chitosan and surface characteristics of cell wall. *Acta Pharmacol Sin*. 2004;25:932–6.
22. 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.
23. Ricard-Blum S. The collagen family. *Cold Spring Harb Perspect Biol*. 2011;3:a004978.
24. Chun TH. Peri-adipocyte ECM remodeling in obesity and adipose tissue fibrosis. *Adipocyte*. 2012;1:89–95.
25. Streuli C. Extracellular matrix remodelling and cellular differentiation. *Curr Opin Cell Biol*. 1999;11:634–40.
26. Niyibizi C, Fietzek PP, van der Rest M. Human placenta type V collagens. Evidence for the existence of an alpha 1(V) alpha 2(V) alpha 3(V) collagen molecule. *J Biol Chem*. 1984;259:14170–4.
27. Noro A, Sillat T, Virtanen I, Ingerpuu S, Bäck N, Kontinen YT, et al. Laminin production and basement membrane deposition by mesenchymal stem cells upon adipogenic differentiation. *J Histochem Cytochem*. 2013;61:719–30.
28. Atkinson JJ, Adair-Kirk TL, Kelley DG, Demello D, Senior RM. Clara cell adhesion and migration to extracellular matrix. *Respir Res*. 2008;9:1.
29. Spiegelman BM, Ginty CA. Fibronectin modulation of cell shape and lipogenic gene expression in 3T3-adipocytes. *Cell*. 1983;35:657–66.
30. Mosher DF, Schad PE. Cross-linking of fibronectin to collagen by blood coagulation Factor XIIIa. *J Clin Invest*. 1979;64:781–7.
31. Khan T, Muise ES, Iyengar P, Wang ZV, Chandalia M, Abate N, et al. Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. *Mol Cell Biol*. 2009;29:1575–91.
32. Spencer M, Unal R, Zhu B, Rasouli N, McGehee RE Jr, Peterson CA, et al. Adipose tissue extracellular matrix and vascular abnormalities in obesity and insulin resistance. *J Clin Endocrinol Metab*. 2011;96:E1990–8.
33. Martinez-Santibanez G, Singer K, Cho KW, DelProposto JL, Mergian T, Lumeng CN. Obesity-induced remodeling of the adipose tissue elastin network is independent of the metalloproteinase MMP-12. *Adipocyte*. 2015;4:264–72.
34. Kiely CM, Sherratt MJ, Shuttleworth CA. Elastic fibres. *J Cell Sci*. 2002;115:2817–28.
35. Holst J, Watson S, Lord MS, Eamegdool SS, Bax DV, Nivison-Smith LB, et al. Substrate elasticity provides mechanical signals for the expansion of hemopoietic stem and progenitor cells. *Nat Biotechnol*. 2010;28:1123–8.
36. Doran MR, Markway BD, Aird IA, Rowlands AS, George PA, Nielsen LK, et al. Surface-bound stem cell factor and the promotion of hematopoietic cell expansion. *Biomaterials*. 2009;30:4047–52.
37. Mullen LM, Best SM, Brooks RA, Ghose S, Gwynne JH, Wardale J, et al. Binding and release characteristics of insulin-like growth factor-1 from a collagen-glycosaminoglycan scaffold. *Tissue Eng Part C Methods*. 2010;16:1439–48.
38. 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.
39. Raeder RH, Badylak SF, Sheehan C, Kallakury B, Metzger DW. Natural anti-galactose alpha1,3 galactose antibodies delay, but do not prevent the acceptance of extracellular matrix xenografts. *Transpl Immunol*. 2002;10:15–24.
40. Daly KA, Stewart-Akers AM, Hara H, Ezzelarab M, Long C, Cordero K, et al. Effect of the alphaGal epitope on the response to small intestinal submucosa extracellular matrix in a nonhuman primate model. *Tissue Eng Part A*. 2009;15:3877–88.
41. van Dongen JA, Getova V, Brouwer LA, Liguori GR, Sharma PK, Stevens HP, et al. Adipose tissue-derived extracellular matrix hydrogels as a release platform for secreted paracrine factors. *J Tissue Eng Regen Med*. 2019;13:973–85.
42. Lin P, Chan WC, Badylak SF, Bhatia SN. Assessing porcine liver-derived biomatrix for hepatic tissue engineering. *Tissue Eng*. 2004;10:1046–53.
43. Badylak SF, Freytes DO, Gilbert TW. Extracellular matrix as a biological scaffold material: structure and function. *Acta Biomater*. 2009;5:1–13.
44. Kokai LE, Schilling BK, Chnari E, Huang YC, Imming EA, Karunamurthy A, et al. Injectable allograft adipose matrix supports adipogenic tissue remodeling in the nude mouse and human. *Plast Reconstr Surg*. 2019;143:299e–309.
45. Young DA, Ibrahim DO, Hu D, Christman KL. Injectable hydrogel scaffold from decellularized human lipoaspirate. *Acta Biomater*. 2011;7:1040–9.
46. Shan X, Choi JH, Kim KJ, Lee YJ, Ryu YH, Lee SJ, et al. Adipose stem cells with conditioned media for treatment of acne vulgaris scar. *Tissue Eng Regen Med*. 2018;15:49–61.
47. Saksela O, Moscatelli D, Sommer A, Rifkin DB. Endothelial cell-derived heparan sulfate binds basic fibroblast growth factor and protects it from proteolytic degradation. *J Cell Biol*. 1988;107:743–51.
48. Santana H, González Y, Campana PT, Noda J, Amarantes O, Itri R, et al. Screening for stability and compatibility conditions of recombinant human epidermal growth factor for parenteral

- formulation: effect of pH, buffers, and excipients. *Int J Pharm.* 2013;452:52–62.
49. Senderoff RI, Wootton SC, Boctor AM, Chen TM, Giordani AB, Julian TN, et al. Aqueous stability of human epidermal growth factor 1-48. *Pharm Res.* 1994;11:1712–20.
 50. Elias AP, Dias S. Microenvironment changes (in pH) affect VEGF alternative splicing. *Cancer Microenviron.* 2008;1:131–9.
 51. Hoener MC, Varon S. Effects of sodium chloride, Triton X-100, and alkaline pH on the measurable contents and sedimentability of the nerve growth factor (NGF) antigen in adult rat hippocampal tissue extracts. *J Neurosci Res.* 1997;49:508–14.
 52. Dong X, Wei X, Yi W, Gu C, Kang X, Liu Y, et al. RGD-modified acellular bovine pericardium as a bioprosthetic scaffold for tissue engineering. *J Mater Sci Mater Med.* 2009;20:2327–36.
 53. Wong ML, Wong JL, Horn RM, Sannajust KC, Rice DA, Griffiths LG. Effect of urea and thiourea on generation of xenogenic extracellular matrix scaffolds for tissue engineering. *Tissue Eng part C Methods.* 2016;22:700–7.
 54. Vavken P, Joshi S, Murray MM. TRITON-X is most effective among three decellularization agents for ACL tissue engineering. *J Orthop Res.* 2009;27:1612–8.
 55. 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, editors. *Topics in tissue engineering.* Vol. 4; 2008. Chap. 4. p. 1–26.
 56. Florini JR, Magri KA. Effects of growth factors on myogenic differentiation. *Am J Physiol.* 1989;256:C701–11.
 57. Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. *Endocr Rev.* 2008;29:535–59.
 58. Aberg D. Role of the growth hormone/insulin-like growth factor 1 axis in neurogenesis. *Endocr Dev.* 2010;17:63–76.
 59. von Heimburg D, Serov G, Oepen T, Pallua N. Fat tissue engineering. In: Ashammakhi N, Ferretti P, editors. *Topics in tissue engineering.* 2003. Chap. 8. p. 1–16.
 60. Park JH, Kim KJ, Rhie JW, Oh IH. Characterization of adipose tissue mesenchymal stromal cell subsets with distinct plastic adherence. *Tissue Eng Regen Med.* 2016;13:39–46.
 61. Bianco P, Robey PG, Simmons PJ. Mesenchymal stem cells: revisiting history, concepts, and assays. *Cell Stem Cell.* 2008;2:313–9.
 62. Maurer MH. Proteomic definitions of mesenchymal stem cells. *Stem Cells Int.* 2011;2011:704256.
 63. Schmelzer E, McKeel DT, Gerlach JC. Characterization of human mesenchymal stem cells from different tissues and their membrane encasement for prospective transplantation therapies. *Biomed Res Int.* 2019;2019:6376271.
 64. Salgado AJ, Reis RL, Sousa NJ, Gimble JM. Adipose tissue derived stem cells secretome: soluble factors and their roles in regenerative medicine. *Curr Stem Cell Res Ther.* 2010;5:103–10.
 65. Dmitrieva LA, Elizova LA. Structural changes in human dentin with the use of modern filling materials. *Stomatologiia (Mosk).* 1991;5:21–3.
 66. Strem BM, Zhu M, Alfonso Z, Daniels EJ, Schreiber R, Beygui R, et al. Expression of cardiomyocytic markers on adipose tissue-derived cells in a murine model of acute myocardial injury. *Cytotherapy.* 2005;7:282–91.
 67. 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.
 68. Shenaq DS, Rastegar F, Petkovic D, Zhang BQ, He BC, Chen L, et al. Mesenchymal progenitor cells and their orthopedic applications: forging a path towards clinical trials. *Stem Cells Int.* 2010;2010:519028.
 69. Murata D, Akieda S, Misumi K, Nakayama K. Osteochondral regeneration with a scaffold-free three-dimensional construct of adipose tissue-derived mesenchymal stromal cells in pigs. *Tissue Eng Regen Med.* 2018;15:101–13.
 70. Dicker S, Le Blanc K, Aström G, van Harmelen V, Götherström C, Blomqvist L, et al. Functional studies of mesenchymal stem cells derived from adult human adipose tissue. *Exp Cell Res.* 2005;308:283–90.
 71. Mori S, Kiuchi S, Ouchi A, Hase T, Murase T. Characteristic expression of extracellular matrix in subcutaneous adipose tissue development and adipogenesis; comparison with visceral adipose tissue. *Int J Biol Sci.* 2014;10:825–33.
 72. Virtue S, Vidal-Puig A. Adipose tissue expandability, lipotoxicity and the metabolic syndrome: an allostatic perspective. *Biochim Biophys Acta.* 2010;1801:338–49.
 73. Mariman EC, Wang P. Adipocyte extracellular matrix composition, dynamics and role in obesity. *Cell Mol Life Sci.* 2010;67:1277–92.
 74. Herrero L, Shapiro H, Nayer A, Lee J, Shoelson SE. Inflammation and adipose tissue macrophages in lipodystrophic mice. *Proc Natl Acad Sci U S A.* 2010;107:240–5.
 75. Zheng W, McLerran DF, Rolland B, Zhang X, Inoue M, Matsuo K, et al. Association between body-mass index and risk of death in more than 1 million Asians. *N Engl J Med.* 2011;364:719–29.
 76. Berrington de Gonzalez A, Hartge P, Cerhan JR, Flint AJ, Hannan L, MacInnis RJ, et al. Body-mass index and mortality among 1.46 million white adults. *N Engl J Med.* 2010;363:2211–9.
 77. Ministry of Food and drug safety of KOREA. Guidelines for evaluating cell therapeutic agents including scaffolds. B1-2014-3-011. 2014.
 78. Noverina R, Widowati W, Ayuningtyas W, Kurniawan D, Afifah E, Laksmiawati DR, et al. Growth factors profile in conditioned medium human adipose tissue-derived mesenchymal stem cells (CM-hATMSCs). *Clin Nutr Exp.* 2019;24:34–44.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.