Acellular Dermal Matrix Grafts for Prevention of Microarterial Thrombophlebitis

Jivianne T. Lee, MD; Sunita Bhuta, MD; Thomas C. Calcaterra, MD; Keith E. Blackwell, MD

Objective: To examine the role of acellular dermal matrix grafts for prevention of microarterial anastomotic thrombophlebitis.

Design: Bilateral femoral artery microvascular anastomoses were created in the field of established wounds infected with Staphylococcus aureus in 12 rats. In each animal, 1 femoral microarterial anastomosis was wrapped with an acellular dermal matrix graft, and the contralateral femoral anastomosis was left unprotected. The incidence of femoral artery thrombosis was determined after 4 days by wound reexploration.

Setting: David Geffen School of Medicine, University of California, Los Angeles.

Main Outcome Measure: The patency of femoral artery anastomoses was determined after 4 days by wound reexploration.

Results: The incidence of femoral artery thrombosis in vessels wrapped with acellular dermal matrix grafts was 17%. The incidence of femoral artery thrombosis in unprotected vessels was 100%. This difference was statistically significant (P<.05).

Conclusion: Acellular dermal matrix grafts seem to have a protective effect in the prevention of acute thrombophlebitis when arterial microvascular anastomoses are performed in infected surgical fields.

Arch Otolaryngol Head Neck Surg. 2007;133:42-45

Infection of the flap recipient wound is well recognized as a potential etiology of free-flap failure.1-3 Urken et al4 reported that recipient wound infection was the most common etiology of free-flap thrombosis in 200 cases of head and neck reconstruction, accounting for 22% of cases of failed reconstruction. Moreover, clinical experience indicates that there is a very low incidence of successful flap salvage when attempting to revascularize a thrombosed free flap within the field of an active infection because most microvascular anastomoses created within the field of an active infection will fail during the early postoperative period. In the series reported by Urken et al,4 no failing flaps that were reexplored in the face of infection were successfully salvaged.

Dermal grafts have been used successfully for many years to provide coverage of the carotid artery and carotid artery bypass grafts in patients with head and neck cancer.5,6 In this application, dermal grafts are thought to lessen the risk of vascular complications by becoming incorporated around the blood vessel and providing a mechanical barrier from the surrounding hostile wound environment. However, to our knowledge, no previous laboratory or clinical investigations have examined the potential use of dermal grafts to provide coverage and protection of microvascular anastomoses.

Acellular dermal matrix grafts (AlloDerm; LifeCell Corp, Branchburg, NJ) are human dermal tissue grafts created by a patented process that eliminates cellular materials from the skin, leaving a dermal matrix that retains a structurally intact basement membrane, intact collagen fibers that support tissue ingrowth, intact elastin fibers to maintain biomechanical integrity, and other biochemical components that promote angiogenesis and cellular ingrowth.7 Previous clinical reports8-10 described successful grafting and integration of acellular dermal matrix grafts in contaminated and infected wounds. The purpose of this study was to examine the potential role of acellular dermal matrix grafts to provide coverage and protection of microvascular arterial anastomoses created in infected wounds.

Methods

The experiment was performed in 12 adult female Sprague-Dawley rats weighing approximately 400 g. All surgical procedures on the animal were performed according to an ap-
proved protocol and under the guidelines of the University of California, Los Angeles, Chancellor's Animal Research Committee and the Animal Welfare Assurance of the Department of Health and Human Services. Based on a power calculation using outcome estimates derived from previously published experiments,11-13 this experiment was originally designed to examine 40 femoral artery anastomoses in 20 rats. However, the experiment was concluded after completion of 24 femoral artery anastomoses in 12 rats when it became apparent that the difference in outcome between the control and experimental anastomoses was already statistically significant. This was done in accordance with accepted practices regarding ethical and humane animal experimentation.

**BACTERIAL PREPARATION AND INOCULATION**

Penicillin-resistant *Staphylococcus aureus* (American Type Culture Collection strain No. 14154, Manassas, Va) colonies were grown on agar plates. The night before inoculation, a suspension culture was made by placing a bacterial culture in 30 mL of tryptic soy broth, which was incubated at 37°C for 15 to 18 hours. Subcultures were made on the morning of the inoculation by placing 500 µL of the overnight suspension culture into 50 mL of fresh tryptic soy broth. The subcultures were incubated for another 2.5 hours and then filtered and centrifuged twice to wash the bacteria. The MacFarland tube method was then used to create a bacterial suspension of $10^8$ cells/mL.

Each rat was anesthetized with an intramuscular injection of ketamine (100 mg/kg) and xylazine (3.2 mg/kg). Using sterile technique, the femoral arteries and veins of both inguinal regions were dissected and exposed. Ten drops of the penicillin-resistant *S. aureus* suspensions ($10^8$ cells/mL) were inoculated into both inguinal wounds using a standard pipette. The inguinal incisions were then closed using 4-0 nylon sutures. Standard postoperative animal care was subsequently administered.

**MICROVASCULAR ANASTOMOSES AND ACELLULAR DERMAL MATRIX GRAFTING**

Three days after inoculation, both inguinal wounds were reexplored with the animals under anesthesia. Establishment of infection was determined using guidelines outlined by Luk and Chow,11 as manifest by the presence of soft tissue inflammation and pus and culture findings from the inoculated bacteria from the wound that were positive for organisms. After establishing the presence of infection, the femoral arteries were again isolated bilaterally. Using an operating microscope, the vessels were stripped of their surrounding adventitia and an Acland microvascular clamp (Accurate Surgical and Scientific Instruments Corp, Westbury, NY) was applied. The femoral arteries then sharply transected, and simple reanastomosis was performed using 8 to 10 interrupted 10-0 nylon sutures per femoral artery. On 1 side of the bilateral inguinal wounds, the site of the femoral artery anastomosis was wrapped with a sheet of acellular dermal matrix (AlloDerm) having a thickness of 0.04 to 0.05 cm and a surface dimension of $2.0 \times 2.5$ mm. The dermal matrix graft was held in place by surface tension, and suture fixation of the graft was not necessary. The dermal matrix grafts were placed into the right inguinal wound in 6 animals and into the left inguinal wound in 6 animals. The contralateral femoral artery anastomoses were left unprotected and exposed to the local wound environment in each animal to serve as a control. Patency of the anastomosis was determined in both antegrade and retrograde directions by strip test immediately after anastomosis and again 1 hour postoperatively. The inguinal wounds were then closed with 4-0 nylon sutures.

**DETERMINATION OF ANASTOMOTIC PATENCY AND HISTOLOGICAL ANALYSIS**

Four days after creation of the femoral artery anastomoses, both inguinal wounds were reexplored with the animals under anesthesia. The patency of the anastomoses was determined by assessing antegrade and retrograde blood flow by strip test. The animals were then humanely euthanized by intentional anesthetic overdose, and the anastomotic sites were excised and placed in formalin. The specimens were then cut, sectioned, and stained for histological analysis using hematoxylin-eosin. Data were analyzed using a 1-tailed Fisher exact test to compare the patency rates of the femoral arteries wrapped with acellular dermal matrix to the patency of the unprotected femoral arteries. Statistical significance was set at a level of $P<.05$.

**RESULTS**

With respect to the exposed unprotected femoral anastomoses, all 12 anastomoses (100%) were found to be thrombosed on reexsploration. Histological analysis of the anastomotic sites revealed the presence of endothelial disruption, thrombus formation, perivascular inflammation, and clusters of bacterial cocci surrounding the vessel (Figure 1). In contrast, only 2 (17%) of 12 femoral arteries that were wrapped with acellular dermal matrix grafts developed thrombosis. The remaining 10 femoral arteries wrapped with acellular dermal matrix grafts (83%) were found to be patent on reexsploration. Histological examination of the patent wrapped femoral arteries showed no evidence of intraluminal thrombosis. Some bacterial colonies were abutting the vessel walls (Figure 2), but the acellular dermal matrix graft seemed to be structurally intact and acting as a physical barrier to separate the femoral artery from a dense inflammatory infiltrate. Data analysis using a 1-tailed Fisher exact test to compare the patency of the unprotected femoral arteries (0/12 or 0%) with the femoral arteries wrapped in acellular dermal matrix (10/12 or 83%) demonstrated a statistically significant difference between the 2 groups ($P<.05$).

**COMMENT**

Established infection in the vicinity of a microvascular anastomosis frequently results in thrombosis. An acute
Luk et al12 showed that the presence of an established inflammatory response may lead to direct damage to blood vessel walls, resulting in platelet aggregation. Soft tissue swelling resulting from an adjacent infection may result in extrinsic compression of a vessel’s lumen. In addition, the local vasodilatation produced by infection may result in a diminution in the rate of blood flow, leading to stasis and thereby increasing the risk of thrombosis. The potential for infection is increased in head and neck reconstruction, where there is frequently contamination of the operative field by saliva. Often, the recipient bed of the flap is fibrotic and hypovascular secondary to previous radiotherapy and surgery, increasing the risk of poor wound healing leading to a postoperative salivary fistula. Contamination of the neck wound by inadvertent communication with a tracheostomy site further contributes to the increased potential for postoperative infections in head and neck microvascular reconstruction.

The current experiment demonstrates that acellular dermal matrix grafts seem to help prevent thrombosis when microvascular arterial anastomoses are performed in infected wounds. A series of previous experiments that examined the impact of infection on microvascular thrombosis performed at the University of Hong Kong provides important insight into the potential protective mechanism provided by acellular dermal matrix grafts, and they also raise possible limitations of the technique. Luk and Chow11 demonstrated that inoculation of the surgical field with a pathologic dose of S aureus at the time of performance of a rat femoral microarterial anastomosis did not adversely affect patency. They showed that during the period when wound contamination develops into infection, the anastomosis becomes well protected from the surrounding inflammation by the development of a distinct layer of connective tissue. In a subsequent investigation, Luk et al12 showed that the presence of an established infection does have an adverse effect of microarterial anastomotic patency. In that study, rat femoral anastomoses were performed 3 to 5 days after inoculating the surgical field with bacteria. In that setting, arterial patency fell to 25%, compared with a patency rate of 81% seen in noninfected controls. Histologic findings showed extensive fibrosis of the adventitia and media in the infected anastomoses, causing constriction of the vessels. In a third study13 using the rat femoral vessel model, the group examined the effect of wound drainage, debridement, and antibiotic therapy when performing microvascular anastomoses in an infected field. They found that this intervention produced patent anastomoses in 95% of microarterial anastomoses but in only 33% of microvenous anastomoses. They concluded that performance of a microvenous anastomosis was not advisable in the presence of an established infection, even after adequate debridement and antibiotic therapy.

The mechanism of acellular dermal homografts in the prevention of microvascular thrombosis in infected surgical fields may be similar to the role of the protective layer of connective tissue that developed around anastomoses performed in contaminated fields in Luk and Chow’s first experiment.11 The acellular dermal matrix grafts may act as a physical barrier to protect the anastomoses from the deleterious effects of the surrounding wound inflammation. However, the current study examined only the role of acellular dermal matrix grafts to prevent arterial thrombosis whereas the third study13 from the University of Hong Kong indicated that infected venous microvascular anastomoses are more prone to experience thrombosis than arterial anastomoses. Therefore, the potential use of acellular dermal matrix grafts in microvascular flap reconstruction in infected surgical fields remains uncertain because we have not examined the effect of dermal matrix grafts in preventing venous thrombosis in the face of infection. Another weakness of the current study arises from the experimental model, in that 1-time wound inoculation with S aureus does not precisely reproduce the most common setting of head and neck wound infections secondary to salivary fistulas, in which there is ongoing wound contamination with a polymicrobial infection.

Based on our current understanding of the process of microvascular thrombosis in the face of infection, the following recommendations can be made regarding the use of acellular dermal matrix grafts in clinical microvascular head and neck reconstruction. Because the incidence of free-flap failure is only about 1% and the incidence of salivary fistula and wound infection is only about 3% in cases of microvascular head and neck reconstruction,14 routine use of acellular dermal matrix grafts to protect microvascular anastomoses is not advocated. Even in those situations in which the risk of a postoperative salivary fistula is high (eg, cases of pharyngoesophageal reconstruction after failed chemotherapy and radiation therapy for advanced cancer of the larynx and hypopharynx), routine use of acellular dermal matrix grafts is not indicated because the risk of free-flap failure is low when salivary fistulae develops during the postoperative period.15 There is even a limited role for the use of acellular dermal matrix grafts for microvascular flap reconstruction of infected wounds because most such wounds are ideally reconstructed in
a delayed fashion after successful treatment of the associated wound infection.

The most likely beneficial role of acellular dermal matrix grafts in clinical microvascular head and neck reconstruction is in the setting of attempted salvage of a failing free flap in the face of an established wound infection. Clinical and laboratory experience indicates a low probability of successful flap revascularization in this setting, even with adequate wound debridement and antibiotic therapy.4,13 Although the current laboratory investigation has not examined the potential role of acellular dermal matrix grafts for protection against venous microvascular thrombosis, the demonstrated benefit of this procedure to prevent arterial microvascular thrombophlebitis justifies its application in this dire clinical situation, pending the completion of laboratory studies to examine the efficacy of acellular dermal matrix grafts for prevention of venous microvascular thrombosis.

In conclusion, acellular dermal matrix grafts seem to have a role for prevention of arterial microvascular thrombosis when anastomoses are performed in the field of an established wound infection.

Submitted for Publication: December 12, 2005; final revision received April 6, 2006; accepted April 26, 2006.

Correspondence: Keith E. Blackwell, MD, 62-132 CHS, 10833 Le Conte Ave, Los Angeles, CA 90095-1624 (kblackwe@ucla.edu).

Author Contributions: Dr Blackwell had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. 
Study concept and design: Lee, Calcaterra, and Blackwell. 
Acquisition of data: Lee, Bhuta, and Blackwell. 
Analysis and interpretation of data: Lee, Bhuta, and Blackwell. 
Drafting of the manuscript: Lee and Blackwell. 
Critical revision of the manuscript for important intellectual content: Bhuta, Calcaterra, and Blackwell. 
Obtained funding: Calcaterra. 
Administrative, technical, and material support: Lee, Bhuta, Calcaterra, and Blackwell. 
Study supervision: Bhuta, Calcaterra, and Blackwell.

Financial Disclosure: At the time that the research described in this article was completed, Dr Blackwell was serving as a member of the Speaker’s Bureau for LifeCell Corp, Branchburg, NJ, which manufactures and sells the acellular dermal matrix grafts used in the experiment.

Funding/Support: Funding support for the research reported in this article was provided by various individual donors who made unrestricted research grants to the University of California to support research in the field of head and neck surgery. The acellular dermal matrix grafts used in the experiments were donated by LifeCell Corp.

REFERENCES