Further Evidence that Human Acellular Dermal Matrix Decreases Inflammatory Markers of Capsule Formation in Implant-Based Breast Reconstruction

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Abstract

Background: Human acellular dermal matrix (HADM; previously termed “acellular cadaveric dermis”) may limit inflammatory changes believed to play a role in capsular contracture, a common complication of implant-based breast reconstruction.

Objectives: Differences between HADM and native breast capsule specimens were evaluated by immunohistochemical analysis of key inflammatory markers involved in capsule formation.

Methods: Twenty consecutive patients underwent immediate, 2-stage, implant-based breast reconstruction with dual-plane HADM. During tissue expander–implant exchange, full-thickness biopsies of biointegrated HADM and native breast capsule (internal control) from the tissue-expander envelope were obtained. Immunohistochemical analysis was performed for endothelial cells (CD31), B cells (CD20), T cells (CD3), macrophages (CD68), collagen I and III, and myofibroblasts (α-smooth muscle actin). Observed levels of marker labeling were semiquantitatively scored from 0 (none) to 3 (severe) by a blinded histopathologist and were statistically analyzed with the Wilcoxon rank sum test.

Results: A bilateral sample was obtained from 1 patient; all other samples were unilateral. Compared with capsule samples from native breast tissue, HADM samples had significantly lower levels of all inflammatory markers (P < .001).

Conclusions: These lower levels of inflammatory markers support previous evidence that HADM may inhibit inflammatory and profibrotic signaling characteristics of breast capsule development and decrease the risk of capsular contracture. Further investigation is needed to determine the mechanism by which HADM inhibits these inflammatory cells, whether HADM reduces the incidence of breast capsular contracture, and if so, the longevity of this effect.

Level of Evidence: 3

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The surgical techniques for postmastectomy patients who undergo breast reconstruction vary and are associated with unique profiles of benefits, risks, and complications. A common procedure is 2-stage reconstruction with placement of a tissue expander or implant, with total submuscular coverage. However, in recent years, partial subpectoral implant positioning and lower-pole coverage with human acellular dermal matrix (HADM) has gained acceptance as an alternative to the total submuscular approach. Suturing of HADM to the inferior margin of the pectoralis major, and along the inframammary fold of the chest wall, provides lower-pole coverage of the implant.

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After reconstructive surgery, a thin layer of scar tissue, or capsule, forms around the breast implant as the result of a normal foreign body reaction. However, pathologic capsule development sometimes occurs and is associated with capsular contracture, the most common complication observed with postmastectomy implant-based breast reconstruction. Baker grade III or IV capsular contracture, marked by pain, hardening, and deformity of the breast, is experienced by approximately 16% to 18% of women who undergo implant-based breast reconstruction; rates are substantially higher among women with known risk factors for contracture, such as exposure to radiation therapy. Implant removal and revisional surgery may be required for these patients, some of whom subsequently undergo autologous breast reconstruction. Given these undesirable outcomes, much focus has been placed on understanding the causative mechanisms of capsule formation and contracture.

Clinical observations from tissue expander–implant exchange procedures performed at our institutions have shown that only limited capsule develops over the portion of tissue expander covered with HADM, in contrast to the area covered with native tissue. In a previous study, we hypothesized that HADM (AlloDerm Regenerative Tissue Matrix, LifeCell Corp, Branchburg, NJ; termed “acellular cadaveric dermis” in our original report) may have an inhibitory effect on capsule formation, and examined biopsy specimens from the tissue-expander envelopes from consecutive patients who underwent tissue expander–implant exchange reconstruction with HADM. In that study, histologic examination demonstrated that HADM samples contained significantly lower levels of staining indicative of granulation tissue formation, blood vessel proliferation, capsule fibrosis, and inflammatory infiltration compared with native breast capsule. The goals of the current analysis, based on tissue specimens obtained from the same cohort of patients, were to further examine the presence of inflammatory cells in the peri-implant breast envelope in regions surrounded by native tissue vs HADM and to further evaluate differences between HADM and native breast capsule specimens by immunohistochemical analysis of key inflammatory markers involved in capsule formation.

METHODS

This observational, retrospective, case-control study was conducted in accordance with the guidelines of the Declaration of Helsinki. Institutional review board approval was obtained from Baylor College of Medicine (Houston, TX). Informed consent was obtained from all patients.

Patient Selection and Implant Procedure

Tissue samples were obtained from the same cohort of patients described in our previous report. Consecutive patients with breast cancer who underwent immediate 2-stage tissue expander–implant exchange breast reconstruction were eligible for inclusion; all reconstructions were performed by a single surgeon (C.B.B.) at 1 of 3 hospitals between January 2007 and January 2008. Patients with history of prior implants, implant infection, or requiring breast irradiation post-expander placement were excluded. In all study patients, a sheet of HADM (AlloDerm), measuring 4 × 12 cm or 4 × 16 cm (thickness, 0.79-2.033 mm) was used to cover the inferior pole of a low- or medium-height tissue expander (Mentor, Santa Barbara, CA), which was placed in the partial subpectoral position. During the initial surgery and later tissue expander–implant exchange, the breast pocket, tissue expander, and implant were irrigated with Adams solution (cefazolin 1 g/gentamycin 80 mg/bacitracin 50,000 U in 500 mL normal saline) before placement and suturing. The inferior edge of the HADM was sutured to the chest wall along the existing inframammary fold or a neo-inframammary fold, and the superior edge was positioned under the pectoralis major in the subaxillary space. All patients received antibiotic treatment while the drains were in place. Patients returned 2 to 10 months later to have their tissue expanders exchanged for a permanent saline or silicone gel implant.

Tissue Biopsies

During the tissue expander–implant exchange procedure, full-thickness biopsies of the tissue-expander envelope were obtained from the lower-pole biointegrated HADM and the upper-pole native subpectoral breast capsule (which served as the internal control).

Tissue specimens were fixed immediately in 10% neutral buffered formalin, embedded in paraffin, sectioned (3 µm), and stained with hematoxylin and eosin. Immunohistochemical analysis was performed to determine the presence of the following inflammatory markers: endothelial cells (CD31), B lymphocytes (CD20), T lymphocytes (CD3), macrophages (CD68), collagen I and III, and myofibroblasts (α-smooth muscle actin).

Quantitation and Data Analysis

Biopsy specimens were masked to tissue type (HADM samples vs native breast capsule) and were semiquantitatively scored by an experienced, blinded histopathologist using a 4-point scale (0 = none, 1 = mild, 2 = moderate, and 3 = severe) to denote the observed level of staining for each aforementioned marker. Scores were compared with the Wilcoxon rank sum test, with ties removed. Statistical significance was defined as $P < .05$. 
RESULTS

Patients

Twenty consecutively treated patients were included in the study. The mean age was 47 years (range, 33-64 years) and the mean time to implant exchange (ie, time to biopsy) was 4.38 months (range, 2-10 months). A bilateral tissue sample was obtained from 1 patient; all other tissue samples were unilateral. No patient received neoadjuvant therapy, and no postoperative complications were reported (eg, seroma, infection, or other implant-related problems).

Histopathologic Evaluation

As observed previously,6 the greatest and most consistently observed differences between the 2 types of capsule samples were significantly lower levels of fibroblast cellularity, granulation tissue, blood vessel proliferation, capsule fibrosis, and chronic inflammation in the HADM samples (P < .001). Further evaluation of key inflammatory markers by immunohistochemical labeling demonstrated that HADM samples (vs native capsule) had significantly diminished levels of all assessed inflammatory markers (P < .005; Figure 1). Figure 2 illustrates immunolabeling of each inflammatory marker examined from HADM samples and native breast capsule. Markers for cells important in the inflammatory response (macrophages, T lymphocytes, B lymphocytes, endothelial cells, and myofibroblasts) were significantly lower in HADM samples than in native controls, as were markers of collagen I and III. Although myofibroblasts, collagen III, and B lymphocytes were significantly lower in the HADM samples, the differences were not as significant as for the other variables.

DISCUSSION

Findings of the present study further support the theory that HADM has certain intrinsic properties that may limit capsule formation. Approximately 4 months after expander placement, HADM samples contained significantly less fibroblast activity, collagen I deposition, and capsular fibrosis than native breast capsule samples from the same patient. These results suggest that incorporating HADM may result in decreased capsule formation.

Although the etiology of capsular contracture remains unknown, inflammation has emerged as a common underlying process, pivotal in the development of both normal breast capsule tissue and capsular contracture.7,8 Fluid and tissue samples from the area surrounding breast implants from patients with clinical signs and symptoms of capsular contracture have been found to contain macrophages and a predominance of T lymphocytes, as well as inflammatory leukotrienes.7,9 Other evidence suggests a role for myofibroblasts, which synthesize collagen, in the development of fibrosis and contracture.10,11 In particular, an increased proportion of collagen I vs collagen III is known to increase the formation of fibrous scar tissue.12 The key role of inflammation and myofibroblasts in capsular contraction is further supported by evidence of a beneficial effect of anti-inflammatory compounds—such as prednisolone and the leukotriene inhibitor zafirlukast—for preventing growth of hypertrophic capsule tissue in animal models13,14 and alleviating clinical signs and symptoms of capsular contracture.15

The current analysis showed that HADM samples contained significantly lower levels of all tissue markers of interest, including T and B lymphocytes, macrophages, myofibroblasts, and collagen types I and III. These findings, which are consistent with results of our previous work5 and a study by Stump et al,11 indicate that HADM may inhibit the inflammatory and profibrotic signaling that is characteristic of breast capsule development and may help decrease the risk of capsular contracture. This possibility is further supported by clinical evidence indicating that the risk of capsular contracture is lower in patients who undergo reconstruction that includes HADM.16

The characteristics of capsular tissue that are associated with the clinical signs and symptoms of contracture have not been well defined, and debate continues about the contributory role of capsule thickness and myofibroblasts. Myofibroblasts are considered important in wound and scar contracture, and thus may be a component of capsular contracture in the breast.10,11 In the present study, levels of myofibroblast markers were significantly lower in the HADM samples. However, other investigators have suggested that the role of myofibroblasts may be transient and related to the onset of capsule contracture, thereafter becoming less prominent in the capsular milieu.10 Further research is needed to determine the role of myofibroblasts in the development of capsule contracture.

On a broader level, our understanding of the mechanisms underlying decreased capsule formation with HADM6 may be elucidated by what is known about adult human wound repair vs animal models of scarless healing. As with any type of foreign body reaction, capsular tissue formed around a breast implant is analogous to the formation of fibrous, acellular, avascular scar tissue. Some evidence suggests that slowing or decreasing the magnitude of certain early repair responses may decrease fibrosis.12 For example, experimental models of fetal scarless wound healing showed that the inflammatory response is of lesser magnitude and shorter duration than the response to adult wounds, and is marked by lower numbers of less-differentiated inflammatory cells (monocytes, neutrophils, macrophages).12 The explanted HADM samples in the current study share some of these features, being marked by lower numbers of immune cells, including CD68+ macrophages, and B and T lymphocytes in comparison to native samples. With evidence of a lesser early inflammatory response, as described previously6 and
confirmed here, it follows that downstream wound healing responses such as angiogenesis, granulation, and remodeling that depend on the chemokines, cytokines, and growth factors (eg, vascular endothelial growth factor) secreted by neutrophils, monocytes, and macrophages at the surgical site, also would be diminished. In fact, we observed evidence of less-robust ongoing revascularization, with fewer endothelial cells in HADM samples than in native controls. In turn, with fewer endothelial cells, which secrete basic fibroblast growth factor (crucial to the formation of granulation tissue and a fibrous capsule), we found less fibrous tissue and fewer α-smooth muscle actin myofibroblasts and collagen I/III fibers in the HADM samples.

The differences in inflammatory response between the HADM and native breast capsule samples may be explained by a prolongation of the inflammatory phase in the native tissue that is not present or is less pronounced with HADM. In the current study, biopsy samples were obtained 4 months (on average) after placement of the tissue expander, at which point the wound-healing process is generally in the remodeling phase. The pronounced amount of inflammatory markers in native breast capsule indicates that wound repair was likely still in the inflammatory phase. However, the decreased inflammatory markers in HADM samples suggest that the inflammatory phase may have been completed in these patients, and that wound healing was at a more advanced remodeling phase. In future preclinical investigations, it would be interesting to examine HADM and native breast capsule tissue for a large range of time points, with the goal of characterizing the remodeling stage of repair in normal wound-healing scenarios, which typically is marked by apoptosis and results in a largely fibrous capsule.

A key question raised by the current findings is whether the wound healing, or foreign body response, is simply of lesser magnitude in HADM samples or whether the nature and time course of cellular responses is fundamentally altered. For example, if a low level of inflammatory signaling persists in HADM-associated tissue beyond the time normally seen in capsule formation in the absence of HADM, would it be possible for a fibrous capsule to form, albeit more slowly, over an extended period? Alternatively, if the HADM milieu is largely noninflammatory, would the foreign body reaction be supplanted by a completely different set of repair processes, possibly allowing for regeneration of normal tissue surrounding the implant? Evidence from studies of wound-repair models and capsular contracture cases suggests that in the periprosthetic region, creating or maintaining expression of several factors, including hyaluronan, tumor necrosis factor–stimulated gene 6, and collagen type III (vs type I), may be critical in preventing fibrosis and/or contracture. Further research on the cellular repair responses that occur over time with HADM incorporation into breast reconstruction may focus on describing these and other factors.

**Limitations**

Potential limitations of the current analysis include (1) lack of clinical outcome data (eg, Baker classification) and longitudinal clinical follow-up; (2) use of semiquantitative scoring of immunohistochemical findings; and (3) analysis of only 1 time point (ie, 4 months after HADM and expander placement). However, our goal was to determine further differences between HADM and native breast capsule specimens that occur in the peri-implant breast envelope at a histopathologic and cellular level, rather than add to the numerous published clinical studies that have evaluated HADM and its associated clinical outcomes. Therefore, we
did not assess clinical outcomes (eg, Baker classification) or follow up on the status of implant capsules. Clinical follow-up would be difficult at best because it has been more than 7 years since the patients underwent surgery, and many have been lost to follow-up. Ideally, future analyses should incorporate both clinical and histologic evaluations.

**Figure 2.** Photomicrographs of inflammatory cell markers in HADM samples vs native breast capsule (control). All original magnifications are ×400. (A) CD31 (endothelial cells) in HADM vs (B) control; (C) CD20 (B lymphocytes) in HADM vs (D) control; (E) CD3 (T lymphocytes) in HADM vs (F) control; (G) CD68 (macrophages) in HADM vs (H) control; (I) collagen type I in HADM vs (J) control; (K) collagen type III in HADM vs (L) control; and (M) α-smooth muscle actin (myofibroblasts) in HADM vs (N) control.
Semiquantitative scoring is our preferred method to assess immunohistochemical findings. Although automated image-analysis systems utilizing pixel ratios could have been considered, these systems are not regarded as standard methodology by many pathologists. In diagnostic and research pathology, a standard method is to assess immunohistochemical staining based on a 4-point graded scale, where 0 = no staining, 1 = minimal/mild staining, 2 = moderate staining, and 3 = severe staining. This scale is quantitative, and in the present study it was validated by a
A blinded pathologist with more than 20 years of experience in diagnostic and research pathology. The system was further validated by repeat grading of the study specimens, demonstrating 100% agreement by the blinded pathologist. Moreover, automated image-analysis systems with pixel ratios are fraught with errors that can be avoided by having board-certified experienced pathologists interpret and analyze the immunohistochemical staining results. Immunohistochemical staining of most tissues results in artifactual staining of background tissue cells with particulate debris, including:

- hemosiderin and hematoidin pigment within the cytoplasm of cells,
- cells with a high peroxisome content that cannot be quenched,
- cells that display a low-intensity (blush-like) reactivity,
- cells that react in a nuclear pattern with an antibody that is considered positive only with cytoplasmic reactivity,
- cells that react in a cytoplasmic pattern with an antibody that is considered positive only with nuclear reactivity,
- stromal components that react with antibodies that should react only with cell nuclei or cytoplasm,
- cells that react with antibodies that should react only with stromal components, and
- other cells that crossreact in a nonspecific manner.

These artifacts are counted as pixels by automated-image analysis systems and give spurious results that invalidate data. To eliminate these potential errors, an experienced board-certified pathologist must accurately assess and grade the immunohistochemical staining of human tissue that is diseased or has undergone surgery. In the case of a pure cell culture or pure tissue type, automated computer analysis may be beneficial. The same holds true for histochemical staining, which is fraught with even more problems associated with artifacts and inappropriate interpretation by automated systems.

In regard to follow-up, it would be of interest to examine HADM and native breast capsule tissue for a wide range of time points to determine if the reduction in cellular markers is due to the fact that repopulation of all cells within the collagen scaffold may be reduced initially.

**CONCLUSIONS**

Compared with native breast capsule specimens, HADM samples contained fewer inflammatory cells at the time of tissue expander–implant exchange in patients undergoing breast reconstruction. Further investigation is needed to determine the mechanism by which HADM inhibits these inflammatory cells, whether HADM reduces the incidence of breast capsular contracture, and, if so, the longevity of this effect.

**Disclosures**

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