Abdominal Wall Allograft

Preclinical Biomechanical Investigation of a Novel Reconstructive Adjunct

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Introduction: Acellular dermal matrices have revolutionized abdominal wall reconstruction; however, device failure and hernia recurrence remain significant problems. Fascia grafts are a reconstructive adjunct with increased tensile strength compared with acellular dermal matrices; however, clinical use is limited by insufficient donor material and donor site morbidity. To this end, we investigate the biomechanical properties of human abdominal wall allografts (AWAs) consisting of the anterior rectus sheath from xiphoid to pubis.

Methods: After cadaveric procurement of 6 human AWAs, the tissue was divided horizontally and a matched-sample study was performed with specimens randomized to 2 groups: fresh, unprocessed versus processed with gamma irradiation and decellularization. Specimens were evaluated for physical properties, DNA content, tensile strength, and electron microscopy.

Results: All AWA donors were male, with a mean age of 55.2 years (range, 35–74 years). Procured AWAs had a mean length of 21.70 ± 1.8 cm, width of 14.30 ± 1.32 cm, and area of 318.50 cm^2, and processing resulted in a 98.3% reduction in DNA content. Ultimate tensile strength was significantly increased after tissue processing, and after subcutaneous implantation, processed AWA demonstrated 4-fold increased tensile strength compared with unprocessed AWAs.

Conclusions: Acellular AWAs represent a novel reconstructive adjunct for abdominal wall reconstruction with the potential of replacing "like with like" without additional donor site morbidity or antigenicity.

Key Words: acellular, abdominal wall allograft, abdominal wall reconstruction, biomechanical properties

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Indications for abdominal wall reconstruction include trauma, necrotizing soft tissue infection, tumor resection, intra-abdominal sepsis, and incisional hernia repair.[1] With over 300,000 ventral hernia repairs performed each year in the United States, and with a hernia recurrence rate of 5% to 40%, there is substantial opportunity to reduce morbidity through advances in hernia repair and abdominal wall reconstruction.[2–4] When reconstructing the abdominal wall, the general and plastic surgeons often face a hostile reconstructive environment with a paucity of good quality autologous tissue or contamination of the surgical field. The technical challenge of abdominal wall reconstruction is further compounded by comorbidities in this patient population that contribute to a high rate of postoperative complications including bulging, hernia recurrence, and wound infection—each of which exceeds 20%.[5] These complications often necessitate reoperation in an environment more challenging than that experienced at the index surgery, where inadequate abdominal wall tissues or bacterial burden make successful repair increasingly difficult.[6,7]

In accordance with concepts of the reconstructive ladder, when primary closure of the abdominal wall cannot be achieved, the technique of local tissue rearrangement with component separation or its derivatives provides a means for abdominal wall reconstruction.[8–10] However, when these techniques have been exhausted, the reconstructive surgeon is faced with a substantial dilemma in restoring the continuity of the abdominal wall with tissue of adequate tensile strength and revascularization potential.[11,12] The emergence of acellular dermal matrices (ADMs) as a key component of the surgical armamentarium of the reconstructive surgeon has revolutionized the management of complex abdominal wall defects. Acellular dermal matrices provide an ample tissue source when autologous tissues are unavailable and offer a construct resistant to infection when reconstruction is performed in a contaminated environment.[13,14] Manufacturers have utilized different proprietary techniques for tissue processing, decellularization, and sterilization, to optimize the ADM properties, as well as host interaction with respect to biocompatibility and foreign body response, to facilitate maximum ADM cellular incorporation and neovascularization. These biomaterial scaffolds serve as a regenerative framework to support host cell integration and new collagen deposition.[15–17] Furthermore, as neovascularization occurs and these materials become incorporated by the host, they show an increase in tensile strength over time. Whereas the application of ADMs has been a major advancement in the field of abdominal wall reconstruction, the incidence of complications including hernia recurrence remains clinically significant.[18–22]

Because they are derived from the dermis, ADMs have a structure and biomechanical profile different from that of abdominal wall fascia. Although ADMs have well-established benefits in resistance to infection and biocompatibility, the innate microstructure of dermis is not ideally suited to the functional stresses exerted on the abdominal wall, which include constant loading from intra-abdominal pressure as well as cyclical loading with respiration and during strenuous physical activity. The impact of these forces on the tensile durability of ADMs has been clinically borne out in multiple studies that document a substantial rate of hernia recurrence and abdominal wall laxity with use of ADMs in abdominal wall reconstruction.[23–25]

Before the introduction of ADMs, there was a long history of abdominal wall reconstruction with fascia flaps.[26–28] The indications for fascia flaps are similar to those for ADMs, including deficient autologous tissue and a contaminated surgical field.[29–31] Animal models of abdominal wall reconstruction with fascia flaps have shown no decrease in tensile strength at 1 year, and human abdominal wall reconstruction with fascia lata allografts has been performed with no major signs of recurrence, laxity, or infection at a mean follow-up of approximately 2 years.[32,33] With the goal of improving options and outcomes in abdominal wall reconstruction, we performed a preclinical biomechanical investigation of an acellular abdominal wall allograft (AWA). This novel reconstructive adjunct has the potential to not only reconstitute deficient tissue on a gross anatomic scale but also restore the structural properties that are essential to long-term durable function of the abdominal wall while avoiding the problems of antigenicity and donor site morbidity.

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MATERIALS AND METHODS

AWA Procurement

Six AWAs were procured from organ donors according to guidelines for human cellular and tissue-based products. Briefly, the anterior rectus sheath fascia was isolated from the xyphoid to the pubis and laterally from semilunar line to semilunar line. The linea alba was preserved during isolation of the construct (Fig. 1). The constructs were then characterized for maximum length, maximum width, surface area, and weight in grams (Table 1). The constructs were then divided transversely perpendicular to the linea alba, and transferred to LifeNet Health on ice for proprietary tissue processing according to approved protocols (Fig. 1).

Experimental Groups and Tissue Processing

Two experimental groups were investigated—control AWAs (Fig. 1; right panel, top) and Matracell-processed acellular AWAs (Fig. 1; right panel, bottom). Both groups are handled in a similar fashion including freezing and gamma irradiation with the exception of Matracell tissue processing to directly compare the effects of tissue acellularization on the physical and mechanical properties of the AWA constructs. After cadaveric procurement of human AWAs, the tissue was sent on ice to LifeNet Health for tissue processing using gamma irradiation for sterilization as well as Matracell tissue processing, which is an efficient 2-day decellularization protocol, which demonstrates a more than 98% reduction in donor DNA with multiple disinfecting agents targeting both cellular and genetic components to produce a safe bio-implant. The protocol includes validated United States Pharmacopeia chapter 71 endpoint microbial testing and does not utilize any animal-derived reagents. The tissue processing protocol has been used previously for commercially available products for orthopedic, cardiac, and vascular procedures.

DNA Quantification of AWAs

The DNA reduction after tissue processing and acellularization were assessed on 1 × 1 cm sections of AWA constructs for DNA using the DNeasy kit (QIAgen). The DNA concentration was determined with a spectrophotometer and was normalized to AWA dry weight, as well as compared with the unprocessed and acellular AWA groups.

TABLE 1. AWA Physical Properties

Demographics of Donor Tissue		
n	8	
Age, y	55.2 ± 5.6	
Sex (% male)	100.0	
Before tissue processing	Mean	SEM
Length, cm	21.7	1.80
Width, cm	14.3	1.32
Surface area, cm^2	318.5	47.34
Mass, g	41.2	5.30
DNA, ng DNA/mg	81.2	12.10
After tissue processing		
% DNA reduction	98.3	0.23

SEM, standard error of the mean.

Tissue Response to Implantation of AWA

Animal experiments were performed under a protocol approved by the University of Virginia Institutional Animal Care (Animal Welfare Assurance No. A3245-01) and Use Committee in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. Rats were housed in a facility accredited by Association for Assessment and Accreditation of Laboratory Animal Care. All procedures were performed under anesthesia, and all efforts were made to minimize pain or suffering. Abdominal wall allograft specimens, processed and unprocessed, were thawed in room temperature phosphate buffered saline (1×), and three 1.0 × 5.5 cm samples of each specimen were sharply cut perpendicular to the linea alba of the donor and placed into an iodophor solution. Three female Sprague-Dawley rats (250–300 g) were utilized for this portion of the study. Animals were anesthetized and maintained under inhaled isoflurane (2%–2.5%). Once anesthetized, the animals were positioned supine, shaved, depilated, and prepared for aseptic surgery with iodophor scrub followed by 70% alcohol and iodophor solution, and then draped for surgery. A 4 cm midline skin incision was created in the mid abdomen and the dissection proceeded through the panniculus carnosus muscle to expose

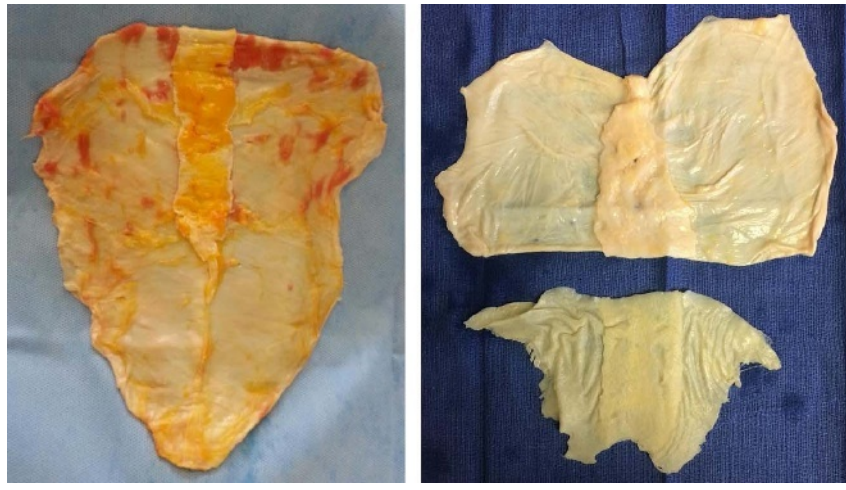

FIGURE 1. Abdominal wall allografts were procured from 8 human donors. AWA before tissue processing (left). AWA is sectioned transversely perpendicular to the linea alba and randomized to 1 of 2 groups (right): (1) unprocessed, frozen; and (2) processed, Allowash XG (LifeNet Health) low-temperature gamma irradiation, 25 kGy, with a sterilization assurance level of 10^{-6}.

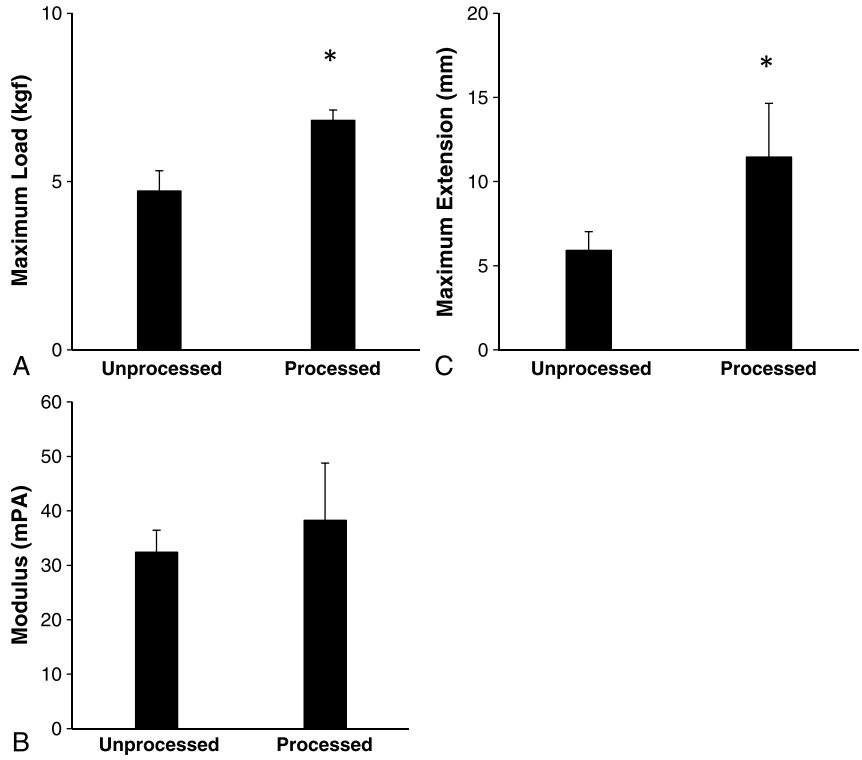

FIGURE 2. Processed and unprocessed AWAs were subjected to mechanical strength testing. Processed AWAs demonstrated a significant increase in ultimate tensile strength ($P < 0.05$, n = 6 per group).

the abdominal wall fascia and linea alba. Samples of human AWA (1.0 × 5.5 cm^2) that had been soaking in iodophor solution for 10 minutes and rinsed with sterile water were placed longitudinally on the anterior surface of the abdominal wall and secured proximally and distally 4-0 Vicryl in interrupted fashion at the cranial and caudal ends. Unprocessed AWA samples were placed into the animals' right side, and processed AWA samples were placed into the in the animals' left side. After implantation, the skin flaps were reapproximated in a tension free manner overlying the implanted AWAs and secured with 4–0 Prolene sutures and the animal was circumferentially bandaged. Animals were also given a subcutaneous injection of buprenorphine (0.5 mg/kg) for pain, recovered and returned to their cages. Female animals were utilized to allow for ease of urination with the bandaging. One week after implantation, the bandages and sutures were removed. Three weeks after implantation, the animals were reanesthetized with inhaled isoflurane (2%–2.5%) and the midline was reopened and the skin was carefully dissected and retracted to expose the AWA implants. The implants were sharply excised. A 2- to 3-mm strip was sharply dissected from the short axis and placed into 10% formalin for histology and scanning electron microscopy. The remainder of the specimen was utilized for material strength testing. Animals were then euthanized with an anesthetic overdose of Euthasol.

Characterization of the Effect of Mode of Tissue Processing on AWA Tensile Strength

The AWA constructs were subjected to repeat baseline characterization after tissue processing, and then, they were dissected into 1 cm by 5 cm strips perpendicular to the linea alba but not including the linea alba in the specimen. Mechanical tensile strength testing was performed on an Instron mechanical tester (Model No. 5943) equipped with a 100-N load cell (Model No. 2530-427) and 1 kg screw action clamps (Norwood, Mass). The AWA strips were loaded onto a 4 × 2 cm^2 sheet

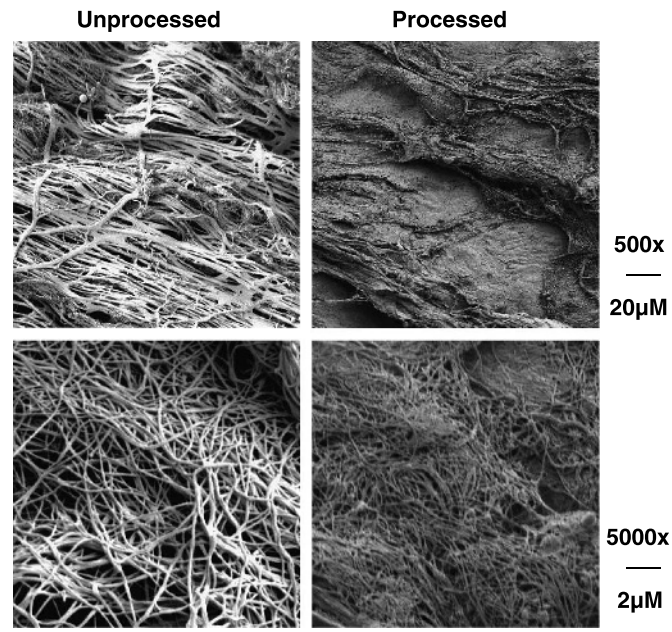

FIGURE 3. Processed and unprocessed AWAs were subjected to scanning electron microscopy to determine the effect of tissue processing on ultrastructural architecture and collagen cross-linking. Processed AWAs demonstrated a notable increase in collagen cross-linking with a significant increase in the number of collagen branch points as well as an increase in the density of collagen fibers per high-power field. Representative images depict the collagen ultrastructure at 500× and 5000×, respectively.

of 100-grit sandpaper pretreated with cyanoacrylate. The AWA strips within the clamp segments were covered with cyanoacrylate followed by sandpaper folded over to ensure solid clamp contact. The AWA strips were loaded into mechanical clamps with a gauge length of 20 mm and clamp length of 15 mm for the studies before tissue implantation. For studies performed after subcutaneous implantation in a rat model, because of tissue availability, samples were loaded with a 1-cm clamp length and 20 mm gauge length. Specimens were prestretched with 10 cycles of 5 mm strain at 10 mm/s and then pulled to failure at 100 mm/min to ensure a midsubstance tear. End of test was determined by 80% decrease from peak load or maximum 10 cm of extension. Primary outcomes include maximum load (N), tensile stress at maximum load (MPa), and elastic modulus.

Scanning Electron Microscopy for Ultrastructural Characterization

Samples of unprocessed and processed AWAs before and after implantation were evaluated through scanning electron microscopy to evaluate the microscopic structure. After the AWA samples were fixed in 10% formalin for 5 days, they were washed (3 × 10 min) with 0.1 M cacodylate buffer and then treated with 2% osmium tetroxide for 60 min. After 2 × 10 min washes with 0.1 M cacodylate buffer and distilled H2O, the samples were dehydrated in a series of 10-minute ethanol treatments (30%, 50%, 70%, 95%, and 100%). Samples were then placed into a critical point dryer with a 15-minute purge time and then mounted on microscope stubs with carbon stickers and sputter coated with gold, 200 seconds at 60 mA. Once coated, the samples were imaged on a Zeiss Sigma HD scanning electron microscope at a voltage of 3.0 kV and a working distance of 13.4 mm.

Histologic Characterization of AWA

AWA constructs (1 × 1 cm sections) were formalin fixed and embedded in paraffin. Sections with a concentration of 5 μM were subjected to staining with hematoxylin and eosin to cellular infiltration after implantation and compared with the unprocessed and acellular AWA groups. Cell counting and quantification were performed using ImageJ software.

Statistical Analysis

All values recorded are presented as mean ± standard error of the mean from independent experiments from given n sizes. Statistical significance of multiple treatments was determined by analysis of variance followed by the Bonferroni post hoc test when appropriate. Statistical significance between 2 groups was determined by using the 2-tailed Student *t* test. *P* values of less than 0.05 are considered significant.

RESULTS

All AWA donors were male, with a mean age of 55.2 years (range, 35–74 years). Physical data for Matracell-processed acellular AWAs are presented in Table 1. Procured AWAs had a mean length of 21.70 ± 1.8 cm, width of 14.30 ± 1.32 cm, and area of 318.50 cm^2. The average weight before and after decellularization was 41.26 g and 22.42 g, respectively. There was a 98.3% reduction in DNA after the Matracell processing of the AWAs.

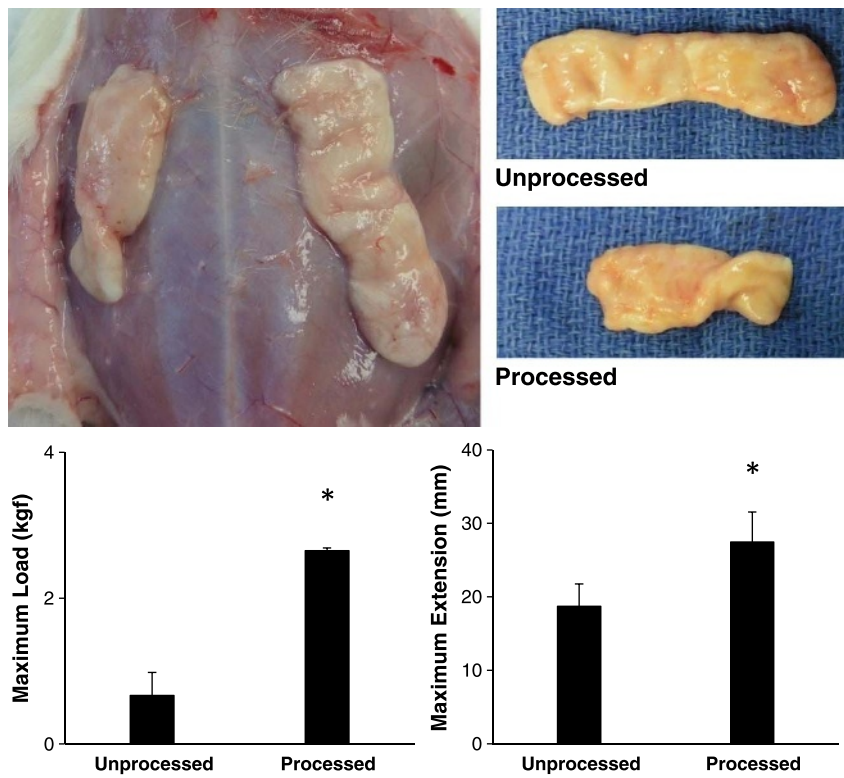

FIGURE 4. Processed and unprocessed AWAs were implanted in a suprafascial position on rat abdominal walls and maintained for 4 weeks to assess the effect of tissue processing on mechanical strength, collagen ultrastructure, and recellularization. Abdominal wall allografts were secured in suprafascial fashion to the abdominal wall. After abdominal wall implantation, processed and unprocessed AWAs strength testing was performed. Processed AWAs demonstrated a significant increase in ultimate tensile strength ($P < 0.05$, n = 3 per group).

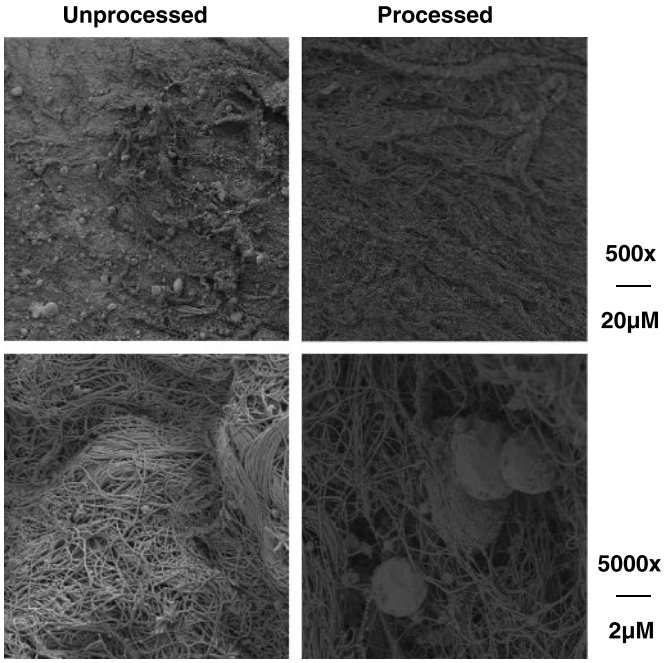

FIGURE 5. After abdominal wall implantation, the processed and unprocessed AWAs were procured and were subjected to scanning electron microscopy to determine the effect of tissue processing on ultrastructural architecture. Processed AWAs demonstrated a notable increase in surface cellularity. Collagen architecture is maintained in both groups after 4 weeks of implantation. Representative images depict the collagen ultrastructure at 500× and 5000×, respectively (n = 3).

Mechanical testing was performed on matched unprocessed and processed AWAs before soft tissue implantation (Fig. 2). The maximum load was 4.72 ± 1.80 kgf in the unprocessed AWA group and 6.8 ± 0.74 kgf in the processed AWA group, which demonstrates a significant increase in ultimate tensile strength after tissue processing ($P < 0.05$, n = 6 per group) (Fig. 2A). Young modulus was 31.39 ± 17.82 mPA for the unprocessed AWA group and 38.25 ± 25.76 mPA for the processed AWA group (Fig. 2B). Maximum extension at failure was 5.91 ± 1.84 mPA for the unprocessed AWA group and 11.46 ± 3.91 mPA for the processed AWA group, which also demonstrated a significant increase after tissue processing ($P < 0.05$, n = 6 per group) (Fig. 2C).

Collagen ultrastructure was assessed at baseline to assess the effect of tissue processing on the composition of the AWA with scanning electron microscopy (Fig. 3). Processed AWAs demonstrated a notable increase in collagen cross-linking with an increase in the number of collagen branch points as well as an increase in the density of collagen fibers per high-power field (Fig. 3).

Mechanical strength testing was then performed after subcutaneous implantation of the AWAs in a rat model as described. After tissue implantation, the maximum load at failure was 0.66 ± 0.15 kgf in the unprocessed AWA group and 2.64 ± 0.54 kgf in the processed AWA group, which demonstrates a significant increase in ultimate tensile strength in the processed group ($P < 0.05$, n = 6 per group) (Fig. 4). Both groups experienced a decrease in maximal tensile strength after 3 weeks of subcutaneous implantation; however, the effect was significantly decreased in the processed AWA group.

After abdominal wall implantation, the processed and unprocessed AWAs were procured and were subjected to scanning electron microscopy to determine the effect of tissue processing on ultrastructural architecture. Processed AWAs demonstrated a notable increase in surface cellularity (Fig. 5). Collagen architecture is maintained in both groups after 3 weeks of implantation. To further confirm the findings of increased surface cellularity, histologic analysis was performed after 3 weeks of subcutaneous implantation. After abdominal wall implantation, processed AWAs demonstrate a significant increase in cellularity in both the center of the construct (0.59 ± 0.28% and 2.34 ± 0.77% for unprocessed AWA and processed AWA, respectively) and the AWA-host tissue border zone (3.53 ± 1.61% and 11.61 ± 4.31% for unprocessed AWA and processed AWA, respectively) ($P < 0.05$, n = 3); however, both groups demonstrated the presence of basophilic cells throughout the construct (Fig. 6).

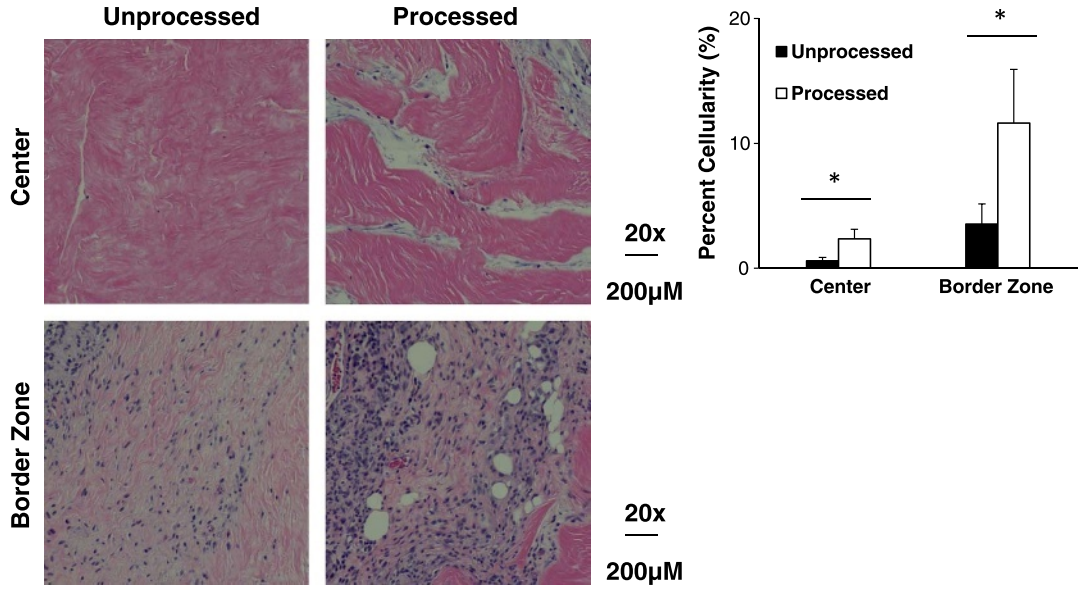

FIGURE 6. After abdominal wall implantation, processed AWAs demonstrate a significant increase in cellularity in both the center of the construct and the AWA-host tissue border zone ($P < 0.05$, n = 3).

DISCUSSION

Given the substantial problem posed by hernia recurrence, with rates approaching 40% in the literature, advances in the field of abdominal wall reconstruction have the potential to impact the care of thousands of patients annually.[2–4] The availability of suitable autologous tissues with the tensile and cellular properties necessary to facilitate healing of the abdominal wall repair and withstand the forces applied to the abdominal wall during the recovery period is generally deficient. This fact is often compounded by a patient population with multiple comorbidities and previous operative procedures, which further compromise available tissues, leading to a postoperative major complication rate approaching 20%.[5] Acellular dermal matrices serve as a regenerative biomaterial scaffold to support host cell integration and collagen remodeling and are used in a variety of reconstructive surgical applications. Acellular dermal matrices have been processed to render the tissues sterile and acellular to facilitate biocompatibility and revascularization. The ADMs for abdominal wall reconstruction has revolutionized the management of complex abdominal wall defects with the provision of an ample tissue source when autologous tissues are insufficient. However, ADMs are derived from dermis and, therefore, have mechanical properties disparate from that of native abdominal wall fascia. In search of a way to replace abdominal wall fascial defects in "like with like" fashion, a variety of autologous approaches using expendable donor fascia through separation of components for local abdominal wall fascia[8,34] or regional flaps using thigh-based fascial flaps[28] have been proposed. However, these techniques are technically demanding, are associated with increased donor site morbidity, and are not always sufficient to restore fascial continuity to the abdominal wall.[35,36]

To this end, we describe and provisionally characterize the utility of human AWA, which consists of the anterior rectus sheath from semilunar line to semilunar line and from xiphoid to pubis, and subjected to tissue processing to render it sterile and acellular. We have demonstrated that our tissue processing technique results in increased tensile strength compared with unprocessed fascia, which is substantiated ultrastructurally with the presence of increased collagen cross-linking. We have performed preclinical characterization in a rat subcutaneous implantation model and have demonstrated that processed acellular AWAs retain tensile strength to a greater degree than unprocessed AWAs and, despite the increased collagen cross-linking and preserved tensile strength, these constructs allow cellular penetration and bio-incorporation.

This study is intended to serve as an initial preclinical evaluation of the biophysical characteristics of AWAs as a potential alternative to other adjuncts available for abdominal wall reconstruction. The tissue procurement and processing described herein result in the procurement of abdominal wall fascial grafts of significant size, 318 cm^2 in surface area, and relatively uniform thickness. The tissue processing described herein, is analogous to tissue processing used for commercially available ADMs (DermACELL, LifeNet Health, Inc, Virginia Beach, Va) with similar reduction in total DNA content and alterations in collagen ultrastructure. Future studies will elucidate the potential of this construct for abdominal wall reconstruction in animal and human models of abdominal wall hernia to determine its bio-incorporation properties, suture pullout strength, and mechanical failure strength before clinical applications.

Limitations of the AWA approach include difficulty associated with procuring the abdominal wall without the creation of fascial defects, in contrast to ADM, which can be procured with the use of a mechanical dermatome. Furthermore, donors must be appropriate as all abdominal walls are not created equal. History of previous abdominal hernia or bulge, history of intra-abdominal surgery, or obesity should be considered contraindications to abdominal wall procurement. The size of the abdominal wall is also limited by the size of the donor, and more importantly, the thickness of the construct is limited by the thickness of the abdominal wall, in contrast to ADM, which can be procured in thicker or thinner fashion to meet specific reconstructive needs. Furthermore, the procurement of AWA fascia is not practical in donors intended for solid intra-abdominal organ donation, because procurement of the fascia would delay procurement of liver, kidney, or pancreas tissues by the organ procurement team.

CONCLUSIONS

Acellular AWAs have potential to serve as a novel reconstructive adjunct for abdominal wall reconstruction with the potential of replacing "like with like" without additional donor site morbidity or antigenicity. There was a decrease in maximum load at failure seen after implantation. Therefore, further testing is required in a clinically relevant model of abdominal wall repair to assess its biomechanical properties after implantation in comparison with existing modalities for abdominal wall reconstruction.

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