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, with the consequence that some surgeons might hesitate to use these materials.

This study was designed to investigate how commonly used commercially available acellular dermal matrices compare in their ability to act as barriers to microbial penetration in vitro. The 4 proprietary dermal matrices studied were AlloDerm (LifeCell; 2008), FlexHD (Musculoskeletal Transplant Foundation; 2008), NeoForm (Mentor Corporation; 2008), and Strattice (LifeCell; 2008).

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 10⁶, 10⁸, or 10⁹ 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 dis-

### TABLE 1. PROPERTIES OF ACCELLULAR DERMAL MATRIX MATERIALS

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

similar skin pathogens are associated with the risk for infection. 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 Trager and Masem et al.

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 ACCELLULAR 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 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 microbiological 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 μL 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.
## 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 $10^4$ to $10^6$ 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 S1 and 208 on plate S5). 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...}

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**Table 2. Results of Pilot Study to Assess Appropriate Microbial Concentration for Comparative Challenge of Acellular Dermal Matrix Materials**

<table>
<thead>
<tr>
<th><em>Staphylococcus aureus</em></th>
<th>Loop Streak Concentration by Length of Incubation, mL</th>
<th>3 d</th>
<th>0.001</th>
<th>0.001</th>
</tr>
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<tbody>
<tr>
<td>Concentration, CFU/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁴</td>
<td>No growth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁵</td>
<td>Uncountable[^a^]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁶</td>
<td>Uncountable[^a^]</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

[^a^]: Indicates too many colonies to count.

**Table 3. Resistance to Microbial Penetration of Selected Acellular Dermal Matrix Materials**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Plate No.</th>
<th>AlloDerm</th>
<th>FlexHD</th>
<th>NeoForm</th>
<th>Strattice</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>UC</td>
<td>185 Colonies</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>UC</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>–</td>
<td>–</td>
<td>UC</td>
<td>–</td>
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<tr>
<td></td>
<td>4</td>
<td>–</td>
<td>–</td>
<td>UC</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>–</td>
<td>–</td>
<td>UC</td>
<td>208 Colonies</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>6</td>
<td>UC</td>
<td>UC</td>
<td>UC</td>
<td>UC</td>
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<tr>
<td></td>
<td>7</td>
<td>–</td>
<td>UC</td>
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<td></td>
<td>8</td>
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<td>UC</td>
<td>UC</td>
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<td></td>
<td>9</td>
<td>–</td>
<td>UC</td>
<td>UC</td>
<td>UC</td>
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<tr>
<td></td>
<td>10</td>
<td>UC</td>
<td>UC</td>
<td>UC</td>
<td>UC</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>11</td>
<td>–</td>
<td>–</td>
<td>UC</td>
<td>–</td>
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<tr>
<td></td>
<td>12</td>
<td>–</td>
<td>–</td>
<td>UC</td>
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<td></td>
<td>13</td>
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<td></td>
<td>14</td>
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<td>–</td>
<td>UC</td>
<td>–</td>
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<tr>
<td></td>
<td>15</td>
<td>–</td>
<td>–</td>
<td>UC</td>
<td>–</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>16</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td></td>
<td>17</td>
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<td>20</td>
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</table>
injury, we used similar setups with the various dermal ma-
erials studied. The results of this study are incon-
clusive. 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 der-
mal substitutes are superior barriers to fungal penetra-
tion 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 use-
ful 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 differ-
ence in morphology, with incubation at 37°C inducing
formation of germ tubes and hyphae, which are the in-
vasive 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 pi-
lot study. Although the different organisms may indeed
behave slightly differently in vitro and in vivo, had dif-
ferent threshold concentrations been used for compari-
sion 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 man-
ner, we used similar setups with the various dermal ma-
trices and similar bacterial concentrations during expo-
sure. Finally, we did not consider the structural and
functional utility of the tested materials, and such con-
siderations 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 der-
mal 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 cavi-
ty, 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 expo-
sures to pathogens rather than once with a larger load as
is typically seen in vitro. Over time, the structural in-
tegrity of the substitutes after in vivo placement may de-
cline, 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 de-
veloped 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, Brei-
denbach and Trager23 defined a concentration of 106
CFU/mL as suggestive of skin infection. Similarly, in an
animal study, Masem et al24 found that skin wounds in-
cubated with S aureus at concentrations of 107 and 108
CFU/mL were impaired in their ability to clear the bacte-ia; 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 107 and 108, suggest-
ing that dermal substitutes are highly resistant to bac-
terial penetration. That very high bacterial concentra-
tions 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, com-
mercially available acellular dermal substitutes are simi-
lar in their resistance to the microbial pathogens S au-
reus 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 concen-
trations of fungal pathogens to better understand in vitro
resistance to fungal penetration, and consider perform-
ing biopsies of in vivo implants for microbiological as-
sessment to determine whether the resistance to infec-
tion was as predicted by laboratory experiments.

We believe this study is an important and early at-
tempt to assess the safety from contamination of artifi-
cial skin substitutes used in dermatology and plastic sur-
gery. Such substitutes are used for the treatment of ulcers,
for the correction of large defects after skin cancer exci-
sions, 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.

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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 consultancies 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).

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REFERENCES


