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REMODELING CHARACTERISTICS AND COLLAGEN DISTRIBUTIONS OF BIOLOGIC SCAFFOLD MATERIALS BIOPSIED FROM POSTMASTECTOMY BREAST RECONSTRUCTION SITES

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Abstract

OBJECTIVE—The study purpose was to evaluate the associations between patient characteristics and the histologic remodeling scores of acellular dermal matrices (ADMs) biopsied from breast reconstruction sites in the first attempt to generate a multivariable risk prediction model of non-constructive remodeling. It was hypothesized that host characteristics and surgical site assessments predict the degree of graft remodeling for ADMs used during breast reconstruction.

METHODS—ADMs were biopsied from the breast reconstruction sites of n=62 patients during a subsequent breast procedure, stained with Hematoxylin and Eosin, and evaluated according to a semi-quantitative scoring system for remodeling characteristics [cell types (CT), cell infiltration (CI), extracellular matrix (ECM) deposition, scaffold degradation (SD), fibrous encapsulation (FE), and neovascularization (NEO)] and a mean composite score (CR). Biopsies were stained with Sirius Red and Fast Green, and analyzed to determine the collagen I:III ratio. Based on univariate analyses between subject clinical characteristics and the histologic remodeling scores, cohort variables were selected for multivariable regression models using a $p < 0.20$.

RESULTS—The CR score model yielded 3 variables: pack-year history, corticosteroid use, and radiation timing ($r^2_{\text{pseudo}}=0.81$). The model for collagen I yielded 2 variables: corticosteroid use and reason for reoperation ($r^2_{\text{pseudo}}=0.78$). The model for collagen III yielded 1 variable: reason for reoperation ($r^2_{\text{pseudo}}=0.35$).

CONCLUSION—These preliminary results constitute the first steps in generating a risk prediction model that predicts the patients and clinical circumstances most likely to experience non-constructive remodeling of biologic grafts used to reconstruct the breast.

Keywords

breast reconstruction; breast augmentation; soft tissue repair; biologic scaffold; acellular dermal matrix; human dermis; tissue remodeling; type I collagen; type III collagen; Sirius Red/Fast Green

INTRODUCTION

The steady increase in post-mastectomy breast reconstruction since 1998 has been driven by prosthetic-based reconstructions.^{1–3,4} Acellular dermal matrices (ADMs) are often used to create an inferolateral sling between the pectoralis major and the inframammary fold during both single-stage and multiple-stage prosthetic breast reconstructions.⁵ Market data suggest that 67.2% of all prosthetic breast reconstructions use ADMs, and 93% of surgeons that

perform at least 25 breast reconstructions of any kind use ADMs during prosthetic breast reconstruction.⁶ These biologic constructs provide scaffolding for neotissue generation, as well as structural strength and shape for the prosthetic breast reconstruction. As the ADM is gradually remodeled by host connective tissue ingrowth, it defines the inframammary fold, stabilizes the position of the pectoralis major by preventing upward migration of the muscle, provides tissue support for the inferior pole of the breast prosthesis, and disperses mechanical forces on the overlying skin envelope.^{5, 7, 8} Potential clinical advantages of ADM reinforcement for prosthetic breast reconstruction include the possibility of single-stage reconstruction, increased initial fill volumes of the implanted prostheses, fewer expansions of the implanted prostheses, decreased incidence of capsular contractures, fewer capsular modifications during second-stage surgeries, decreased rates of revision, and improved aesthetic outcomes.^{9–11}

Quantifying the influence of host comorbidities and treatments on the tissue remodeling of breast reconstruction sites may identify modifiable risk factors for non-constructive tissue remodeling. This information may be used to generate a risk prediction model that reliably predicts the patients and clinical circumstances for which non-constructive remodeling of a breast reconstruction site with ADM reinforcement is most likely to occur. The risk prediction model could inform preoperative patient counseling, and aid surgical decision-making regarding the most appropriate reconstruction options for patients in the context of their clinical circumstances. Therefore, the purpose of this prospective study was to evaluate the multivariable associations of patient characteristics to the histologic remodeling scores of ADMs biopsied from breast reconstruction sites in the first attempt to generate a risk prediction model of non-constructive remodeling. We hypothesized that higher collagen type I:III ratios and more favorable histologic remodeling scores of ADM biopsies would directly correlate with indwelling duration of mesh, and inversely correlate with subject age at the time of mesh implantation, body mass index (BMI) at the time of scaffold implantation, tobacco use, corticosteroid treatment, chemotherapy treatment, and breast radiation therapy. Furthermore, we hypothesized that higher collagen type I:III ratios and more favorable histologic remodeling scores would demonstrate greater association with peripheral biopsies of the ADM sling (superior pole and inferior pole biopsies) compared to central biopsies of the ADM sling.

MATERIALS & METHODS

Patient Selection & Specimen Collection

The study protocol was approved by the Human Research Protection Office (HRPO) at Washington University in St. Louis (Institutional Review Board # 201101959), and meets the corresponding ethical guidelines for human research conduct. The study was also registered with clinicaltrials.gov (identification # NCT01060046). Sixty-two subjects (n=62) with an acellular dermal matrix implanted during a previous breast reconstructive procedure and scheduled for a subsequent breast procedure were identified and consented for the study between August 2007 and February 2013.

Specimens of the ADMs were procured during the subsequent breast procedure. Specimens measuring 1 cm² each were procured from the superior, central, and/or inferior aspects of

the implanted ADM, as well as from the subcapsular breast tissue of each patient (Figure 1). For patients who had undergone bilateral breast reconstruction with ADMs and a subsequent bilateral breast procedure, specimens were obtained from both breasts (subset n=15). All specimens were immediately preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μ m, and stained with Hematoxylin & Eosin (H&E) and Sirius Red/Fast Green (SR/FG).

Remodeling Characteristics

Hematoxylin & Eosin (H&E) stained slides were evaluated for the degree of six remodeling characteristics, including: cellular infiltration, cell types, host extracellular matrix (ECM) deposition, scaffold degradation, fibrous encapsulation, and neovascularization. A single slide of each specimen was evaluated under light microscopy at 100x magnification by a pathologist using a semi-quantitative scoring system adapted from Valentin et al¹² for biologic scaffolds and presented previously.^{13–15} Assigned to each specimen was a single score for each remodeling characteristic ranging in value from 0 to 3, with higher scores representing more favorable remodeling characteristics (Figure 2). A composite remodeling score was then calculated as the mean of the six component remodeling scores for each specimen. Specimens from the subcapsular breast tissue of each patient served to provide the control values for the six component remodeling scores and the composite remodeling score for that patient.

Collagen Distribution

Sirius Red/Fast Green (SR/FG) stained slides were prepared and evaluated according to methods presented previously.^{14–16} In brief, Sirius Red stains collagen fibers, whereas Fast Green stains non-collagenous proteins for contrast. Under polarized light, Sirius Red-stained collagen I fibers appear bright red while Sirius Red-stained collagen III fibers appear pale green. Each slide was photographed under cross-polarized light using an Axioskop 40® microscope (Carl Zeiss®, Thornwood, NY) equipped with a Zeiss Axiocam® at a magnification of 400x (n=10 photographs per specimen). Axiovision 4.7® (Zeiss®) software was utilized to semi-quantitatively evaluate both the areas (μ m²) that appeared red under cross-polarized light (for collagen I) and the areas that appeared green under cross-polarized light (for collagen III) on each slide. A collagen I:III ratio was then calculated. To obtain control values of collagen I and III for a given patient, the collagen I area, the collagen III area, and collagen I:III ratio were calculated from the subcapsular breast tissue specimen of that patient.

Statistical Analysis

Data from the REDCap® study database were securely exported to SAS® version 9.3 (SAS Institute, Inc.®; Cary, North Carolina) to perform all statistical analyses. The following dependent variables were analyzed as continuous variables: composite remodeling score, collagen I area, collagen III area, and collagen I:III ratio. Given the low sample size and sparseness of the data, the following dependent variables with greater than 2 ordinal categories were collapsed based on the data distribution and analyzed as dichotomous variables: cell type score (<2 or >2), cell infiltration score (<3 or $\geq$ 3), ECM deposition score (<3 or $\geq$ 3), scaffold degradation score (<3 or $\geq$ 3), fibrous encapsulation score (<2 or $\geq$ 2),

and neovascularization score (<3 or ≥3). As for the independent variables, breast cancer status, smoking status, corticosteroid therapy, breast radiation therapy history, breast specimen radiation therapy, and indication for subsequent breast procedure at T2 were treated as dichotomous variables; smoking history, chemotherapy, and breast radiation therapy timing were treated as categorical variables; and host age at T1, BMI at T1, duration of *in vivo* scaffold specimen dwelling, and pack-year history were treated as continuous variables.

Data for all continuous variables were assessed for approximate symmetry of distribution before univariate analyses ensued. Continuous cohort characteristics were summarized as mean (standard deviation (SD)) or median (25th percentile (q1), 75th percentile (q3)) in the case of skewed distributions. Trivariate linear or logistic regression models within the generalized linear mixed model framework were implemented.^{17, 18}

Based on linear or logistic regression analyses for each independent variable, predictive variables were selected for multivariable modeling using a threshold $p < 0.20$. The multivariable models were then reduced via backward elimination using a threshold $p < 0.10$. Due to the preliminary nature of the study with the aim of generating scientific hypotheses that will be critically evaluated in future research, liberal significance criteria were used and significance values were not adjusted for multiple comparisons. While there is no universally accepted measure of model fit for generalized linear mixed models, a likelihood ratio-based pseudo- r^2 value was calculated to gauge the goodness-of-fit of the final multivariable models.¹⁹ Continuous dependent variables were analyzed using the Proc MIXED procedure and dichotomous dependent variables were analyzed using the Proc GLIMMIX procedure.

RESULTS

Patient Characteristics and Specimen Characteristics

Under an IRB-approved protocol, ADM specimens were biopsied from the breast reconstruction sites of 62 female subjects at the time of a subsequent breast procedure. As shown in Table 1, the subjects had a mean age at T1 of 49.3 ± 10.9 years (range: 23.8–77.2 years). A total of 48 subjects provided specimens from a unilateral breast and 14 subjects provided specimens from bilateral breasts. Of the female subjects, 52 subjects had been diagnosed with breast cancer in a unilateral breast, 4 subjects had been diagnosed with breast cancer in bilateral breasts, and 6 subjects had never been diagnosed with breast cancer. Indications for ADM implantation were: breast reconstruction following mastectomy for cancer of that breast ($n=44$), or breast reconstruction following prophylactic mastectomy ($n=32$). Of the initial breast reconstruction procedures, all of the surgical sites were clean ($n=76$). Indications for the subsequent breast procedure at the time of specimen collection were: exchange of the tissue expander for a permanent implant ($n=62$), concern for surgical site infection ($n=4$), devascularization of tissue ($n=2$), or other reason ($n=8$). Of these subsequent breast procedures, the surgical sites were clean ($n=73$), clean-contaminated ($n=0$), contaminated ($n=3$), or infected ($n=0$).

Specimens procured for this study were biopsied from the superior (n=76), central (n=42), and inferior (n=76) region of the ADM, and submuscular capsule (n=76) (Figure 1). All of the ADM specimens procured from this sample were LifeCell® Alloderm® Regenerative Tissue Matrix (n=270; LifeCell Corporation®; Branchburg, NJ), a commercially-available cadaveric human dermis allograft. Median (quartile 1, quartile 3) duration of ADM specimen implantation was 231.5 days (155 days, 402 days; range: 56–2129 days). A total of 270 ADM specimens had been in direct contact with a tissue expander of one of the following types while implanted: Allergan® textured tissue expander (n=201; Allergan® Incorporated, Irvine, CA), Allergan® smooth tissue expander (n=0), Mentor® textured tissue expander (n=15; Mentor® Worldwide LLC, Santa Barbara, CA), Mentor® smooth tissue expander (n=38), or other tissue expander type (n=0) [n=16 missing expander type data]. Of the specimens procured, 156 specimens were obtained from a breast that had been diagnosed with breast cancer whereas 114 specimens were obtained from a breast that had never been diagnosed with breast cancer.

Remodeling Characteristics

Global Assessment of Scaffold Remodeling by Biopsy Site—The frequency or mean values for each dependent variable by biopsy site for this cohort are represented in Table 2. The overall difference in mean values for collagen III area and for collagen I:III ratio among the biopsy sites trended toward but did not reach significance, whereas a significant overall difference in mean composite score existed among the biopsy sites (p values not shown; threshold of significance for overall difference in means was $p = 0.05$).

Cell Type Score—The cohort variables whose associations with Cell Type Score were evaluated in multivariable models were subject age at T1 (univariate $p=0.13$), smoking history (univariate $p=0.15$), breast specimen radiation therapy (univariate $p=0.04$), and breast radiation therapy timing (univariate $p=0.03$). Of these four variables, breast specimen radiation therapy remained in the logistic regression model for Cell Type Score after the backward elimination process.

This variable did not differ in its effect across the biopsy locations. Compared to specimens that had never been exposed to radiation therapy while implanted, specimens that had been exposed to radiation therapy while implanted were estimated to have a 4.39 (95% CI 1.10–17.59) times significantly greater odds of having a Cell Type Score ≥ 2 ($p=0.04$) (n=148 specimens from n=58 subjects, of which n=13 subjects contributed specimens from bilateral breasts).

Cell type scores were dichotomized as ≤ 2 and >2 , based on the presence or absence of inflammatory cells (Figure 2). Univariate analyses revealed that subject age upon ADM implantation ($p=0.13$), smoking ($p=0.15$), breast radiation therapy history ($p=0.04$), and radiation timing (univariate $p=0.03$) significantly correlated with the cell type scores. Of these four variables, breast radiation remained in the logistic regression model for cell type score after the backward elimination process. Compared to ADM specimens never exposed to radiation therapy, previously radiated ADM specimens were estimated to have significantly greater odds of having the presence of inflammatory cells ($p=0.04$).

Cell Infiltration Score—The cohort variables whose associations with Cell Infiltration Score were evaluated in multivariable models were subject BMI at T1 (univariate $p=0.11$), chemotherapy (univariate $p=0.05$), corticosteroid therapy (univariate $p=0.08$), breast radiation therapy history (univariate $p<0.01$), breast radiation therapy timing (univariate $p=0.04$), and smoking status (univariate $p=0.11$). Of these five variables, breast radiation therapy history remained in the logistic regression model for Cell Infiltration Score after the backward elimination process.

This variable did not differ in its effect across the biopsy locations. Compared to specimens from subjects who had a positive history of ever receiving radiation therapy to the breast, specimens from subjects who had a negative history of ever receiving radiation therapy to the breast had a 0.18 (95% CI 0.06–0.51) significantly lower odds of having a Cell Infiltration Score <3 ($p<0.01$) ($n=149$ specimens from $n=58$ subjects, of which $n=9$ subjects contributed specimens from bilateral breasts).

When cell infiltration scores were dichotomized to scores <3 and scores ≥ 3 , we were able to distinguish ADM specimens by the presence or absence of cellular penetrance to their center (Figure 2). Note that references to the center pertain to the center of the ADM biopsy specimen, and not necessarily the center of the entire piece of ADM implanted into the subject. Subject BMI at the time of ADM implantation ($p=0.11$), chemotherapy ($p=0.05$), corticosteroid therapy ($p=0.08$), breast radiation therapy history ($p<0.01$), radiation timing ($p=0.04$), and smoking ($p=0.11$) were found to significantly correlate with cell infiltration scores from the histologic analysis. Of these five variables, breast radiation therapy history remained in the logistic regression model for cell infiltration score after the backward elimination process.

Extracellular Matrix Deposition Score—The cohort variables whose associations with ECM Deposition Score were evaluated in multivariable models were subject BMI at T1 (univariate $p=0.09$), breast radiation therapy history (univariate $p=0.09$), breast specimen radiation therapy (univariate $p=0.16$), chemotherapy (univariate $p=0.01$), and corticosteroid therapy (univariate $p=0.13$). Of these five variables, chemotherapy remained in the logistic regression model for ECM Deposition Score after the backward elimination process.

This variable did not differ in its effect across the biopsy locations. Compared to specimens from subjects who had received chemotherapy while the mesh was implanted, specimens from subjects who had received chemotherapy while the mesh was not implanted had a significantly lower odds of having an ECM Deposition Score <3 (OR=0.21; 95% CI 0.05–0.96) ($p=0.04$). Compared to specimens from subjects who had received chemotherapy while the mesh was implanted, specimens from subjects who had never received chemotherapy had a significantly lower odds of having an ECM Deposition Score <3 (OR=0.22; 95% CI 0.08–0.67) ($p<0.01$). Compared to specimens from subjects who had received chemotherapy while the mesh was not implanted, specimens from subjects who had never received chemotherapy did not demonstrate a statistically significant different odds of having an ECM Deposition Score <3 ($p=0.96$) ($n=136$ specimens from $n=53$ subjects, of which $n=11$ subjects contributed specimens from bilateral breasts).

Scaffold Degradation Score—The cohort variables whose associations with Scaffold Degradation Score were evaluated in multivariable models were subject age at T1 (univariate $p=0.15$), duration of scaffold indwelling (univariate $p=0.09$), smoking history (univariate $p=0.09$), smoking status (univariate $p=0.07$), and chemotherapy (univariate $p=0.06$). Of these five variables, subject age at T1 and smoking history remained in the logistic regression model for Scaffold Degradation Score after the backward elimination process (Table 3).

Neither of these two variables differed in its effect across the biopsy locations. Controlling for smoking history, a one-year increase in the subject age at T1 was associated with an increased odds for a higher Scaffold Degradation Score (OR=1.04; 95% CI 0.99–1.08) ($p=0.10$). Compared to specimens from subjects who had a positive smoking history, specimens from subjects who had a negative smoking history had a significantly higher odds of having a Scaffold Degradation Score <3 (OR=2.84; 95% CI 0.99–8.11) ($p=0.05$), controlling for subject age at T1. Data were analyzed from $n=149$ specimens from $n=58$ subjects, of which $n=13$ subjects contributed specimens from bilateral breasts).

Fibrous Encapsulation Score—Among the specimens with data available for the Fibrous Encapsulation Score ($n=149$ from $n=58$ subjects, of which $n=13$ subjects contributed specimens from bilateral breasts), 147 specimens had a Fibrous Encapsulation Score=3, 1 specimen had a Fibrous Encapsulation Score=2, and 1 specimen had a Fibrous Encapsulation Score=0. The sparseness of the data distribution for the Fibrous Encapsulation Score precluded further univariate analyses and multivariable modeling.

Neovascularization Score—The cohort variables whose associations with Neovascularization Score were evaluated in multivariable models were smoking history (univariate $p=0.06$), smoking (univariate $p=0.05$), chemotherapy (univariate $p=0.01$), corticosteroid therapy (univariate $p=0.09$), breast radiation therapy history (univariate $p=0.01$), breast specimen radiation therapy (univariate $p=0.03$), and breast radiation therapy timing (univariate $p=0.04$). Of these seven variables, smoking history, breast radiation therapy history, and chemotherapy remained in the logistic regression model for Neovascularization Score after the backward elimination process (Table 4).

None of these three variables differed in its effect across the biopsy locations. Compared to specimens from subjects who had a positive history of ever being a tobacco smoker, specimens from subjects who had a negative history of ever being a tobacco smoker were estimated to have a significantly greater odds of having a Neovascularization Score <3 (OR=3.12; 95% CI 1.20–8.12) ($p=0.02$), controlling for breast radiation therapy history and chemotherapy. Compared to specimens from subjects who had a positive history of ever having radiation therapy to the breast, specimens from subjects who had a negative history of ever having radiation therapy to the breast were estimated to have a 0.31 (95% CI 0.10–0.90) times significantly lower odds of having a Neovascularization Score <3 ($p=0.03$), controlling for smoking history and chemotherapy. Compared to specimens from subjects who had received chemotherapy while the mesh was implanted, specimens from subjects who had received chemotherapy while the mesh was not implanted had a significantly lower odds of having a Neovascularization Score <3 (OR=0.26; 95% CI 0.07–0.93) ($p=0.04$),

controlling for smoking history and breast radiation therapy history. The distinguishing histologic feature of a neovascularization score <3 was the absence of blood vessels penetrating the center of the ADM specimen (Figure 2). Compared to specimens from subjects who had received chemotherapy while the mesh was implanted, specimens from subjects who had never received chemotherapy had a significantly lower odds of having a Neovascularization Score <3 (OR=0.45; 95% CI 0.16–1.23) or failure of vascular penetrance to the center of the ADM specimen, while controlling for smoking history and breast radiation therapy history ($p=0.07$). Compared to specimens from subjects who had received chemotherapy while the mesh was not implanted, specimens from subjects who had never received chemotherapy did not have a significantly different odds of having a Neovascularization Score <3 ($p=0.81$), controlling for smoking history and breast radiation therapy history. Data were analyzed from $n=136$ specimens from $n=53$ subjects, of which $n=12$ subjects contributed specimens from bilateral breasts).

Composite Remodeling Score—The cohort characteristics whose associations with Composite Remodeling Score were evaluated in multivariable models were pack-year history (F-test, 1df, univariate $p<0.01$), breast radiation therapy timing (F-test, 6df, univariate $p=0.02$), and corticosteroid therapy (F-test, 1df, univariate $p<0.01$). The model selection process for Composite Remodeling Score yielded a model with all of these variables: breast radiation therapy timing (F-test, 6df, $p=0.06$), pack-year history (F-test, 1df, $p=0.09$), and corticosteroid therapy (F-test, 1df, $p<0.01$) (Table 5).

Separate estimates are reported for breast radiation therapy timing, due to statistically discernible differences in the effect across the three biopsy locations (F-test, 4df, $p=0.08$, respectively). Pack-year history was significantly related to Composite Remodeling Score in all three biopsy locations. Controlling for breast radiation therapy timing, and corticosteroid therapy, a one-year increase in the pack-year history of the subject was associated with a 0.01 unit significant decrease in the Composite Remodeling Score (F-test, 1df, $p=0.09$). Breast radiation therapy timing was significantly related to Composite Remodeling Score only for specimens from the superior and central biopsy locations. In the superior biopsy location, specimens from breasts that had been exposed to radiation therapy more than 180 days prior to procurement of the mesh specimen were associated with mean Composite Remodeling Scores 0.62 units significantly lower than specimens from breasts that had been exposed to radiation therapy less than or equal to 180 days prior to procurement of the mesh specimen (F-test, 1df, $p=0.01$), controlling for pack-year history, and corticosteroid therapy. In the superior biopsy location, specimens from breasts that had not been exposed to radiation therapy were associated with mean Composite Remodeling Scores 0.63 units significantly higher than specimens from breasts that had been exposed to radiation therapy more than 180 days prior to procurement of the mesh specimen (F-test, 1df, $p<0.01$), controlling for pack-year history, and corticosteroid therapy. In the central biopsy location, specimens from breasts that had not been exposed to radiation therapy were associated with mean Composite Remodeling Scores 0.37 units significantly higher than specimens from breasts that had been exposed to radiation therapy more than 180 days prior to procurement of the mesh specimen (F-test, 1df, $p=0.04$), controlling for pack-year history, and corticosteroid therapy. Corticosteroid therapy was significantly related to Composite

Remodeling Score in all three biopsy locations. Mesh specimens exposed to corticosteroid therapy were associated with Composite Remodeling Scores 1.04 units significantly lower across all three biopsy locations compared to mesh specimens that were never exposed to corticosteroid therapy (F-test, 1df, $p<0.01$), controlling for pack-year history, and breast radiation therapy timing. Note that the sample contained specimens from 5 breasts among 4 patients who received corticosteroid therapy while the mesh was implanted; thus, the results of analyses with the corticosteroid therapy variable must be interpreted with caution. The pseudo- r^2 value for the Composite Remodeling Score model was 0.81, indicating moderate predictive capability for this model (n=168 specimens from n=55 subjects, of which n=11 subjects contributed specimens from bilateral breasts).

Mean Collagen I Area—The cohort variables whose associations with mean collagen I area were evaluated in multivariable models were BMI at T1 (F-test, 1 df, univariate $p=0.19$), smoking history (F-test, 1df, univariate $p=0.10$), chemotherapy (F-test, 2df, univariate $p=0.01$), corticosteroid therapy (F-test, 1df, univariate $p=0.01$), breast radiation therapy history (F-test, 1 df, univariate $p=0.03$), and indication for subsequent breast surgery (F-test, 1df, univariate $p=0.09$). Of these six variables, only corticosteroid therapy (F-test, 1df, $p=0.01$) and indication for subsequent breast surgery (F-test, 1df, $p=0.03$) remained in the multivariable model for mean collagen I area after the backward elimination process (Table 6).

Neither of these two variables differed in its effect across the biopsy locations. Specimens from breasts that were exposed to corticosteroid therapy while the mesh was implanted were estimated to have a mean collagen I area 5990.81 units significantly lower than specimens from breasts that were never exposed to corticosteroid therapy while the mesh was implanted, controlling for the indication for the subsequent breast surgery. Note that the sample contained specimens from 5 breasts among 4 patients who received corticosteroid therapy while the mesh was implanted; thus, the results of analyses with the corticosteroid therapy variable must be interpreted with caution. Specimens from breasts for which the reported reason for the subsequent breast surgery was infection, tissue devascularization, or other reason were estimated to have a mean collagen I area 2755.04 units lower compared to specimens from breasts for which the reported reason for the subsequent breast surgery was exchange of the tissue expander for a permanent implant, controlling for corticosteroid therapy. Note that the sample contained specimens from 14 breasts among 10 patients for whom the indication for the subsequent breast surgery was infection, tissue devascularization, or other reason; thus, the results of analyses with the indication for subsequent breast surgery variable must be interpreted with caution. The pseudo- r^2 value for the mean collagen I area model was 0.78, indicating moderate predictive capability for this model (n=131 specimens from n=40 subjects, of which n=11 subjects contributed bilateral specimens).

Mean Collagen III Area—The cohort variables whose associations with mean collagen III area were evaluated in multivariable models were breast cancer status (F-test, 1df, univariate $p=0.13$), breast specimen radiation therapy (F-test, 1df, univariate $p=0.15$), and indication for subsequent breast surgery (F-test, 1df, univariate $p=0.12$). Of these variables, indication

for subsequent breast surgery (F-test, 1df, $p=0.07$) remained in the model for mean collagen III area after backward elimination process (Table 7).

This variable did not differ in its effect across the biopsy locations. Specimens from breasts for which the reported reason for the subsequent breast surgery was infection, tissue devascularization, or other reason were estimated to have mean collagen III area scores 1016.15 units significantly lower than specimens from breasts for which the reported reason for the subsequent breast surgery was exchange of the tissue expander for a permanent implant. Note that the sample contained specimens from 14 breasts among 10 patients for whom the indication for the subsequent breast surgery was infection, tissue devascularization, or other reason; thus, the results of analyses with the indication for subsequent breast surgery variable must be interpreted with caution. The pseudo- r^2 value for the mean collagen III area model was 0.35, indicating poor predictive capability for this model ($n=158$ specimens from $n=48$ subjects, of which $n=13$ subjects contributed bilateral specimens).

Mean Collagen I:III Ratio—The collagen I:III ratio was analyzed on the natural log scale. The cohort variables whose associations with mean collagen I:III ratio were evaluated in multivariable models were pack-year history (F-test, 1df, univariate $p=0.17$), and duration of scaffold indwelling (F-test, 1df, univariate $p=0.17$). Neither of these two variables remained in the model for mean collagen I:III ratio after the backward elimination process ($n=129$ specimens from $n=39$ subjects, of which $n=11$ subjects contributed bilateral specimens).

DISCUSSION

Non-constructive tissue remodeling of postmastectomy breast reconstruction sites may lead to reconstruction complications and failures. Complications of breast reconstruction can delay adjuvant therapy due to the presence of nonhealing wounds, and may require hospital readmissions, pharmacotherapy, and additional procedures. For these reasons, complications of postmastectomy breast reconstruction can have significant implications for the timing, effectiveness, and overall cost of a patient's breast cancer treatment.

Inadequate tissue perfusion remains the primary etiology of most postmastectomy breast reconstruction complications. Previous studies have underscored the importance of the host biologic response at the interface between the prosthetic surface and the breast tissue to constructive tissue remodeling following prosthetic breast reconstruction.²⁰ Evidence further suggests that the host biologic response to acellular dermal matrix (ADM) reinforcement influences the potential for breast reconstruction success.^{5, 21–30} Currently, the literature lacks a risk prediction model that reliably predicts the patients and clinical circumstances for which non-constructive remodeling of a postmastectomy breast reconstruction site with ADM reinforcement is most likely to occur. The ability to preoperatively predict the extent of neovascularization and constructive tissue remodeling in the ADMs used for prosthetic breast reconstruction would be of great potential value in surgical decision-making for postmastectomy patients.

After their cell type scores were dichotomized to ≤ 2 and >2 (Figure 2), ADMs that were radiated during their implantation were noted to have significantly higher odds of containing inflammatory cells in the biopsied specimens ($p=0.04$). At the doses used for cancer therapy, radiation has a pro-inflammatory effect,³¹ which may disrupt normal ADM remodeling and affect its integration with a mastectomy flap. Moreover, specimens harvested from patients that had never had radiation to their breast were characterized by significantly greater cellular penetrance, or infiltration into the center of ADM biopsies ($p<0.01$). While studies examining a dose-response relationship between radiation and ADM cellular infiltration are not readily available, this relationship has significant clinical implications. We previously reported less ADM integration in patients exposed to post-mastectomy radiotherapy.¹⁵ These data, however, also suggest that patients radiated prior to ADM insertion are also at increased risk of poor cellular penetrance when an ADM is used. This is a common scenario in patients who have had breast conservation therapy, recur, and then require mastectomy with reconstruction. Our data indicate that exposure of the implanted ADM scaffold to chemotherapy is associated with less uniform ECM deposition. Previous *in vitro* studies suggest that chemotherapeutic agents used in breast cancer impact ECM protein expression as a mechanism for limiting tumor metastasis.³² The effect of chemotherapy on ECM deposition may alter subsequent ADM remodeling when used for prosthetic breast reconstruction and translate to limited clinical efficacy.

Increased age at the time of ADM implantation was associated with scaffold degradation. That is, the older the patient at the time of ADM implantation, the more likely that the original ADM was near-completely degraded. While other studies looking at the impact of age on scaffold degradation and ADM remodeling are lacking, a reduction in cellular proliferation, and in particular fibroblasts,³³ have been shown to be age-related and clinically, advanced age is a risk factor for ADM explantation when used in prosthetic breast reconstruction.³⁴ Not surprisingly, our data suggest that a history of smoking is associated with less extensive bioabsorption and constructive remodeling of the ADM. Smoking limits mastectomy skin flap perfusion, and is associated with an increased rate of breast reconstruction failure with tissue expanders overall.^{28, 35, 36} Interestingly, our data also demonstrated more widespread ADM scaffold vascularity in smokers. We speculate that an increase in neovascularization of the ADM in smokers may be a compensation mechanism for diminished oxygen carrying capacity.

Consistent with our previous findings,¹⁵ radiation was also associated with less uniform vascular penetrance of the ADM scaffolds. The findings of other studies on the impact of radiation on angiogenesis are variable, with some showing an increase in neovascularization, and others a decrease.³⁷ Chemotherapy, administered specifically during the period of ADM implantation, limited vascular penetrance into the scaffold. Previous work demonstrates that taxanes and anthracyclines can downregulate vascular endothelial growth factor and other signaling molecules and may explain a reduction in angiogenesis.^{38–41} Taken together, our data indicates that radiation and chemotherapy can impair vascular penetrance of an implanted ADM scaffold in breast reconstruction.

The composite remodeling score, derived from the other six parameters used to assess constructive remodeling of the ADM, was impacted by several variables. Interestingly, this

score was the only dependent variable for which the effect differed by biopsy location. As the time interval from conclusion of radiation to biopsy increased, there was less constructive remodeling of the scaffold biopsied from the superior and central ADM sling. It is possible that a more tenacious fibrotic response with time since the radiation exposure may influence the potential for tissue remodeling. Although the results must be interpreted with caution due to limited sample sizes, specimens exposed to smoking ($p=0.09$) or corticosteroid therapy ($p<0.01$) were associated with less favorable composite remodeling scores controlling for other associations when compared to specimens that did not have these exposures. These data indicate that smoking and corticosteroid exposure are independently associated with less host tissue ingrowth into the ADM. This is consistent with angiogenic impairment in smokers,⁴² and the antiproliferative properties of corticosteroids.⁴³

The quantities of both Type I and III collagen were significantly reduced in the context of corticosteroid administration during the period of ADM implantation. Type I collagen fibers have greater tensile strength and are prominent in remodeled scar tissue. By contrast, Type III collagen fibers have less tensile strength, are produced by immature fibroblasts, and are found in a higher proportion in the earlier phases of scar remodeling. Several studies confirm the downregulation of collagen deposition in the context of corticosteroid administration.^{44–46,47} Importantly, ADM biopsies demonstrating reduced deposition of both Type I and III collagen occurred more frequently in the context of an implant exchange performed to manage a complication like wound necrosis or infection rather than for an uncomplicated implant exchange. These data suggest an association between the degree of Type I and III collagen deposition in the scaffold and complications in ADM-assisted staged prosthetic breast reconstructions. Moreover, our findings of a link between poor ADM remodeling and clinical outcomes supports a recently published randomized prospective trial stating that “poor acellular dermal matrix integration is associated with an increased risk of infection”.^{48, 49}50–52

All specimens except those from 10 patients (14 breasts) were procured during the routine exchange of tissue expanders for permanent implants. Therefore, the data contributed by these specimens were not likely biased toward non-constructive remodeling. No clinical evidence of surgical site contamination or infection was present at the time of ADM implantation for any of the study subjects. However, specimens were procured from 3 breasts with clinical evidence of surgical site contamination at the time of ADM biopsy. For specimens from these 3 breasts, the presence of pathogens during the period of ADM indwelling may have led to a local inflammatory response that biased the histologic results toward non-constructive remodeling.

These preliminary results are the first steps in generating a risk prediction model that reliably predicts the patients and clinical circumstances for which non-constructive remodeling of a postmastectomy breast reconstruction site with ADM reinforcement is most likely to occur. Ultimately, this risk prediction model will be further developed, validated, and applied to predict the patients most likely to experience breast reconstruction failure and the mean time to reconstruction failure.

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Figure 1.
Diagram of acellular dermal matrix biopsy sites following postmastectomy breast reconstruction. [Diagram courtesy of Johnny P. Costello.]

Score	0	1	2	3
Cell Type	Inflammatory cells present, no fibroblasts present	Primarily inflammatory cells, few fibroblasts present	Primarily fibroblasts, few inflammatory cells present	Fibroblasts only, no inflammatory cells present
Cellular Infiltration (inflammatory cells include neutrophils, macrophages, and foreign body giant cells)	Zero cells in contact with scaffold	Cells contact periphery, but do not infiltrate the scaffold	Cells infiltrate scaffold, but none reach scaffold center	Cells penetrate into the scaffold center
Extracellular Matrix (ECM) Deposition	No host ECM deposition	Host ECM deposited at scaffold periphery	Host ECM deposited inside scaffold, but not the scaffold center	Host ECM deposited into scaffold center
Scaffold Degradation	Original scaffold intact; borders remain clearly demarcated	Scaffold partially degraded; layers separated by cells, blood vessels, host tissue, etc.	Scaffold extremely degraded; difficult to distinguish scaffold from host tissue	Scaffold completely degraded; no evidence of original scaffold
Fibrous Encapsulation	Extensive fibrous encapsulation (50-100% of scaffold periphery)	Moderate fibrous encapsulation (25-49% of scaffold periphery)	Mild fibrous encapsulation (0<x<25% of scaffold periphery)	No fibrous encapsulation (0% of scaffold periphery)
Neovascularization	Zero blood vessels present	Vessels present to mesh periphery only, no penetration into scaffold	Vessels infiltrate scaffold, but none reach the scaffold center	Vessels penetrate into scaffold center

Figure 2.
Histologic semi-quantitative scoring system for biologic scaffold remodeling.

Table 1**Overall Cohort Variables.**

	Descriptive Statistic
Cell Types Score >2, N (%) *	113 (44.7)
Cell Infiltration Score 3, N (%) *	218 (85.8)
Extracellular Matrix Deposition Score 3, N (%) *	207 (81.5)
Scaffold Degradation Score 3, N (%) *	147 (57.9)
Neovascularization Score 3, N (%) *	179 (70.5)
Composite Remodeling Score, mean (SD) *	2.69 (0.35)
Collagen I Area, mean (SD) *	7145.3 (4550.2)
Collagen III Area, mean (SD) *	3207.4 (3012.7)
Collagen I:III, mean (SD) [natural log scale] *	1.13 (1.32)
Age at Time of Mesh Implantation, years, mean (SD)	49.3 (10.9)
BMI at Time of Mesh Implantation, mean (SD)	27.6 (5.9)
Mesh Indwelling Duration, days, median (q1, q3)	231.5 (155, 402)
Smoking History, N (%)	22 (36.1)
Smoking Status, N (%)	
Never Smoked	39 (63.9)
Quit 30 Days Before Mesh Explantation	16 (26.3)
Current Smoker	6 (9.8)
Pack-Year History, median (q1, q3)	0 (0, 5)
History of Breast Cancer, N (%)	56 (90.3)
Breast Radiation Therapy History, N (%)	21 (33.9)
Breast Specimen Radiation Therapy, N (%) §	12 (15.8)
Breast Radiation Therapy Timing, N (%) §	
Breast Did Not Receive Radiation §	60 (81.1)
Last Radiation Treatment 180 Days Prior to Mesh Explantation §	7 (9.4)
Last Radiation Treatment >180 Days Prior to Mesh Explantation §	7 (9.4)
Chemotherapy, N (%)	
Never Received Chemotherapy	24 (42.1)
Received Chemotherapy While Mesh Not Implanted	10 (17.5)
Received Chemotherapy While Mesh Implanted	23 (40.4)
Corticosteroid Therapy, N (%)	4 (6.9)
Tissue Expander Type, N (%)	
Smooth	9 (15.3)
Textured	50 (84.7)
Indication for Subsequent Breast Surgery, N (%)	
Exchange of Tissue Expander for Permanent Implant	52 (83.9)
Infection, Devascularized Tissue, or Other	10 (16.1)
Gross Appearance to Surgeon at Time of Biopsy	
Not At All or Minimally Incorporated into Host Tissue	4 (7.4)

	Descriptive Statistic
Mostly Incorporated into Host Tissue	10 (18.5)
Fully Incorporated into Host Tissue	40 (74.1)

The * denotes variable descriptive statistics provided at the observation (biopsy) level; whereas the $\bar{s}$ denotes variable descriptive statistics provided at the breast level. All other variable descriptive statistics are provided at the subject level.

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Table 2a

Score Frequencies of Ordinal Dependent Variables by Biopsy Site; n (%).

Dependent Variable	Superior Biopsy (n=58 specimens from 62 subjects)				Central Biopsy (n=34 specimens from 62 subjects)				Inferior Biopsy (n=57 specimens from 62 subjects)			
	0	1	2	3	0	1	2	3	0	1	2	3
Cell Type Score*	0	1 (1.4)	48 (68.6)	21 (30)	0	0	27 (64.3)	15 (35.7)	0	2 (2.9)	43 (61.4)	25 (35.7)
Cell Infiltration Score	1 (1.4)	2 (2.8)	13 (18.3)	55 (77.5)	0	0	11 (26.2)	31 (73.8)	0	1 (1.4)	5 (7.2)	64 (91.4)
ECM Deposition Score	1 (1.4)	6 (8.5)	12 (16.9)	52 (73.2)	0	2 (4.7)	12 (28.6)	28 (66.7)	1 (1.4)	2 (2.9)	7 (10)	60 (85.7)
Scaffold Degradation Score	2 (2.8)	11 (15.5)	27 (38.0)	31 (43.7)	0	8 (19.0)	13 (31.0)	21 (50.0)	1 (1.4)	7 (10.0)	18 (25.7)	44 (62.9)
Fibrous Encapsulation Score	0	0	0	71 (100)	0	0	1 (2.4)	41 (97.6)	1 (1.4)	0	0	69 (98.6)
Neovascularization Score	1 (1.4)	7 (9.9)	25 (35.2)	38 (53.5)	0	3 (7.1)	17 (40.5)	22 (52.4)	1 (1.4)	4 (5.7)	10 (14.3)	55 (78.6)

* n=57 specimens from 62 subjects for Cell Type Score at the Superior Biopsy site.

Table 2b

Mean Values of Continuous Dependent Variables by Biopsy Site.

Dependent Variable	Superior Biopsy (n=76 specimens from 62 subjects)		Central Biopsy (n=42 specimens from 62 subjects)		Inferior Biopsy (n=76 specimens from 62 subjects)	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Composite Remodeling Score	2.61	(0.37)	2.60	(0.37)	2.72	(0.40)
Collagen I Area	7714.36	(5060.98)	9077.36	(3694.54)	6621.54	(4498.98)
Collagen III Area	2536.15	(2584.99)	3959.37	(3111.54)	3049.82	(3120.36)
Collagen I:III Ratio [natural log scale]	1.50	(1.34)	1.08	(1.23)	1.08	(1.43)

Table 3

Multivariable Associations Between Cohort Characteristics and Scaffold Degradation Score <3 (n=149 specimens from n=58 subjects).

Central Biopsy*			
Effect	OR Estimate	95% CI	P-value
Age at T1, years	0.97	0.93–1.01	0.10
Smoking History			
Positive History	(reference)	-	-
Negative History	2.84	0.99–8.11	0.05

* An estimate being displayed only for the central biopsy location indicates a common effect estimated across all three biopsy locations.

Table 4

Multivariable Associations Between Cohort Characteristics and Neovascularization Score <3 (n=136 specimens from n=53 subjects).

Effect	Central Biopsy *		
	OR Estimate	95% CI	P-value
Smoking History			
Positive History	(reference)		
Negative History	3.12	1.20–8.12	0.02
Breast Radiation Therapy History			
Positive History	(reference)		
Negative History	0.31	0.10–0.90	0.03
Chemotherapy			
Never Received Chemotherapy	0.45	0.16–1.23	-
Received Chemotherapy While Mesh Not Implanted	0.26	0.07–0.93	0.04
Received Chemotherapy While Mesh Implanted	(reference)		

* An estimate being displayed only for the central biopsy location indicates a common effect estimated across all three biopsy locations.

Table 5

Multivariable Associations Between Cohort Characteristics and Composite Remodeling Score (n=168 specimens from n=55 subjects).

Effect	Central Biopsy*		Superior Biopsy		Inferior Biopsy	
	Estimate (Standard Error)	P-value	Estimate (Standard Error)	P-value	Estimate (Standard Error)	P-value
Pack-Year History, pack-years	-0.01 (0.01)	0.09				
Breast Radiation Therapy Timing [§]		-				
Breast Did Not Receive Radiation Therapy	0.12 (0.14)	0.43	0.01 (0.16)	0.96	0.01 (0.12)	0.97
Last Radiation Treatment 180 Days Prior to Mesh Biopsy	(reference)		(reference)		(reference)	
Last Radiation Treatment > 180 Days Prior to Mesh Biopsy	-0.26 (0.20)	0.22	-0.62 (0.23)	0.01	-0.17 (0.16)	0.34
Corticosteroid Therapy						
Did Not Receive Corticosteroid Therapy While Mesh Implanted	(reference)					
Received Corticosteroid Therapy While Mesh Implanted	-1.04 (0.26)	<0.01				

* An estimate being displayed only for the central biopsy location indicates a common effect estimated across all three biopsy locations.

[§] The comparison of the “Breast Did Not Receive Radiation Therapy” and “Last Radiation Treatment > 180 Days Prior to Mesh Biopsy” categories resulted in estimates (standard error) and p-values equal to 0.3711 (0.1650), p-value=0.0380; 0.6252 (0.1781), p-value=0.0012; and 0.1693 (0.1219), p-value=0.2024 for the Central, Superior, and Inferior biopsy locations, respectively.

Table 6

Multivariable Associations Between Cohort Characteristics and Mean Collagen I Area (n=131 specimens from n=40 subjects).

Central Biopsy*		
Effect	Estimate (Standard Error)	P-value
Corticosteroid Therapy		
Did Not Receive Corticosteroid Therapy While Mesh Implanted	(reference)	
Received Corticosteroid Therapy While Mesh Implanted	−5990.81 (2174.65)	<0.01
Indication for Subsequent Breast Surgery		
Exchange of Tissue Expanders for Permanent Implants	(reference)	
Infection, Devascularized Tissue, and Other	−2755.04 (1182.60)	0.03

* An estimate being displayed only for the central biopsy location indicates a common effect estimated across all three biopsy locations.

Table 7

Multivariable Associations Between Cohort Characteristics and Mean Collagen III Area (n=158 specimens from n=48 subjects).

Central Biopsy*		
Effect	Estimate (Standard Error)	P-value
Indication for Subsequent Breast Surgery		
Exchange of Tissue Expanders for Permanent Implants	(reference)	
Infection, Devascularized Tissue, and Other	-1016.15 (543.19)	0.07

* An estimate being displayed only for the central biopsy location indicates a common effect estimated across all three biopsy locations.