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Breast

# Acellular Dermal Matrix Sterility: Does It Affect Microbial and Clinical Outcomes Following Implantation?

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**Introduction:** The use of acellular dermal matrices (ADMs) in breast reconstruction is a controversial topic. Recent literature has investigated the effects of ADM sterilization on infectious complications, although with varying conclusions. Previous work by our group showed no difference between aseptic and sterilized products immediately out of the package. In this study, we investigate the microbiologic profiles of these agents after implantation.

**Methods:** In this prospective study, we cultured samples of ADM previously implanted during the first stage of tissue expander-based immediate breast reconstruction. A 1 cm2 sample was excised during the stage II expander–implant exchange procedure, and samples were incubated for 48 hours in tryptic soy broth. Samples with growth were further cultured on tryptic soy broth and blood agar plates. Patient records were also analyzed, to determine if ADM sterilization and microbial growth were correlated with infectious complications.

**Results:** In total, 51 samples of ADM were collected from 32 patients. Six samples were from aseptic ADM (AlloDerm), 27 samples were from ADM sterilized to 10–3 (AlloDerm Ready-to-Use), and 18 samples were from products sterilized to 10–6 (AlloMax). No samples demonstrated bacterial growth. Only 5 patients experienced postoperative complications, of whom only 1 patient was infectious in nature. We failed to demonstrate a statistically significant correlation between sterility and postoperative complications.

**Conclusions:** Our findings showed no difference in microbial presence and clinical outcomes when comparing ADM sterility. Furthermore, no samples demonstrated growth in culture. Our study brings into question the necessity for terminal sterilization in these products. *(Plast Reconstr Surg Glob Open 2019;7:e2355; doi: 10.1097/GOX.0000000000002355; Published online 7 August 2019.)*

## INTRODUCTION

In a recent study, which polled plastic surgeons who perform breast reconstruction, over 84% of participants stated that they utilize acellular dermal matrices (ADMs) in their practice.[1] Those who do use ADM when performing alloplastic breast reconstruction are provided the benefits of better inframammary fold definition, improved cosmesis, reduced rates of capsular contracture, greater intraoperative fill, and fewer postoperative fills and decreased amount of time needed to reach the second stage of breast reconstruction.[2–4] Unfortunately, concerns have remained among plastic surgeons regarding the risks associated with ADM use, notably the increased risks of seroma and infection. In fact, 70% of plastic surgeons believe that ADM use increases seroma rates, whereas 16% believe that it is associated with increased risk for surgical site infections.[1] There have been several studies on this topic, some of which have confirmed these fears,[2,5–7] whereas others have failed to show any significant difference.[8,9]

In an effort to reduce the risk of infection with these products, several ADM producing biomedical companies began to introduce sterilized materials. Previously, ADMs

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were aseptically processed, an approach which limits the contamination from the environment. To avoid the damages that may occur with terminal sterilization, aseptic processing relies upon washing with detergents and antibiotics, alongside the decellularization process. This type of processing typically confers a sterility assurance level (SAL) of $10^{-3}$. Terminal sterilization is usually accomplished by using gamma irradiation or steam sterilization and confers an SAL of $10^{-6}$.

Previous work by Mendenhall et al[10] has demonstrated the ill effects of terminal sterilization. Specifically, collagen fibers within the acellular dermal matrix tend to show a higher degree of disorganization when viewed under electron microscopy. In terms of the clinical benefits of utilizing terminal sterilization, the literature is mixed, with some studies showing decreased rates of infection[11] and others showing either no difference[12] or even increased rate of infection and seroma.[13,14]

In an earlier study performed by our group, we investigated the in vitro effects of sterilization when compared with aseptic processing.[15] A total of 92 samples of ADM were sterilely harvested out of the package before implantation. The samples were taken from both sterile and aseptically processed ADM. Samples were then cultured in growth medium, whereas the patients were followed postoperatively to correlate with clinical outcomes. Only one sample showed growth, producing *Escherichia coli* in culture, which was believed to be secondary to contamination. No significant differences were noted between the sterile and aseptic groups in terms of postoperative infection or seroma.

The purpose of this study is to determine if the sterilization process confers any benefits to patients in vivo. Much as in our previous study, the aim is to culture samples of ADM and follow clinical outcomes. We hope to determine if there is any presence of microbial growth after implantation and if this correlates with both postoperative seroma and infection.

## METHODS

After receiving approval from our Institutional Review Board, patients were prospectively enrolled into the trial. We recruited all women undergoing the exchange procedure for breast reconstruction following placement of tissue expanders with an acellular dermal matrix. Patients were excluded preoperatively if they did not speak English as their primary language or were cognitively impaired. We also excluded patients intraoperatively if an adequate sample was unable to be obtained.

All ADM samples were harvest under sterile conditions in the operating room during the expander for implant exchange procedure. A 1 cm × 1 cm sample was harvested from each operated breast. Thus, if a patient had a unilateral reconstruction, 1 sample was harvested and, in the case of bilateral reconstructions, 2 samples were harvested. The surgeons participating in the study placed the implants in the sub-pectoral plane, with the ADM serving as an inferolateral sling. Four surgeons participated in the study, and the ADM used by each surgeon was based on their personal preference, as determined by previous experience and training. The ADM brands included in the study were AlloDerm, AlloDerm Ready-To-Use (LifeCell, Branchburg, NJ), and AlloMax (Bard, Warwick, RI).

To perform our microbial analysis, we used a 2-stage culturing system. First, we incubated all ADM samples in tryptic soy broth (BD Biosciences, San Jose, CA). These samples were shaken at 225 rpm at 37°C for 24 hours using a bacterial shaker (Benchmark Inc., Edison, NJ). If the samples failed to show any growth, they were cultured in the same conditions for an additional 24 hours. Samples that did show growth, as demonstrated by a lack of translucency in the medium, were streaked onto tryptic soy agar, MacConkey agar, and 5% blood agar plates using sterile disposable inoculation loops. These agar plates were cultured at 37°C and observed for growth at 24 and 48 hours. Any samples that failed to demonstrate bacterial growth after 48 hours, whether in the tryptic soy broth or after being plated, were recorded as negative. Samples that did show growth when streaked on culture plates were sent for further genotyping. A diagram of the protocol is shown in Figure 1.

The second portion of our study was to provide clinical correlation between growth patterns and postoperative

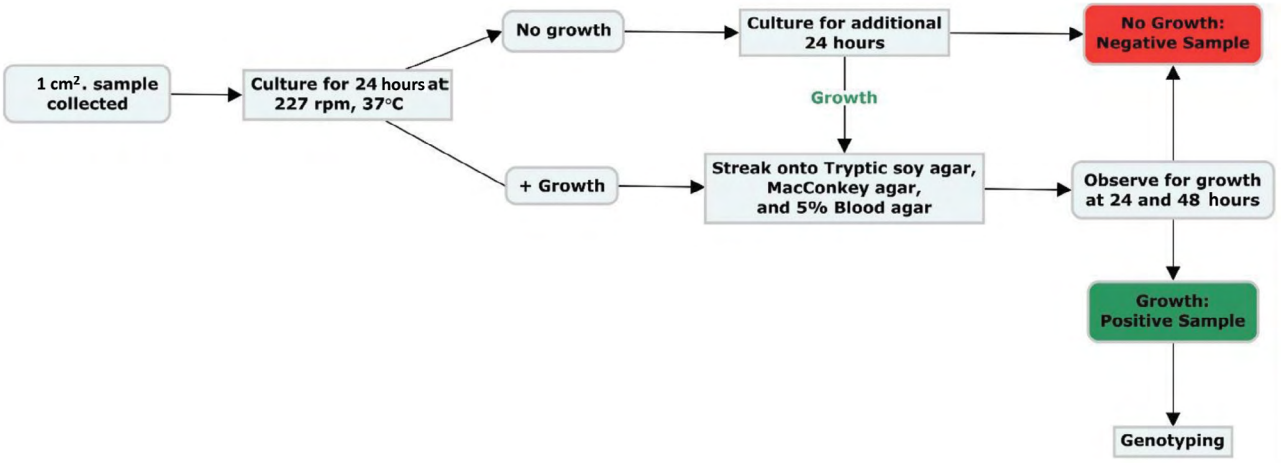


**Fig. 1.** Culture Protocol.

**Table 1. Patient Demographics and Comorbidities**

| | AlloDerm (n = 6) | AlloDerm RTU (n = 27) | AlloMax (n = 18) | *P* |
|---|---|---|---|---|
| Age (y) | 48.7 | 49.9 | 55.4 | 0.146 |
| Body Mass Index (BMI) | 23.5 | 26.6 | 25.5 | 0.239 |
| Hypertension | 0 (0.0%) | 1 (3.7%) | 7 (38.9%) | 0.03 |
| Hyperlipidemia | 0 (0.0%) | 1 (3.7%) | 7 (38.9%) | 0.03 |
| Diabetes | 0 (0.0%) | 0 (0.0%) | 2 (11.1%) | 0.148 |
| Hypothyroidism | 0 (0.0%) | 7 (25.9%) | 3 (16.7%) | 0.325 |
| History of breast surgery | 0 (0.0%) | 4 (14.8%) | 5 (27.8%) | 0.258 |
| Active smoking | 2 (33.3%) | 2 (7.4%) | 2 (11.1%) | 0.243 |
| Former smoker | 0 (0.0%) | 11 (40.7%) | 6 (33.3%) | 0.243 |
| Preoperative chemotherapy | 2 (33.3%) | 4 (14.8%) | 4 (22.2%) | 0.552 |
| Preoperative radiation | 0 (0.0%) | 1 (3.7%) | 2 (11.1%) | 0.473 |
| Postoperative chemotherapy | 0 (0.0%) | 9 (33.3%) | 7 (38.9%) | 0.196 |
| Postoperative radiation | 0 (0.0%) | 1 (3.7%) | 0 (0.0%) | 0.636 |
| Nipple sparing mastectomy | 0 (0.0%) | 2 (7.4%) | 0 (0.0%) | 0.396 |

outcomes. Patient charts were reviewed after 6 months postoperatively to assess for complications. Our primary endpoints were cellulitis, deep space infection, and seroma formation. The patients were split into groups based on which type of ADM had previously been surgically implanted. Statistical analysis of the patient's comorbidities and outcomes was completed with $\chi^2$ analysis for categorical variables and analysis of variance for continuous variables. We analyzed the data at the breast level, and all statistics were performed with SPSS version 25.0 (IBM, Armonk, NY).

## RESULTS

Our study population included 51 samples from 32 patients. All samples were collected over a 2-year period from June 2015 to June of 2017. A total of 6 samples of AlloDerm were collected from 3 patients, 27 samples of AlloDerm Ready-to-Use (RTU) were collected from 17 patients, and 18 samples of AlloMax were collected from 12 patients. Patient demographics and comorbidities are show in Table 1. The only statistically significant difference between the groups was the higher rate of hypertension and hyperlipidemia in the AlloMax group.

We additionally tracked patient charts for complications, which occurred following tissue expander placement, but before final implant placement. As shown in Table 2, there was a low rate of complications, with only 2 cases of seroma and 1 patient experiencing cellulitis. One of the patients experiencing seroma and the sole patient with cellulitis were in the AlloDerm RTU group, whereas the other patient experiencing seroma was in the AlloMax group. There was no statistically significant difference between the rate of complications and the ADM used by the surgeon.

**Table 2. Postoperative Complications Following Tissue Expander Placement**

| | AlloDerm | AlloDerm RTU | AlloMax | *P* |
|---|---|---|---|---|
| Cellulitis | 0 (0.0%) | 1 (3.7%) | 0 (0.0%) | 0.636 |
| Deep infection | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | Not Applicable |
| Dehiscence | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | NA |
| Flap necrosis | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | NA |
| Seroma | 0 (0.0%) | 2 (7.4%) | 1 (5.6%) | 0.782 |
| Hematoma | 0 (0.0%) | 0 (0.0%) | 1 (5.6%) | 0.393 |

None of the samples harvested during our study produced a positive culture. There were 5 patients who experienced complications following implant placement. One patient suffered a small hematoma, which did not require operative intervention. Three patients experienced a seroma, 2 of whom had received AlloMax and 1 who had received AlloDerm RTU. Only the patient with AlloDerm RTU required drainage, the other 2 patients were treated conservatively. Another patient in the AlloDerm RTU group had 3 episodes of cellulitis with 3 seromas. The patient was treated with office drainage 3 times for the seromas, was given oral antibiotics for 2 of the episodes of cellulitis, and was admitted for Intravenous antibiotics for the third episode of cellulitis. Unfortunately, this patient eventually had the implant removed, although cultures of the implant and deep tissue did not show signs of infection on culture. An additional patient had her implant removed for personal reasons, and thus, this complication was not included in our cohort. Overall, we did not note a statistically significant difference in the rate of seroma ($P = 0.676$), cellulitis ($P = 0.636$), or hematoma ($P = 0.393$) between the groups.

## DISCUSSION

Surgeons have been using ADMs in a wide range of functions because it was first introduced in 1995. In this study, we focus on breast reconstruction, where its benefits have been shown to aid in both cosmetic and temporal outcomes for patients undergoing alloplastic breast reconstruction.[2,4,16] Furthermore, with the advent of the prepectoral technique, the importance of ADM usage has grown as well. Unfortunately, with these benefits comes risk. A systematic review by Phillips et al[17] demonstrated that breast reconstructions utilizing ADM had infection rates as high as 31%. The breast, in general, is considered a clean site (Class 1 wound) with an average surgical site infection risk of 5% (2–16%).[18] Therefore, having such a high rate of infectious complications is alarming. A meta-analysis by Smith et al[7] showed that ADM utilization was also associated with a higher rate of seroma and skin necrosis.

Further work in the clinical realm has failed to consistently demonstrate a benefit to using sterile processing. In a retrospective study completed by Weichman et al,[11] the authors found using sterilized ADM not only lead to reduced rates of major and minor infection but even lead to an equivalent complication to complete submuscular coverage. Additionally, Venturi et al[19] performed a prospective study to investigate the safety of sterilized ADM in breast reconstruction. This study included 65 consecutive tissue expander-based reconstructions, with complications occurring in only 3 breasts. Given these results, the authors believed that sterile ADM conferred a safer complication profile for patients. These results are contrasted by Yuen et al[13] who found that sterile ADMs lead to higher rates of seroma and cellulitis. Similarly, Hoffman et al[20] compared FlexHD, a sterile ADM, to AlloDerm. Their work also showed a higher rate of major and minor infection in the sterilized ADM group, although failed to show a difference in seroma, hematoma, and return to the operating room.

More recent work on this topic has focused on systematic reviews and meta-analysis in an effort to increase study numbers and provide greater statistical significance. In one study by Lyons et al,[6] the authors found that ADM sterility did not have any effect on seroma, infection, or explanation rates. A meta-analysis by Macarios et al[12] compared sterile and aseptic products, again finding no statistically significant difference in the rates of cellulitis, seroma, or explantation. It seems that these larger studies have failed to identify any outcome-based improvement related to sterile ADM use.

The usage of terminal sterilization was originally proposed as a means to limit infection and contamination when working with ADMs. In a study by Mendenhall et al,[10] the authors used fluorescent in situ hybridization to study the presence of bacterial DNA on ADMs under microscope. The study showed that there was trace bacterial DNA in all samples included, although the amount of DNA was nearly double in aseptically processed ADMs when compared with sterilely processed products. That being said, there was no statistically significant difference when studying human ADMs and no difference was noted in culture. In the same study, the authors saw increased disorganization in the collagen fibers of ADMs, which were sterilely processed, although this did not seem to affect stem cell ingrowth. Additional studies have also demonstrated reliable matrix incorporation after sterilization.[19]

There remains much uncertainty as to why ADMs confer increased risk of complications. Previous work into the microbial properties of ADMs has demonstrated that both sterile and aseptic products are resistant to the ingrowth of both *Staphylococcus aureus* and *Streptococcus pyogenes*, 2 prevalent organisms in the human skin.[21] A study of wound biomarkers present in the drains placed in patients during alloplastic breast reconstruction also demonstrated no difference between those patients with ADM when compared with those without.[22] It has become clear that the reaction between several factors in vivo leads to infections related to the usage of these products.

Several mechanisms have been proposed for how ADMs may lead to infection, including contamination during production, contamination during placement, and seeding from the body postoperatively. When reviewing the results of our previous study investigating microbial growth of ADMs before implantation,[15] we believed that the clinical effect of pre- or perioperative seeding was unlikely. With all culture results failing to show growth, in vivo study was felt to be the next appropriate step. The results of our current study again failed to demonstrate growth after culturing specimens that had been previously implanted into our patients. Furthermore, during the 6-month postoperative period, only 4 of the 51 breasts included experienced a complication, of which only 3 were infectious in nature. These complications were not statistically significantly correlated with any of the 3 ADMs used in our study population.

We believe that the results of this study indicate that the sterilization process does not provide a benefit in terms of reducing infectious complications. Although it was not investigated in our study, it is possible that sterilizing may weaken the ADM and could contribute to implant malposition. As stated earlier, previous studies found ADM incorporation and stem cell ingrowth to be unaffected by sterilization, although collagen disorganization may play a more important role in preventing stretching of the ADM or suture tear through, although this latter point is hypothetical. Without a reduction in infectious outcomes while considering the possibility of a weakened ADM, the surgeon is forced to question the value of using a terminally sterilized product compared with aseptically processed products. It is our opinion that the surgeon's experience, need for inferolateral coverage, and presence of well vascularized flaps are the most important factors to determine which ADM should be used

There are several limitations to our study. First and foremost, we have a limited sample size. With only 51 samples included in the study, this study may be underpowered. We believe that a protocol including more patients and breasts would be better able to uncover statistically significant differences between the ADM groups. Another limitation is that only 3 different ADM products were included. Although previous studies have usually been limited to 2 products,[11–13,20] and 71.6% of plastic surgeons who use ADM choose AlloDerm for breast reconstruction,[1] there are still several options on the market that should be investigated. Finally, only 4 surgeons participated in our study and ADM usage was determined by surgeon preference rather than randomization.

## CONCLUSIONS

The use of ADMs in breast reconstruction has been shown to not only provide better cosmetic results but also assist in the filling process. This unfortunately bears an associated risk of increased rates of postoperative infections complications. Although terminal sterilization of these products theoretically should aid in reducing postoperative infections, our results fail to demonstrate a difference between sterile and aseptic processing. We believe that

the sterilization process is unlikely to confer any benefit to the patient or reconstructive process, and plastic surgeons should not rely upon an increased SAL when determining which ADM is appropriate to use for implant-based breast reconstruction.

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