

## Special Topic

# Preparation, Characterization, and Clinical Implications of Human Decellularized Adipose Tissue Extracellular Matrix (hDAM): A Comprehensive Review

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## Abstract

Fat grafting is commonly employed by plastic and reconstructive surgeons to address contour abnormalities and soft-tissue defects; however, because retention rates and thus volume filling effects are unpredictable, there is a search for new and innovative approaches. Initial studies on the use of human decellularized adipose tissue extracellular matrix (hDAM) show promise for its use not only in tissue engineering, but also in fat grafting. In this review, we examine and analyze the literature for the preparation, characterization, and use of hDAM and its derivatives in tissue engineering and plastic surgery applications. All studies reviewed involve physical, chemical, and/or biological treatment stages for the preparation of hDAM; however a distinction should be made between detergent and nondetergent-based processing, the latter of which appears to preserve the native integrity of the hDAM while most-efficiently achieving complete decellularization. Methods of hDAM characterization vary among groups and included simple and immunohistochemical staining, biochemical assays, 3-dimensional (3D) imaging, and mechano-stress testing, all of which are necessary to achieve a comprehensive description of this novel tissue. Finally, we examine the various preclinical models utilized to optimize hDAM performance, which primarily include the addition of adipose-derived stem cells or cross-linking agents. Overall, hDAM appears to be a promising adjunct in fat-grafting applications or even possibly as a stand-alone soft-tissue filler with off-the-shelf potential for commercial applications.

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Plastic and reconstructive surgeons are continually seeking alternatives for and/or strategies to improve the clinical results attained by soft-tissue filling. Autologous fat grafting is routinely implemented to treat a variety of soft-tissue defects and contour abnormalities. However, unpredictable resorption rates that range anywhere from 25% to 80%<sup>1,2</sup> often result in suboptimal volumizing effects that necessitate repeat harvesting and grafting procedures. One such strategy for improvement is cell-assisted lipotransfer (CAL), whereby the regenerative components such as adipose-derived stem cells (ADSCs) are isolated from, and then added back to, the lipoaspirate prior to lipofilling.<sup>3</sup> Various studies have demonstrated the regenerative benefits of ADSC enrichment,<sup>4</sup> while others have shown that retention of adipose tissue after CAL can be superior to fat grafting

alone.<sup>1,3,5</sup> However, these techniques are limited by their need for a separate harvesting procedure, extended cell-

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culturing times, and not being currently approved for use in the United States (US) by the Food and Drug Administration (FDA).

In general, there are 2 theories to describe the pathophysiology of fat-graft outcome. The first is that once grafted, there is at least partial survival of the adipocytes to achieve a lipofilling effect. The second is that the grafted adipocytes undergo necrosis, and it is the remaining stromal and stem cells that recruit host tissue to fill the void through adipogenesis, neovascularization, and fibrosis.<sup>2</sup> A critical review of the literature, however, supports the notion that both of the above factors contribute to the volume effects observed with fat grafting. Debate will continue on the subject, but a recent study has demonstrated that the majority of neovascularization and adipogenesis observed in fat grafting occurs through recipient tissue recruitment.<sup>6</sup> Recently, several groups have started to examine the use of hDAM as a scaffold for tissue engineering; this shows great promise as a vehicle for adipose stem-cell delivery as well as a construct that promotes soft-tissue regeneration.<sup>7-25</sup> The term hDAM is synonymous with decellularized adipose tissue (DAT), adipose derived matrix (ADM), and acellular adipose matrix (AAM).

Frequently, substantial amounts of adipose tissue are discarded as medical waste during abdominoplasty, liposuction, body contouring, and breast reduction procedures. This has led to burgeoning research that, until now, has focused on the optimization of fat-graft harvesting and processing, or on its cellular components such as the stromal vascular fraction (SVF). However, the noncellular component, or the hDAM, may be as important. For instance, Young et al examined the use of commercially available soft-tissue fillers, synthetic and natural polymers, with and without extracellular (ECM)-based materials for adipose tissue engineering and found that ECM-based products had the greatest potential to promote de novo adipogenesis and hence promote long-term retention. They also identified several qualities that would enhance the adipo-inductive properties of grafted material, including minimal in vivo immunogenicity, the ability to induce angiogenesis in vivo, and a composition similar to native adipose ECM.<sup>26</sup> The focus of this review is hDAM, which meets all of the above criteria, and may serve as an ideal soft-tissue filler both alone or as an adjunct to fat grafting.

To date, several studies have been published on early research that examines hDAM as a scaffold for ADSC supplementation to enhance adipogenesis.<sup>7,11-13,17,27-33</sup> Additionally, evidence suggests that the addition of cross-linking agents to hDAM may serve in lieu of, or as an adjunct to, achieve the superior preclinical results already observed with this soft-tissue filler.<sup>27,32</sup> In this review, we examine how different research groups isolate, characterize, and use hDAM for tissue-engineering applications. We then discuss the potential for hDAM as an off-the-shelf lyophilized adjunct in

allogeneic and autologous fat grafting to facilitate both adipogenesis and angiogenesis and to improve volume retention. In addition, hDAM has the potential as a stand-alone filler for soft-tissue reconstruction without the need for autologous fat or ADSCs because of the inductive properties of the ECM to recruit host cells and facilitate de novo adipogenesis.

## METHODS

### Study Design

A PubMed literature search was conducted in May 2015 using the terms “decellularized adipose tissue” and “acellular adipose matrix,” along with an advanced search combining the title elements of “adipose” and “matrix.” This search strategy was refined after conducting individual searches for terms previously used to describe decellularized adipose tissue extracellular matrix. Referenced articles from the selected studies were further evaluated for potential inclusion in our analysis. In total, 224 unique studies were identified.

### Selection Criteria and Data Collection

Abstracts were assessed if their title implied the use, incorporation, or analysis of adipose-derived extracellular matrix. Articles were generally excluded if the tissue described was not derived from adipose tissue, the method of decellularization was not adequately reported, or the article was not available in the English language. The full text of each article was read critically by a research assistant (V.B. or E.A.) to ensure that each manuscript included the methodology for sample preparation and at least 1 form of structural analysis. Each summary and article were then read and cross-referenced for completeness (D.B.). In total, 34 articles were included for review.

## RESULTS

### Decellularization of Adipose Tissue

The optimal method of adipose tissue matrix preparation includes the removal of all cellular components, and hence immunogenicity, while maintaining an ideal 3D configuration of the ECM and key components such as type IV collagen and laminin (Table 1).<sup>10-12,34</sup> It is widely accepted that the method of hDAM isolation will impact the ECM makeup and configuration, which carry functional and pathophysiological implications with regard to its regenerative capacity.<sup>12,35</sup> Additionally, Gilbert et al demonstrated that not all cellular components are removed in various decellularization processes used in commercially available products. Measurable amounts of DNA were found in many of these tissues using histological staining and gel electrophoresis.<sup>36</sup>

Table 1. Optimized Human Decellularized Adipose Tissue Extracellular Matrix (hDAM) Preparation Protocols

Group	Classification	Key Steps/Components	Processing Time	Advantages	Disadvantages
Flynn <sup>7</sup>	Nondetergent-based	(1) 3 freeze/thaw cycles (2) Trypsin digestion (3) Polar solvent extraction (4) Specialized rinsing buffer (5) Specialized enzymatic digestion (6) Specialized rinsing buffer (7) Polar solvent extraction	~120-156 hours <sup>7,9</sup>	<ul style="list-style-type: none"> <li>Retention of native hDAM structures including LN, Col IV</li> <li>Complete decellularization consistently observed</li> </ul>	<ul style="list-style-type: none"> <li>Time consuming</li> <li>Maximum fat parcel size that can be processed limited to 800 mg<sup>9</sup></li> </ul>
Choi et al <sup>10</sup>	Detergent-based	(1) Tissue homogenization (2) Salt-solution treatment (3) SDS incubation (4) DNase/RNase treatment	~54 hours	<ul style="list-style-type: none"> <li>DNA and RNA almost completely undetectable</li> <li>Grafts showed no signs of inflammatory response in vivo</li> </ul>	<ul style="list-style-type: none"> <li>Processing led to 24% decrease in acid/ pepsin-soluble collagen and 21% decrease in soluble elastin</li> <li>LN and Col IV not analyzed</li> </ul>
Young et al <sup>13,27</sup>	Detergent-based	(1) Tissue thawed (2) SDS incubation (3) Specialized enzymatic digestion (4) Lyophilization	~88-124 hours	<ul style="list-style-type: none"> <li>Complete decellularization based on IHC staining</li> <li>Good preservation of Col I-IV with some preservation of LN</li> </ul>	<ul style="list-style-type: none"> <li>High retained DNA content compared with other studies</li> <li>Demonstrated good host integration in in vivo model</li> </ul>
Brown et al <sup>12</sup>	Detergent-based	(1) Tissue thawed (2) Mechanical massaging (3) Trypsin digestion (4) Mechanical massaging (5) Triton X-100 incubation (6) Ethanol/peracetic acid treatment (7) N-propanol polar solvent extraction (8) Lyophilization	~24 hours	<ul style="list-style-type: none"> <li>Relatively quick assay</li> <li>Results in retention of collagen (mainly Col III) and GAGs</li> </ul>	<ul style="list-style-type: none"> <li>LN completely removed by decellularization process</li> <li>Some lipid droplets remained in matrix</li> </ul>
Wang et al <sup>11</sup>	Detergent-based	(1) 3 freeze/thaw cycles (2) Extensive wash step (3) Salt-solution treatment (4) Overnight wash (5) Salt-solution treatment (6) Trypsin digestion (7) Polar solvent extraction (8) Triton X-100 incubation	~214 hours	<ul style="list-style-type: none"> <li>Complete decellularization with extremely low DNA content observed</li> <li>Maintenance of collagen, VEGF and GAGs and removal of MHC-I</li> </ul>	<ul style="list-style-type: none"> <li>Long preparation time required</li> <li>LN completely removed during processing</li> </ul>

Col, collagen; DNA, deoxyribonucleic acid; DNase, deoxyribonuclease; GAG, glycosaminoglycans; IHC, immunohistochemistry; LN, laminin; MHC, major histocompatibility complex; RNA, ribonucleic acid; RNase, ribonuclease; SDS, sodium dodecyl sulfate; VEGF, vascular endothelial growth factor.

In order to compare and validate results of different studies assessing the efficacy of hDAM as a scaffold for tissue engineering, a standardized method of decellularization and characterization is desired to limit variation in the composition, purity, and configuration of the hDAM being evaluated.

Most published work on the decellularization of adipose tissue consists of physical, chemical, and biological treatment stages.<sup>10</sup> Flynn was the first to describe

comprehensive methods for the decellularization of adipose tissue. This 5-day protocol starts with the mechanical disruption of the tissue achieved through multiple freeze-thaw cycles in hypotonic buffer. An overnight enzymatic digestion is followed by a 2-day polar solvent extraction in isopropanol to remove lipid content. Next, the tissue is exposed to a number of wash and enzymatic digestion steps including DNase. Finally, the tissue is subjected to 1

final polar solvent extraction before the tissue is ready for use.<sup>7</sup> Flynn was able to achieve complete decellularization of adipose tissue samples up to 25 g in mass that, after hydration, typically represented 30% to 45% of the original tissue mass. Hematoxylin and eosin (H&E) staining and scanning electron microscopy (SEM) confirmed the absence of cells and cellular debris.<sup>7</sup>

Brown et al compared 3 different methods for the decellularization of adipose tissue. The first protocol, originally developed for the decellularization of liver tissue, included “mechanical massaging” prior to exposure to the strong detergent Triton X-100. An n-propanol incubation step was eventually added due to its cost-effective ability to remove residual lipid components. The final 2 methods involved the use of various enzymes or detergents and acids, respectively, but both methods produced wet materials that were high in lipid content as well as DNA of high base pair length. Decellularization was assessed by H&E and 4',6-diamidino-2-phenylindole (DAPI) staining as well as DNA quantification. Despite the finding that the first method was superior in terms of preservation of native collagen structure, secretion of basic fibroblastic growth factor, and an observed increase in the retention of glycosaminoglycans (GAGs), all 3 methods resulted in the complete removal of laminin, a key component of native adipose extracellular matrix.<sup>12</sup>

Choi et al employed a tissue homogenizer for mechanical disruption prior to treating the tissue with a hyperosmolar salt solution. The lipoaspirate was then treated with the detergent sodium dodecyl sulfate (SDS), followed by the enzymes DNase and RNase. A near complete elimination of DNA content was quantified via gel electrophoresis, and decellularization was confirmed with acridine orange (AO) and propidium iodide (PI) staining followed by SEM. The presence of laminin, however, was not analyzed in this study.<sup>10</sup>

Wang et al prepared hDAM using a modified protocol they previously developed to decellularize musculofascial ECM. Their protocol utilized very similar components to the Flynn methods, including a polar solvent extraction; however they swapped the enzymatic digestion step for a Triton X-100 incubation. H&E and DAPI staining confirmed the absence of cellular content that was later confirmed via SEM. DNA was also extracted and quantified to reveal a low  $2.1 \text{ ng} \cdot \text{mg}^{-1}$  of processed tissue. While SEM demonstrated that this extraction technique was very successful at decellularization while maintaining native 3D architecture, laminin was unable to be detected in the final tissue.<sup>11</sup>

The most comprehensive comparison of hDAM-isolation methods was published by Sano et al in 2014. Particularly, they compared purely mechanical, acid-based, and detergent-based methods to the original Flynn protocol and applied variations to each technique in an effort to define the optimal implementation. The mechanical-based method A was the

least successful in that even after 18 freeze-thaw cycles, all of the cells and cellular components still remained, yet the matrix structures were destroyed. The acid-based (method B) and enzyme-based (method C) protocols were successful at maintaining the matrix architecture; however, cells and cellular fragments remained even after extending the incubation periods eightfold. The only method found to be completely successful at decellularization was the Flynn protocol; however, Sano et al could only achieve complete decellularization after extending the enzymatic digestion steps. Additionally, they found that 0.8 g of adipose tissue was the upper limit of en bloc tissue that could be processed at any given time. This method did result in the preservation of collagen IV and laminin.<sup>9</sup>

Optimally, the decellularization process will strike a balance between efficiency of cell removal and maintenance of native matrix structure. Flynn avoided detergents like SDS because their retention can be cytotoxic to cells and results in significant alteration, swelling, or irreversible macroscopic degradation to matrix components.<sup>7</sup> Poon et al also avoided detergents because of the increased risk of matrix protein denaturation and degradation.<sup>25</sup> Wu et al used peracetic acid in combination with Triton X-100 to create a form of hDAM. A low concentration of the acid (0.1%) resulted in samples with high DNA content and incomplete lipid removal, while a high concentration (5%) resulted in a significant degradation of collagen. The group determined that a 3% peracetic acid step yielded the optimal effect; however, they did not assay for the presence of laminin.<sup>32</sup>

## Characterization of hDAM

Just as there is a need for a standardized decellularization protocol, there is also a need for a set of characterization assays that would further allow for a more accurate comparison of studies and large-scale production of hDAM (Table 2). Though there is a great deal of variability among the studies reviewed, each implements at least 1 form of simple histological staining and SEM to assess for the presence of cells and cellular components and to visually characterize the matrix. Others go further by employing techniques such as DNA isolation/quantification, immunohistochemistry (IHC), biochemical assays, and mechanostress testing to further characterize hDAM. In this section, we summarize these options and discuss their clinical implications.

Simple histological staining is the quickest and most direct way to assess for decellularization. The majority of studies reviewed utilize H&E staining, which allows for the direct visualization of cell membranes, proteins, and nuclei.<sup>7-9,11-14,30,32,36</sup> Additional methods commonly used include Masson's trichrome staining, which, in addition to staining cell fragments, allows for the detailed detection of

**Table 2.** Characterization of hDAM: Currently Methods

Technique	Types	Detects/Confirms
Histological stains	Hematoxylin & eosin Oil red O Masson's trichrome	Cell and vascular architecture Lipid and adipocyte content Collagen structure
Immunohistochemistry	Hoescht DAPI AO/PI Receptors/CD markers	Nucleic acid and retrained cellular components Presence and location of markers (eg, CD31, Col IV, LN)
Biochemical assays	DNA extraction kits RT-PCR Western blot	Quantification and identification of specific components (eg, DNA, genes, GAGs, elastin)
Electron microscopy	Scanning transmission	3D ultrastructure of extracellular matrix
Mechano-stress testing	Young modulus Storage and loss modulus	Measure of elasticity/mechanical integrity Rheological properties

3D, 3 dimensional; AO/PI, acridine orange/propidium iodide; CD, cluster of differentiation; Col, collagen; DAPI, 4',6-diamidino-2-phenylindole; DNA, deoxyribonucleic acid; GAG, glycosaminoglycan; LN, laminin; RT-PCR, reverse transcriptase polymerase chain reaction.

collagen,<sup>7,14,32</sup> and oil red O staining, which allows for the identification of adipocytes and lipid content.<sup>11,13,14,32</sup>

Those studies that did not use H&E staining employed some form of nucleic acid staining for the detection of retained cellular components in the form of Hoechst, DAPI, or AO/PI,<sup>10,29</sup> or 1 of these fluorescent stains was used in combination with H&E.<sup>8,11-13,30,32,36</sup> Perhaps the even greater utility of immunohistochemical staining is the ability to detect specific cellular and structural markers like CD31<sup>8,32</sup> and VEGF<sup>11</sup> or important extracellular components such as collagen, laminin, fibronectin, and vitronectin. Interestingly, only roughly half of the groups publishing on this topic actually implemented IHC to quantify the presence of type IV collagen and laminin,<sup>7,9,11-13</sup> the primary constituents of adipose extracellular matrix basement membrane.<sup>7,12,26</sup> Additionally, IHC can be used to detect the presence or absence of major histocompatibility complex class I (MHC-I), the absence of which indicates the removal of alloantigenicity.<sup>11</sup>

Biochemical assays are powerful tools for the assessment of hDAM constituents. One of the more commonly studied aspects includes the extraction, characterization, and quantification of DNA,<sup>8,10-13,29</sup> a surrogate marker of decellularization. It has been suggested that a DNA content < 50 ng · mg<sup>-1</sup> of tissue is optimal for proper decellularization.<sup>8,12</sup> Other assays in this category allow for the assessment of components like GAGs,<sup>13</sup> elastin,<sup>10</sup> and specific collagen content.<sup>32</sup> Additionally, gel electrophoresis can be

used to quantify specific matrix components such as fibronectin and laminin.<sup>25</sup>

Finally, 3D and mechanical properties must be taken into consideration when defining the structure of hDAM. Virtually every study has employed SEM or transmission electron microscopy.<sup>7,9-14,29,32</sup> In addition to enabling the confirmation of decellularization, this high-level visualization also allows one to assess the ultrastructural characteristics of the ECM.<sup>9</sup> Mechano-stress testing is another component that is often overlooked but critical in the evaluation of hDAM. ECM scaffolds that are too soft may result in structural collapse, whereas those that are too rigid may lead to irritation and scar-tissue formation.<sup>37</sup> Kochhar et al recently reported that their detergent-based hDAM demonstrated viscoelastic properties similar to standard lipoaspirate and is therefore an ideal candidate to substitute for standard lipofilling.<sup>38</sup> These mechanical properties are usually communicated as rheological or elasticity data in the form of the storage and loss modulus<sup>27,38</sup> or Young's modulus,<sup>10,33,37</sup> respectively. Finally, matrix porosity is a component thought to influence cellular autocrine and paracrine function.<sup>39</sup> Reporting on this structural element can be achieved using SEM,<sup>9</sup> ethanol displacement,<sup>40</sup> and capillary flow porometry.<sup>18</sup>

## DISCUSSION

Several groups are currently testing hDAM in vitro and in vivo for potential clinically-translatable, tissue-engineering applications. However, most institutions are using a different means for hDAM preparations, employing adipose matrices of differing compositions, thus making comparison of functional assay results difficult if not impossible. Natural ECMs possess the inherent property of biological recognition, which includes the crucial properties of ligand presentation for cellular interactions, susceptibility to cell-triggered proteolytic enzymes, and subsequent remodeling needed for morphogenesis.<sup>41</sup> At this point, data are limited that indicates what constitutes the optimal ECM components for regenerative purposes, but 1 preclinical study suggests that the presence of collagen IV and laminin, key constituents of the basement membrane, is associated with increased adipogenesis.<sup>34</sup> The majority of methods reviewed employ detergents, such as SDS or Triton X-100, which effectively remove laminin. However, the original protocol developed by Flynn<sup>7</sup> appears to be the only methodology that preserves all key structures of the basement membrane, and this finding was confirmed by Sano et al.<sup>9</sup>

In her initial study, Flynn demonstrated that hDAM seeded with ADSCs exhibited increased expression of peroxisome proliferative activated receptor gamma (PPAR<sub>γ</sub>) and human CCAAT-enhanced binding protein alpha (CEBP<sub>α</sub>), both key regulators of adipogenesis, when compared with

control adipogenic-differentiated ADSCs. Interestingly, GAPDH activity (a marker of adipose tissue maturation) was found to be inversely proportional to the body mass index of the ADSC donors.<sup>7</sup> Turner and Flynn went further by designing composite hydrogel hDAM scaffolds that can serve as microcarriers for ADSCs.<sup>31</sup> They implanted this hDAM subcutaneously in rats with or without ADSC supplementation. Macroscopically, there was no difference between the retained grafts, which were both superior to the gelatin-carrier control group. When analyzed under a microscope, both the seeded and unseeded hDAM grafts exhibited good integration with host tissue, whereas the gelatin microcarriers were found to be encapsulated by dense fibrous tissue.<sup>42</sup> Because the seeded hDAM microcarriers exhibited the highest degree of cellularity and angiogenesis when compared with the unseeded graft, the researchers conducted further experiments on this model and found that the addition of the cross-linking agent methacrylated chondroitin sulphate increased its overall performance.<sup>28</sup>

Cross-linking appears throughout the literature in various forms as a method to augment the beneficial properties of decellularized adipose tissue matrix. Lu et al employed a modified version of the Flynn protocol to obtain murine decellularized adipose tissue extracellular matrix (mDAM). Cross-linking of heparin to the matrix was achieved using 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) and N-hydroxysuccinimide to create a delivery vehicle for basic fibroblast growth factor (bFGF). Their enhanced mDAM resulted in the formation of highly vascularized adipose tissue after 6 weeks compared with mDAM alone in a mouse model. Additionally, the explanted mDAM exhibited higher levels of CEBP $\alpha$ , adiponectin and glucose transporter-4 expression, key components for adipogenesis.<sup>43</sup> Wu et al investigated the efficacy of an injectable hDAM, which was cross-linked with hexamethylene diisocyanate and EDC to improve volume retention. They demonstrated that their injectable hDAM, which was derived using acids and detergent, could support growth and differentiation of ADSCs in vitro. Additionally, their rat model indicated that cross-linking increased the hDAM's resistance to enzymatic degradation promoting host-cell migration with adipose-tissue development and vascularization without the need for ADSC supplementation. Long-term in vivo studies demonstrated their acellular injectable developed into newly formed vascularized adipose tissue.<sup>32</sup> Young et al also compared their detergent-based hDAM gel for soft-tissue filling in a mouse model, supplemented with either ADSCs or transglutaminase (TG), a cross-linking agent. Interestingly, they found that both were superior to hDAM alone and that the addition of TG was as effective as ADSCs at improving neovascularization and the soft-tissue-filling effect.<sup>27</sup>

A group in South Korea is also extensively studying the properties and applications for hDAM. Initially, this

group investigated the efficacy of a purely mechanically based hDAM powder for soft-tissue-filling applications.<sup>17</sup> Lipoaspirate was subjected to multiple washes with distilled water, homogenization, and centrifugation, forming a gel-like tissue suspension that was freeze dried and ground to a fine powder. After sterilization, the gel was cultured and injected into nude mice. Their data indicated that hDAM powder seeded with ADSCs displayed superior proliferation, viability, and distribution in vitro while demonstrating improved neovascularization and adipogenesis in vivo when compared with hDAM powder alone. In a subsequent study, Choi et al used the previous protocol to fabricate hDAM scaffolds into various shapes including round dishes, hollow tubes, and beads. Interestingly, in vitro testing revealed that the frequency of ADSC attachment was directly correlated to the scaffold pore size, a parameter for which the magnitude is directly proportional to the freezing temperature used for scaffold preparation.<sup>18</sup> Unfortunately, these studies do not comment on the efficacy this preparation had on matrix decellularization.<sup>17,18</sup> Subsequently, the group modified their original protocol to develop a decellularization method for hDAM that incorporated tissue homogenization followed by detergent-based processing. Biochemical analysis revealed an hDAM that exhibited a 24% decrease in acid/pepsin-soluble collagen and a 21% decrease in soluble elastin; however they report no significant change in matrix composition, and ex vivo analysis revealed host-cell engraftment with minimal inflammatory cell infiltration.<sup>10</sup> More recently, this group has been investigating the use of various forms of hDAM for tissue-specific applications.<sup>16,29,44</sup>

There are additional studies that demonstrate the capacity of hDAM to fill soft tissue defects when supplemented with ADSCs. One group used ultrasonic homogenization followed by pancreatin digestion to create an hDAM as well as an ECM from porcine small intestine mucosa. The hDAM was superior for promoting ADSC viability and proliferation when co-cultured in vitro, induced the upregulation of adipose-related genes such as PPAR $\gamma$  and leptin, and also demonstrated superior adipogenesis in an in vivo model.<sup>14</sup> Wang et al created a detergent-based hDAM that they extensively characterized in terms of complete decellularization, low DNA count, porosity, and maintenance of collagen. This hDAM, which was devoid of laminin, showed comparable results to native tissue fat grafting after supplementation with  $4 \times 10^5$  cells/mL of ADSCs in an in vivo model. Their hDAM alone, similar to the previously mentioned detergent-based adipose ECMs, did not promote adipogenesis.<sup>11</sup> This reinforces the notion that if hDAM is not processed in a manner that preserves the key components of the basement membrane, an external cross-linking agent or the addition of stem cells is needed for hDAM to serve as a soft-tissue filler.

Preliminary studies have shown great potential for the use of hDAM in a variety of tissue-engineering approaches

with obvious clinical ramifications, and these applications are not limited to soft-tissue filling. For instance, 1 group demonstrated the ability to significantly improve the healing of full-thickness wounds using sheets of detergent-based hDAM impregnated with ADSCs.<sup>45</sup> Another group combined hDAM with mDAM to construct adipose tissue-derived acellular matrix thread that, when seeded with ADSCs, was able to improve erectile function after 3 months in a rat model for cavernous nerve injury.<sup>30</sup>

In terms of plastic and reconstructive surgery applications, we feel 1 approach should be at the forefront of current investigation: the use of hDAM as a stand-alone injectable filler to substitute for traditional fat grafting in situations requiring volumetric replacement. This provides a major advantage to current processes as it obviates the need for same-setting fat harvesting while still using natural human adipose-derived scaffold as a filler. Though the majority of studies reviewed focus on hDAM as a framework for ADSC delivery, we feel a further emphasis is needed on the regenerative benefits of hDAM alone, a product that could be lyophilized, stored, and used in an off-the-shelf manner. For instance, Turner et al created a nondetergent-based, unsupplemented hDAM that exhibited good host-tissue integration after implantation in rats when compared with the control.<sup>42</sup> Similarly, Poon et al created a detergent-free porcine decellularized adipose tissue extracellular matrix (pDAM) hydrogel that was capable of promoting adipogenesis in rats.<sup>22,25</sup> Not only does this product lack immunogenicity, but its properties would align it under section 361 of the Public Health Service Act by the US FDA as being a “minimally manipulated” tissue, much like the many brands of acellular dermal matrix used today.

The potential volumizing effects observed when using hDAM as a stand-alone filler have yet to be adequately studied. Turner et al did not detect any macroscopic or microscopic differences between seeded and unseeded hDAM grafts in a rat model; both were superior to the control but were not compared with fat grafting.<sup>42</sup> In the animal portion of the study performed by Poon et al, pDAM alone stimulated adipogenesis equivalent to 30% of the original volume grafted, compared with 48% retention observed in the group grafted with ADSC-supplemented pDAM after 8 weeks; but again, no comparison was made to traditional lipotransfer.<sup>25</sup> If the latter study is reproducible, then it should come as no surprise that cell supplementation augments the retention of grafted adipose tissue, because this was convincingly demonstrated by Kolle et al.<sup>1</sup> Unfortunately, removing the unpredictability in fat grafting using this strategy results in a process that requires an initial harvest procedure, extensive time in cell culture, and approval as an investigational new drug in the US.

A large quantity of adipose tissue is discarded as medical waste during various surgical procedures including liposuction, reduction mammoplasty, abdominoplasty, and

various other body contouring procedures. Rather than being discarded, this adipose tissue could serve as a reservoir of hDAM. As soon as it is harvested, the adipose tissue can be frozen and sent to a central processing facility where decellularization could proceed. This is practical because the first stage in many decellularization protocols is the physical treatment stage consisting of multiple freeze-thaw cycles.

A limitation of this review was the possible exclusion of relevant articles pertaining to hDAM. For instance, 2 articles of interest were published in the Chinese literature and not analyzed due to the lack of a translation into English.<sup>46,47</sup> Additionally, our literature search was limited to PubMed. While this is an extremely inclusive resource, it is not exhaustive. It is possible that some relevant articles were not detected using our methodology.

## CONCLUSIONS

Currently, there is no universally accepted, standardized protocol for the decellularization of adipose tissue. Various agents including salts, enzymes, and detergents are used in the decellularization process, but only the nondetergent-based methodologies appear to maintain all the components of adipose basement membrane. Sano et al determined that a modified version of the Flynn protocol was most efficient at both decellularization and maintaining the native constituents of the ECM<sup>9</sup>; however in vitro and in vivo data comparing clinical efficacy of the various forms of hDAM preparation are lacking. Furthermore, full characterization that includes simple and immunohistochemical staining, biochemical assays, 3D imaging, and mechano-stress testing will enable researchers to make more informed decisions when attempting to produce hDAMs that most closely mimic native adipose tissue. In addition to demonstrating that ECM stiffness alone can promote adipogenesis in vitro,<sup>24</sup> a number of groups have demonstrated significant preclinical results with cross-linked, detergent-based hDAM products that suggest 3D architectural elements and mechanotransduction may be as important for in vivo performance as the protein constituents. Once these structural elements are elucidated, hDAM can even be incorporated as bioink for 3D printing applications where the porosity and viscoelastic properties can be manipulated as needed for adipogenesis or for other potential applications.<sup>20,21</sup>

Fat grafting is one of the most commonly employed techniques in plastic and reconstructive surgery to address contour abnormalities and soft-tissue defects. However, fat-graft retention rates are unpredictable, despite numerous harvesting and processing techniques described in the literature. There is a need for a novel approach to fat grafting, and we feel that hDAM is an untapped resource in this setting. Human decellularized adipose tissue extracellular matrix carries the potential of being an off-the-shelf filler to

stimulate de novo adipogenesis and neovascularization without the need for harvesting fat from the patient. Although more work needs to be done, initial studies show great promise for the use of hDAM in the setting of soft-tissue filling.

## Disclosures

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