Effects of a Fibrin Sealant on Skin Graft Tissue Adhesion in a Rodent Model

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No sponsorships or competing interests have been disclosed for this article.

Abstract

Objective. To establish a rodent model for skin grafting with fibrin glue and examine the effects of fibrin glue on the adhesive strength of skin grafts without bolsters.

Study Design. Animal cohort.

Setting. Academic hospital laboratory.

Subjects and Methods. Three skin grafts were created using a pneumatic microtome on the dorsum of 12 rats. Rats were evenly divided into experimental (n = 6) and control (n = 6) groups. The experimental group received a thin layer of fibrin glue between the graft and wound bed, and the control group was secured with standard bolsters. Adherence strength of the skin graft was tested by measurement of force required to sheer the graft from the recipient wound. Adhesion strength measurements were taken on postoperative days (PODs) 1, 2, and 3.

Results. The experimental group required an average force of 719 g on POD1, 895 g on POD2, and 676 g on POD3, while the average force in the control group was 161 g on POD1, 257 g on POD2, and 267 g on POD3. On each of the 3 PODs, there was a significant difference in adherence strength between the experimental and control groups (P = .036, P = .029, P = .024).

Conclusion. There is a significant difference in the adherence strength of skin grafts to the wound bed in the early postoperative period of the 2 groups. In areas of high mobility, using the fibrin sealant can keep the graft immobile during the critical phases of early healing.

Keywords
skin graft, sealant, complications, shear force, fibrin glue

Received July 14, 2015; revised January 20, 2016; accepted February 3, 2016.

Skin grafting is a common procedure often used in the reconstructive ladder. The best technique of securing the graft to the recipient site in areas of high mobility such as head, neck, and upper extremity, where motion of the repair is apt to disrupt the graft, remains debatable. Traditional bolstering techniques and vacuum-assisted closure (VAC) devices have been used to immobilize grafts and prevent potential space formation.1,2 However, these techniques can be cumbersome when used in head and neck grafts or patients who do not require hospitalization. Fundamentally, imbibition and graft success are dependent on a technique that provides immobility.3

Use of fibrin sealants in skin grafting has been shown to increase graft take at infected sites and reduce wound contraction in several descriptive studies.4,5 Furthermore, several studies, using planimetric computer analysis, have demonstrated that use of fibrin sealant can reduce seroma or hematoma formation, increase clinical appearance of graft take, and decrease graft mobility.5-8

Adherence of a skin graft to the wound bed in a highly mobile area is essential to the success of the repair.1,9 Our group looks to quantify strength of skin graft tissue adherence with the use of a fibrin sealant compared with traditional bolster technique in a highly mobile dorsal skin graft rat model. The amount of force required to displace the graft from the recipient site is examined.

Methods

Animals

Twelve male Sprague-Dawley rats weighing approximately 300 to 400 g were used. The protocol was approved by the Thomas Jefferson University Institutional Animal Care and Use Committee. Animals were housed in a 12-hour light/dark cycle with food and water ad libitum. Rats received 0.2 mL of buprenorphine sustained release following the skin grafting procedure and were monitored daily following all surgical procedures.

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Experimental Design

The animals were split evenly into 2 groups. The experimental group received a thin layer of the fibrin sealant (Artiss; Baxter Healthcare Corp, Deerfield, Illinois) between the graft and wound bed. Packaged syringe sprayer was stored in a freezer and thawed just prior to use. The Artiss (Baxter Healthcare) syringe unit has a thrombin and fibrinogen chamber, which is connected to an Easy spray unit (Baxter Healthcare), providing a controlled pressurized stream of air. The nasal was held 10 cm from the wound surface, and 1 mL was applied in a thin mist created by the pressurized air. The skin graft was then laid on the wound, and a sponge was used to apply pressure for 2 minutes. The control group received standard technique tie-over bolsters over each graft without fibrin sealant application. Three grafts of approximately equal size were made on each rat. Each rat would have 1 of the 3 grafts tested for adherence strength on postoperative days (PODs) 1, 2, and 3.

Skin Grafting Procedure

The animals were anesthetized using isoflurane 3% delivered to effect and 0.1 cc/kg ketamine/acepromazine solution. The dorsums of the rats were then shaved. A pneumatic dermatome was set to a 0.016-inch shave graft depth. Using a sterile technique, the dermatome was applied over the shaved area to create a graft approximately 8 × 2 cm (Figure 1A). This graft was then divided into 3 equal parts. The wound bed was rinsed with normal saline and wiped clean. In the experimental group, the fibrin sealant was sprayed evenly over the entire wound bed. The grafts were then placed on the fibrin sealant and held in place for 2 minutes (Figure 1B).

The rats of the control group were grafted in the same fashion, and the wound site was rinsed and cleaned without the addition of the fibrin sealant. The grafts were placed on the wound bed, and tie-over bolsters made of Xeroform (Covidien, Dublin, Ireland) dressing were placed on the top of each graft (Figure 1C). Neck collars were placed on each rat in both groups to prevent auto-digestion of the graft or disruption of the bolster.

Outcome Measurements

Skin graft adherence strength was measured using a novel adhesion strength testing system (Figure 2A). This system was designed to quantify the amount of force required to disrupt the graft on each postoperative day. The rat was placed at the center of the system and anesthetized using isoflurane. A 5-0 Prolene suture was placed in the skin graft. The suture was then looped and tied onto the vertical arm of the testing system (Figure 2B). The vertical arm was attached to the horizontal movement arm of the system, which was freely mobile on a hinged platform. The distal end of the horizontal arm was attached to a cable, which ran through a pulley system and was hooked at the end to attach weight. Addition of weight to the pulley system caused movement of the horizontal and vertical arms. When the rat was anesthetized and the suture loop was in place on the vertical arm, the horizontal arm was moved toward the pulley until the slack in the suture was removed. The nongrafted skin of the animals was pinned at 2 points to the device to provide countertension to the skin. At this point, weights were sequentially added to the hooked end of the cable. As each weight was added, 2 seconds were given to determine if there was disruption of the graft from the wound bed. Once enough weight was added to the cable so...
like the graft sites of the mobile head, neck, and upper extremity. We found that there is a significant difference in skin graft adhesion between the grafts treated with fibrin sealant and those with a standard bolstering technique at the completion of inhibition or POD3. This critical process in the healing of skin grafts requires immobility of the graft, and this was achieved in our model. Grafts treated with the fibrin sealant required greater force to remove the graft from the wound bed. These findings indicate that there is greater strength of adhesion between the graft and wound bed for the skin grafts treated with fibrin sealant, minimizing graft disruption. Although not part of our primary end points, we believe that the use of the fibrin sealant reduced the risk of potential space formation and decreased operative time compared with the bolster group. Uncontrollable factors such as differences in the mobility of individual rats and absolute thickness of fibrin glue application may be factors that could explain the variations within. Reduced adherence strength on POD3 compared with POD2 in the fibrin sealant arm may be related to the effects of the adhesive properties breaking down and the natural biologic adherence of the graft to the wound bed. This transition may be seen as a decrease in adherence strength, but other studies have demonstrated long-term viability.6 Even when the analysis was performed with exclusion of data points ± 1 SD or data points ± 2 SD, the 2 arms were still statistically different.

Previous studies have looked at graft viability with fibrin sealant in burn wounds in porcine models.5 They showed that there was a statistically significant increase in graft viability and a decrease in graft mobility with fibrin sealant compared with staples but did not demonstrate an increase in adherence strength. Foster et al8 also showed significantly decreased hematoma/seroma formation, indicating that the use of fibrin sealant created greater graft take and less potential space than staples. Both of these studies used planimetry software, or photographic measurements, to determine the total surface area of the test sites and areas of graft loss. Planimetry uses photographic measurement to calculate the estimated wound area and areas of graft loss. At best, this technique is an estimated measure of graft take, which is based on subjective appearance of the graft. Our report corroborated the findings of these studies but used direct force measurements to determine the degree of tissue adhesion to compare the efficacy of fibrin sealant vs tie-over bolster. This technique provides a mechanism for the observed findings. As health care costs require further justification and added expense becomes standard, this study will bolster the rationale behind the clinical observations. Further study into economic impact would be needed to derive further conclusions on the cost/benefit ratio.

Suturing and stapling has also been used to immobilize skin grafts. Gibran et al11 examined skin graft adhesion and hematoma/seroma formation in burn patients by comparing fibrin sealants with staples and found that the fibrin sealant cohort had subjectively better tissue adherence and statistical significance in reduced hematoma/seroma formation. Another review of sutures and fibrin sealant remarked that fibrin sealant can more easily eliminate dead spaces and control bleeding

Figure 2. (A) Novel adhesion strength testing system. Weights were applied to a pulley, with additive force in a directionally consistent vector. (B) Application of the testing system on the skin graft, with 2 spikes holding countertension.

that the graft was disrupted, the weight was recorded as the force necessary to disrupt that graft. Antibiotic ointment was applied to the wound area and the neck collar was reapplied. This process was repeated on POD2 and POD3. Statistical analyses of adherence strength were performed using a 2-sided t test. The level of significance was set at P < .05.

Results

The 3 skin grafts on each of the 12 rats were tested using the adhesion strength testing system. The force required to remove the skin grafts varied in each POD (Figure 3). Skin grafts applied with fibrin sealant required an average force of 719 g on POD1, 895 g on POD2, and 676 g on POD3 to remove the skin grafts. The average force to remove the grafts in the control group was 161 g on POD1, 257 g on POD2, and 267 g on POD3. On each of the 3 postoperative days, there was a statistically significant difference in adherence strength between the experimental and control groups (P = .036, P = .029, P = .024).

Discussion

This is the first report to quantify the adhesive strength of fibrin sealant in skin grafts in an animal model of high tissue mobility. By using the dorsum of the rat, the grafts experienced twisting, arching, and physical contact, much like the graft sites of the mobile head, neck, and upper extremity.
better than sutures.\textsuperscript{12} Fibrin sealants are safe and nonhazardous\textsuperscript{5,11} to the host, encouraging wound healing.

Practical application of fibrin sealants needs further testing, but the implication of this study gives a foundation for further investigation. In select cases, such as skin grafts overlying muscle only on flaps of the head and neck, the use of fibrin sealant without traditional bolstering also allows for continual visual inspection of the graft, where tie-over bolsters may not be advisable due to risk of compromise to the underlying flap that is supporting the graft. Although this study does not look at the traditional 7-day end point, at which time the bolsters are removed, it does give confidence to the surgeon of early adherence, when immobility of the graft is critical to allow for imbibition and inosculation. The main complications include seromas and flap breakdown or prolonged hospital stays for drain management.

Our study did not evaluate graft take beyond the 3 days of observation. While we cannot accurately state that the use of fibrin sealant in this study had any effect on overall graft take, we are confident that our fibrin sealant application technique does confer viability based on previous studies using similar techniques.

Limitations of this study include a small sample size. We used 12 total rats to measure the efficacy of the fibrin sealant. This number provided a statistically significant result with fewer animals than other studies.\textsuperscript{5,8} One or more of the 3 sites of skin grafting on each rat may be prone to movement or disruption of the graft based on the skin mobility and arching of the rat. The adhesion testing system was designed and built by our team and may not convey true physiologic stresses imparted on skin grafts.

**Conclusion**

There is a significant increase in the adhesion strength of skin grafts to the wound bed in the fibrin sealant group. In areas of high mobility, using the fibrin sealant can keep the graft immobile during the critical phases of early healing. Therefore, we believe that fibrin sealant used in skin grafting techniques could minimize graft disruption and complications and has merit to be evaluated further in the large rotational flap setting.

**Author Contributions**

Mark D. Balceniuk, collected data, analyzed data, wrote article; Nicholas A. Wingate, collected data, analyzed data, wrote article; Howard Krein, design, analyzed data, revised article; Joseph Curry, conception, design, revised article, approval; Ryan Hefelfinger, analyzed data, revised article; Adam Luginbuhl, design, analyzed data, revised article, final approval.

**Disclosures**

**Competing interests:** None.

**Sponsorships:** None.

**Funding source:** None.
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