Resistance of Acellular Dermal Matrix Materials to Microbial Penetration

Elizabeth N. Fahrenbach, MD; Chao Qi, PhD; Omer Ibrahim, MD; John Y. Kim, MD; Murad Alam, MD, MSCI

Importance: Acellular dermal matrices have many current and potential applications, but their long-term safety has not been extensively studied. In particular, limited information exists regarding such materials’ resistance to infection.

Objective: To assess the resistance to microbial penetration of common acellular dermal matrix materials used in reconstruction after skin cancer excision, treatment of chronic ulcers and burns, breast reconstruction, hernia repairs, and other applications.

Design: Comparative in vitro study of 4 commercially available dermal substitutes for their ability to act as barriers to penetration by common skin pathogens.

Setting: University-based dermatology and plastic surgery departments and a hospital microbiology laboratory.

Materials: Four commercially available dermal substitutes, including AlloDerm (LifeCell), FlexHD (Musculoskeletal Transplant Foundation), Strattice (LifeCell), and NeoForm (Mentor Corporation).

Intervention: We tested the 4 dermal matrix materials with the following 4 organisms commonly implicated in wound infections: Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, and Candida albicans. Each material was inoculated with the same concentration of each pathogen.

Main Outcome Measure: The number of bacterial colonies grown on blood agar plates.

Results: AlloDerm and rehydrated FlexHD were found to be the best barriers to penetration by P aeruginosa. AlloDerm, FlexHD, and Strattice also prevented penetration by S aureus and S pyogenes; NeoForm was less effective in withstanding these organisms. The results of this study were inconclusive with regard to C albicans penetration.

Conclusions and Relevance: Three of the 4 commonly used acellular dermal matrix materials are resistant to in vitro penetration by S aureus and S pyogenes and partially resistant to P aeruginosa. Resistance to fungal pathogens is uncertain. Antimicrobial differences across matrix materials may influence their selection for particular uses, such as treatment of refractory leg ulcers or reconstruction after skin cancer excision.

within 2 to 3 months; in a more vascular context (eg, the face), the acellular dermis can resorb completely within 6 months.

Although acellular dermal matrices have many current and potential applications, their long-term safety has not been extensively studied. In particular, information is limited regarding such materials’ resistance to infection,12-14 with the consequence that some surgeons might hesitate to use these materials.

This study was designed to investigate how commonly used commercially available dermal matrices compare in their ability to act as barriers to microbial penetration in vitro. The 4 proprietary dermal matrices studied were AlloDerm (LifeCell),18 FlexHD (Musculoskeletal Transplant Foundation), NeoForm (Mentor Corporation),20 and Strattice (LifeCell).21

For each, we assessed the ability of the matrix to resist infections by the 4 organisms most commonly implicated in burn wound infection, including Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, and Candida albicans.

**Methods**

**Establishment of Appropriate Microbial Concentration for Tissue Challenge**

A pilot study was performed to determine the appropriate microbial concentration to use in an in vitro comparison of the ability of various dermal matrices to act as barriers to microbial penetration. For this purpose, AlloDerm was selected because it is a legacy product that has been available for nearly 2 decades. Using a single matrix limits the generalizability of this study because the other materials might have substantially different thresholds for microbial permeability. However, using AlloDerm alone minimized complexity and reduced the cost and time required for completion of the pilot study.

Six 1 × 2-cm patches of AlloDerm were rehydrated following the directions on the package insert and placed on 6 separate blood agar plates. Each patch was inoculated with 50 µL of a solution containing S aureus at a concentration of 10⁷, 10⁶, or 10⁵ colony-forming units (CFU)/mL, with 2 patches for each concentration.

**Staphylococcus aureus** was the representative pathogen used to establish a threshold concentration for comparability. Staphylococcus aureus was the sole pathogen used to quantify the threshold of bacterial breach for dermal substitutes because prior quantitative culture investigations of cutaneous pathogens have established that approximately similar concentrations of similar skin pathogens are associated with the risk for infection.22,23 Therefore, the study as designed elicited specific bacterial threshold data only for S aureus, with the selection of the concentration of the other pathogens based on extrapolation from the work of Breidenbach and Trager22 and Masem et al.23

One set of plates (1 for each concentration) was incubated for 3 days and the other set for 7 days. After the incubation period, the patches were carefully peeled from the surface of the media, and a single 3-mm punch biopsy specimen was sampled to obtain growth medium from below the dermal matrix patch. The samples from the 3-day incubation group were incubated in 5 mL of brain-heart infusion (BHI) broth at 37°C overnight. The samples from the 7-day incubation group were incubated in 5 mL of BHI and shaken for 2 hours at 37°C.

After incubation in BHI broth, the blood agar plates were inoculated with calibrated loops (0.01 and 0.001 mL) in the way that is used for quantitative culture of urine specimens. One colony from the 0.01-mL loop streaking represents 100 CFU/mL and 1 colony from the 0.001-mL loop streaking represents 1000 CFU/mL. This procedure yielded 6 plates for the 3- and 7-day incubation groups (2 for each bacterial concentration). The plates were incubated overnight, and colony counts were performed the following day.

**Resistance of Acellular Matrices to Microbial Penetration**

The commonly used commercially available acellular dermal matrices chosen for this study were listed earlier, and their properties are defined in Table 1. The dermal matrix materials that require rehydration before implantation (AlloDerm, NeoForm, and Strattice) were prepared as prescribed by the package inserts. The FlexHD material does not require rehydration before implantation and thus was not rehydrated. After rehydrating, NeoForm and Strattice required sectioning because they were not available in the small 1 × 2-cm patches that we used in this study. The patches of AlloDerm, NeoForm, and Strattice were then placed over sterile gauze for 10 to 15 minutes to allow excess moisture to be wicked away. The prehydrated FlexHD material was not placed over gauze before placement on the agar plate.

Twenty 1 × 2-cm patches of each dermal substitute were placed on top of blood agar culture medium, yielding 80 plates. Because our pilot study identified 10⁶ CFU/mL as the appropriate threshold for the microbacterial dose, 4 solutions of this concentration were created for S aureus, P aeruginosa, S pyogenes, and C albicans. The 20 patches of AlloDerm were then inoculated with 1 mL of solution containing 10⁶ CFU/mL of S aureus (plates A1–A5), P aeruginosa (plates A6–A10), S pyogenes (plates A11–A15), or C albicans (plates A16–A20). This process was repeated for the remaining dermal matrices, and plates were labeled F1 through F20 (FlexHD), N1 through N20 (NeoForm), and S1 through S20 (Strattice).

The 80 patches of acellular dermal matrices inoculated with bacteria or C albicans were incubated for 3 days in air at 37°C. After the incubation period, the dermal matrix patches were carefully peeled from the underlying blood agar plate. A 3-mm punch biopsy specimen of the culture medium below each patch was obtained. The punch specimens were placed in separate tubes with 5 mL of BHI broth and shaken for 2 hours at 37°C. For each sample of broth, a blood agar plate was inoculated with a 0.001-mL calibrated loop in the way that is performed for quantitative urine cultures. These plates were incubated overnight, and a colony count was performed the following day.

Because this study did not involve human subjects or access to human tissue or medical records, the study was not subject to Northwestern University institutional review board oversight.

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**Table 1. Properties of Acellular Dermal Matrix Materials**

<table>
<thead>
<tr>
<th>Matrix Material (Manufacturer)</th>
<th>Method of Preservation</th>
<th>Biological Origin</th>
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<tbody>
<tr>
<td>AlloDerm (LifeCell) cryopreserved</td>
<td>Cadaveric human tissue</td>
<td></td>
</tr>
<tr>
<td>FlexHD (Musculoskeletal Transplant Foundation) prehydrated</td>
<td>Cadaveric human tissue</td>
<td></td>
</tr>
<tr>
<td>NeoForm (Mentor Corporation) cryopreserved</td>
<td>Porcine skin tissue</td>
<td></td>
</tr>
<tr>
<td>Strattice (LifeCell) cryopreserved</td>
<td>Cadaveric human tissue</td>
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</table>
**RESULTS**

**PILOT STUDY**

The results of the acellular dermal substitute pilot study are displayed in Table 2. AlloDerm faltered as a barrier to bacterial penetration by $10^6$ to $10^8$ CFU/mL after 3 and 7 days of incubation. An extended incubation time (7 days) did not facilitate bacterial penetration at the concentration of $10^4$ CFU/mL.

**COMPARATIVE STUDY**

The results of the colony count from the comparative study are displayed in Table 3. AlloDerm acted as the best barrier to bacterial penetration. *Staphylococcus aureus* and *S pyogenes* were unable to penetrate AlloDerm, and *P aeruginosa* penetrated 2 of 5 patches of AlloDerm. FlexHD was next in bacterial resistance, with *S aureus* unable to penetrate any of the 5 patches but *P aeruginosa* completely penetrating all of the FlexHD samples in uncountable numbers. Strattice performed well against gram-positive organisms, preventing penetration of *S pyogenes* and allowing penetration of relatively few *S aureus* organisms (185 colonies counted on plate 51 and 208 on plate 53). However, unlike AlloDerm, Strattice was not able to prevent penetration of *P aeruginosa*. NeoForm exhibited the least ability to act as a barrier to bacterial penetration, with uncountable numbers of bacterial colonies found for *S aureus*, *P aeruginosa*, and *S pyogenes*.

**COMMENT**

From the pilot study, we determined that a bacterial concentration of $10^6$ CFU/mL, or the threshold dose at which bacterial breach occurred, would be an appropriate microbial concentration with which to evaluate the barrier function of the 4 acellular dermal substitutes to be studied. Based on the pilot data, a 3-day incubation period was determined to be sufficient for bacterial penetration (ie, it was adequate time for the microbes to penetrate...
the surface of the dermal substitute and reach the blood agar plate below), were this process to occur.

The pilot study also suggested methodological improvements that would benefit the main comparative study. Accordingly, we implemented the following 3 changes for the main study: (1) before overlaying the rehydrated patch of dermal matrix material on the blood agar plate, excess moisture was removed by briefly drying the patches between 2 layers of sterile gauze; (2) to prevent runoff of the bacterial solution on the plate, a 1-μL rather than a 50-μL aliquot of bacterial suspension was used to inoculate the dermal matrix material; and (3) in the interest of time, shaking the agar biopsy specimens in BHI broth for 2 hours was substituted for incubating the agar punches in BHI broth overnight.

The comparative study was designed to compare the in vitro resistance with microbial penetration of common commercially available acellular dermal matrix materials. The results of the comparative study indicate that, among the group of 4 materials studied, AlloDerm provided the best barrier to *P aeruginosa*. NeoForm, FlexHD, and Strattice all demonstrated good to perfect resistance to penetration by *S aureus* and *S pyogenes* at the bacterial concentrations studied. NeoForm demonstrated the least ability to act as a barrier to microbial penetration because penetration occurred in all plates for each of the 3 bacterial organisms. This study did not detect any differences in the ability of acellular dermal matrices to prevent penetration by *C albicans*. That *P aeruginosa* was able to penetrate the most patches overall in this study may be attributed to its motility as a flagellated organism.

Regarding the difference in fungal resistance across the materials studied, the results of this study are inconclusive. The cultures showed no evidence of *C albicans* penetration for any of the dermal substitutes studied, and this finding may indicate that all 4 of the acellular dermal substitutes are superior barriers to fungal penetration in vitro or that the concentration of *C albicans* chosen to inoculate the patches was inappropriately low to provide useful results. A further comparative study using higher concentrations of *C albicans* inoculant may be useful for discriminating between materials. Such a future study would need to control for the fact that, for *C albicans*, strain and culture conditions can make a difference in morphology, with incubation at 37°C inducing formation of germ tubes and hyphae, which are the invasive structures of *C albicans* and hence relatively more likely to penetrate dermal substitutes.

A limitation of this study is that *S aureus* was the only pathogen studied for a threshold concentration in the pilot study. Although the different organisms may indeed behave slightly differently in vitro and in vivo, had different threshold concentrations been used for comparison of bacterial breach, one could argue that the bar regarding matrix resistance to a particular pathogen was unfairly raised or lowered. For the sake of uniformity and to compare matrices and pathogens in a standardized manner, we used similar setups with the various dermal matrices and similar bacterial concentrations during exposure. Finally, we did not compare the structural and functional utility of the tested materials, and such considerations may strongly influence material selection in a clinical setting. Specifically, certain materials may be better suited for particular clinical applications.

We cannot translate our in vitro results directly to the likely insults that would threaten the integrity of dermal substitutes during in vivo challenges. On one hand, the cumulative burden of pathogenic organisms in vivo is likely to be lower than the high levels simulated in vitro. Assuming that no contamination occurs at the time of placement, if substitutes are placed deep into a body cavity, limited opportunities exist for future exposures to organisms. However, in the rare instances when in vivo substitutes are thus exposed, they may be more likely to be stressed repeatedly with smaller quantitative exposures to pathogens rather than once with a larger load as is typically seen in vitro. Over time, the structural integrity of the substitutes after in vivo placement may decline, thus rendering them more susceptible to bacterial or fungal penetration. Although the risks involved with in vivo and in vitro exposures differ, the model we developed appears to be a reasonable approximation of in vivo risk of infection.

Overall, the bacterial concentration required for breach of these dermal substitutes was markedly higher than the concentrations typically associated with skin infection. In an investigation of complex extremity wounds, Breidenbach and Trager defined a concentration of 10⁴ CFU/mL as suggestive of skin infection. Similarly, in an animal study, Masem et al found that skin wounds incubated with *S aureus* at concentrations of 10⁴ and 10⁵ CFU/mL were impaired in their ability to clear the bacteria; skin tissues were not damaged at concentrations of 10⁵. The bacterial concentration of 10⁶ CFU/mL used in the main portion of our study was higher than these previously reported thresholds of 10⁴ and 10⁵, suggesting that dermal substitutes are highly resistant to bacterial penetration. That very high bacterial concentrations are able to disrupt nonliving dermal substitutes is not surprising. Indeed, it is reassuring that the dermal substitutes we examined are at least as resistant, if not more resistant, to infection and disruption than normal living, vascularized skin tissue.

**CONCLUSIONS**

We found that 3 of the 4 tested commonly used, commercially available acellular dermal substitutes are similar in their resistance to the microbial pathogens *S aureus* and *S pyogenes* but less consistent in their resistance to *P aeruginosa*. Further studies would corroborate these results with a larger sample size, study different concentrations of fungal pathogens to better understand in vitro resistance to fungal penetration, and consider performing biopsies of in vivo implants for microbiological assessment to determine whether the resistance to infection was as predicted by laboratory experiments.

We believe this study is an important and early attempt to assess the safety from contamination of artificial skin substitutes used in dermatology and plastic surgery. Such substitutes are used for the treatment of ulcers, for the correction of large defects after skin cancer excisions, and for deeper reconstructions, such as breast re-
constructions after mastectomies. The concern precipitating this study was that deeply implanted materials may represent a nidus for infection or be susceptible to breakdown after relatively small bacterial insults. Our results show that 3 of the 4 commonly used dermal substitutes are, in vivo, highly resistant to bacterial breach. This resistance is comparable in magnitude to that of living skin and subcutaneous tissues. Modest differences between bacterial resistance of different dermal substitutes are potentially useful pilot data for further studies and helpful for manufacturers seeking to improve further the utility of dermal substitutes.

Accepted for Publication: September 6, 2012. Published Online: February 20, 2013. doi:10.1001/jamadermatol.2013.1741

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Author Contributions: All the authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Fahrenbach, Qi, and Alam. Acquisition of data: Fahrenbach, Qi, and Kim. Analysis and interpretation of the data: Ibrahim, Kim, and Alam. Drafting of the manuscript: Fahrenbach, Ibrahim, Kim, and Alam. Critical revision of the manuscript for important intellectual content: Qi, Ibrahim, Kim, and Alam. Obtained funding: Alam. Administrative, technical, or material support: Qi and Kim. Study supervision: Kim and Alam.

Conflict of Interest Disclosures: Dr Alam serves on the medical advisory board of Lasering. Dr Kim is a consultant for Mentor Corporation and the Musculoskeletal Transplant Foundation (MTF). Dr Kim receives honoraria for his consultations at Mentor Corporation and the MTF. Northwestern University has a clinical trials unit that receives grants from very many corporate and governmental entities to perform clinical research, and Dr Alam has been the principal investigator on studies funded in part by Allergan, Bioform, Medicis, and Ulthera. Dr Alam receives royalties from Elsevier for technical books he has edited (<$5000 per year).

Funding/Support: This study was supported by departmental research funds from the Department of Dermatology, Northwestern University.

REFERENCES


