The Effect of 2 Sealants (FloSeal and Tisseel) on Fasciocutaneous Flap Revascularization

Scott Jorgensen, BS; Daphne A. Bascom, MD, PhD; Aaron Partsaferas, BS; Mark K. Wax, MD

Background: Fasciocutaneous tissue transfer is one of the most common procedures performed in head and neck reconstructive surgery. These composite tissues can be transferred as either a free flap or a pedicled flap. Free tissue transfer has become the reconstructive modality of choice following head and neck oncologic ablation. Synthetic tissue adhesives and hemostatic agents are rapidly gaining popularity in reconstructive surgery. Their ability to decrease bleeding and promote flap sealing and healing has led to a proliferation in their use. To our knowledge, the short-term effect of these substances on healing, as measured by flap revascularization, has not been systematically investigated.

Methods: Fifty-six male Sprague-Dawley rats were divided into 3 groups: a control group, a matrix hemostatic sealant (FloSeal) group, and a 2-component fibrin sealant (Tisseel) group. In each group, the rats had a 3 × 6 cm fasciocutaneous flap based on the inferior epigastric artery elevated and exposed to 2 hours of primary ischemia. In the control group, 0.2 mL of isotonic sodium chloride solution was placed between the flap and its bed, while in the experimental groups, 0.5 mL of FloSeal or 0.2 mL of Tisseel was applied to the wound before closure. Each group was then divided into groups of 5 rats. Each of these groups then had their pedicle divided on postischemic day 4, 5, 6, or 7. The percentage survival of the flap was measured 7 days after pedicle ligation.

Results: There was no statistical difference in flap survival of rats treated with isotonic sodium chloride solution, Tisseel, or FloSeal. Ligation of the flap pedicle on days 4, 5, 6, or 7 did not result in any difference in flap survival among the 3 groups.

Conclusion: The FloSeal and Tisseel demonstrate no short-term detrimental effect on flap survival nor do they seem to affect revascularization in a fasciocutaneous free flap model.

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Composite free tissue transfer has proved to be the best modality for reconstructing complex bone and soft tissue defects in the head and neck. Many different donor sites are available for harvesting these composite tissue components. The multitude of available flaps allows the design of a donor site that offers the best composite tissue match. Contemporary free tissue transfer has a success rate of between 90% and 100%.

Numerous factors can complicate the outcome of microsurgical free tissue transfer. Many of these occur during the initial surgical procedure and are preventable or correctable by a meticulous surgical technique. Other intraoperative or postoperative complications, such as seroma, hematoma, or fistula formation, may contribute to flap failure or may make the flap more susceptible to an ischemic insult. Flaps that are less tolerant to ischemia may fail, with devastating results to the patient. Many thrombin-based agents have become available for commercial use. These agents come in 2 main forms: (1) preparations that contain only thrombin and (2) agents that contain thrombin and fibrin. Agents containing thrombin only require endogenous fibrin to form a fibrin clot. Agents containing fibrin and thrombin form a clot when mixed together in a dry wound bed. They are primarily used as tissue glues. A matrix hemostatic sealant (FloSeal; Fusion Medical Technologies Inc, Fremont, Calif) and a 2-component fibrin sealant (Tisseel; Baxter International, Deerfield, Ill) have increasingly been used in head and neck reconstruction. Their ability to improve hemostasis, weld tissues together to prevent seromas, and decrease the frequency and severity of cerebrospinal fluid leaks has prompted their increasing use in reconstructive surgery.
Fifty-six adult male Sprague-Dawley rats, weighing between 200 and 300 g (Baxter International, Deerfield, Ill), were used. The National Institutes of Health guide for the care and use of laboratory animals was followed throughout the study. This protocol was approved by the Institutional Animal Care and Use Committee at Oregon Health and Science University. Rats were housed in cages in pairs with access to food and water ad libitum. Their condition was observed daily.

General anesthesia was induced using an isoflurane non-rebreathing inhalation mask. Once the animals were anesthetized, they were placed in a supine position on a warming pad at 40°C. Hair removal from the belly was accomplished by shaving. The skin was then prepared with a povidone-iodine solution, and the animals were anesthetized. The deep femoral artery and vein were ligated and transected to shunt all femoral flow to and from the raised flap.

The rats were divided into 3 groups: (1) a control group, (2) a FloSeal group, and (3) a Tisseel group. All animals were anesthetized and the flaps elevated in the standard fashion, as described herein. All animals received the same preoperative and postoperative care and were treated with buprenorphine hydrochloride, 0.1 mg/kg subcutaneously, twice a day for 3 days for analgesia. Following elevation of the flap, all animals had a 20-g vascular Heititz clip applied to the artery and vein in the vascular pedicle for 2 hours. During this ischemic time, the flap was sutured back in place, and the animals were allowed to ambulate and were given food and water ad libitum. After the vascular clip was applied, the animals received 1 of 3 treatments. In the control group, 0.2 mL of isotonic sodium chloride solution was placed between the flap and the abdominal bed before closure. In the Tisseel group, 0.2 mL of Tisseel (500 IU/mL of thrombin, 75-115 mg/mL of fibrinogen, and 3000 IU/mL of fibrinolysis inhibitor) was placed between the flap and its bed. In the FloSeal group, 0.5 mL of FloSeal (4000 IU of bovine thrombin per 5 mL of FloSeal) was placed between the flap and the wound bed before wound closure. All wounds were then closed with 4-0 polyglycolic acid (Dexon), and the animals were awakened.

Table 1. Number of Animals in Each Treatment Group*

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Pedicle Severed, d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
</tr>
<tr>
<td>FloSeal</td>
<td>5</td>
</tr>
<tr>
<td>Tisseel</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
</tr>
</tbody>
</table>

*FloSeal (Fusion Medical Technologies Inc, Fremont, Calif), matrix hemostatic sealant; Tisseel (Baxter International, Deerfield, Ill), 2-component fibrin sealant.

Table 2. Flap Survival in the 3 Treatment Groups*

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>63.0 ± 32.3</td>
</tr>
<tr>
<td>FloSeal</td>
<td>38.1 ± 25.4</td>
</tr>
<tr>
<td>Tisseel</td>
<td>40.8 ± 24.2</td>
</tr>
</tbody>
</table>

*Data are given as mean ± 1 SD percentage survival. FloSeal (Fusion Medical Technologies Inc, Fremont, Calif), matrix hemostatic sealant; Tisseel (Baxter International, Deerfield, Ill), 2-component fibrin sealant.

Initially, free flaps are dependent on their vascular pedicle for survival. Long-term survival is facilitated by revascularization from the surrounding tissue bed. Factors that interfere with this revascularization can contribute to long-term wound healing problems and decrease the ability of the flap to tolerate division of its vascular pedicle or pedicle thrombosis.

This study evaluates the effects of FloSeal and Tisseel on revascularization of the rat fasciocutaneous flap.

## METHODS

Fifty-six adult male Sprague-Dawley rats, weighing between 200 and 300 g, were used. The National Institutes of Health guide for the care and use of laboratory animals was followed throughout the study. This protocol was approved by the Institutional Animal Care and Use Committee at Oregon Health and Science University. Rats were housed in cages in pairs with access to food and water ad libitum. Their condition was observed daily.

General anesthesia was induced using an isoflurane non-rebreathing inhalation mask. Once the animals were anesthetized, they were placed in a supine position on a warming pad at 40°C. Hair removal from the belly was accomplished by shaving. The skin was then prepared with a povidone-iodine solution, and a standard 3x6-cm fasciocutaneous flap, based on the inferior epigastric artery and vein, was raised. A standard template was drawn for each animal. Vascular dissection included the femoral vessels proximal and distal to the origin of the epigastric pedicle. The distal femoral vessels were tied and transected to shunt all femoral flow to and from the raised flap. The deep femoral artery and vein were ligated and transected. The femoral nerve was left intact throughout the procedure. A loosely tied silk loop was placed around the vascular pedicle to facilitate later pedicle identification.

The rats were divided into 3 groups: (1) a control group, (2) a FloSeal group, and (3) a Tisseel group. All animals were anesthetized and the flaps elevated in the standard fashion, as described herein. All animals received the same preoperative and postoperative care and were treated with buprenorphine hydrochloride, 0.1 mg/kg subcutaneously, twice a day for 3 days for analgesia. Following elevation of the flap, all animals had a 20-g vascular Heititz clip applied to the artery and vein in the vascular pedicle for 2 hours. During this ischemic time, the flap was sutured back in place, and the animals were allowed to ambulate and were given food and water ad libitum. After the vascular clip was applied, the animals received 1 of 3 treatments. In the control group, 0.2 mL of isotonic sodium chloride solution was placed between the flap and the abdominal bed before closure. In the Tisseel group, 0.2 mL of Tisseel (500 IU/mL of thrombin, 75-115 mg/mL of fibrinogen, and 3000 IU/mL of fibrinolysis inhibitor) was placed between the flap and its bed. In the FloSeal group, 0.5 mL of FloSeal (4000 IU of bovine thrombin per 5 mL of FloSeal) was placed between the flap and the wound bed before wound closure. All wounds were then closed with 4-0 polyglycolic acid (Dexon), and the animals were awakened. Table 1 provides the number of animals in each treatment group by the day the pedicle was severed. Division of the pedicle was accomplished on anesthetized animals by opening the inferior aspect of the incision, identifying the epigastric and femoral vessels, and then ligating them. The wound was closed with 4-0 polyglycolic acid. An assessment of percentage of flap survival was undertaken at 7 days. A viable flap was characterized by warm, pink, hair-bearing skin. Nonviable flaps were characterized by a dry, hard, hairless eschar. All flaps were of a standard size, with an individual template drawn for each animal. Flaps were evaluated for survival at 7 days following the intervention. The template was reapplied and the necrotic area etched into the templates. The percentage survival of the flap was calculated by dividing the area of viable flap by the total area of the flap and multiplying by 100.

Animals were excluded from the study if they died intraoperatively or during the immediate postoperative period. Animals who autocannibalized the flap were killed humanely and excluded from the data analysis.

The primary outcome measure was percentage survival of the flap. The difference in survival between groups was statistically analyzed using a 1-way analysis of variance with Tukey and Scheffe post hoc tests of significance. The data were analyzed using Statistical Product and Service Solutions software (SPSS Inc, Chicago, Ill). P<.05 was considered significant.

## RESULTS

All flaps survived the initial 2-hour ischemic insult. The mean±1 SD percentage survival for the flaps among groups is presented in Table 2. Among groups, there was no statistically significant difference in flap survival when the pedicle was severed on days 4, 5, 6, or 7 (Figure 1). In the Tisseel group (Figure 2), there was a statistically significant difference in flap survival when comparing day 4 with day 7 (P=.01) and day 5 with day 7 (P=.02). The FloSeal and Tisseel demonstrated no significant difference in revascularization during a 7-day period when compared with the control group. Among groups, no animal developed a hematoma under the flap. Seroma formation was equal among all groups.

## COMMENT

Free tissue transfer is often used to restore form and function to the patient following head and neck surgery. Successful transfer depends on a patent vascular pedicle,
until revascularization from the periphery of the surgical bed can occur. Revascularization is an integral component of wound healing and long-term flap survival. One component of this revascularization is the migration and proliferation of capillary endothelial cells. Endothelial cells in the wound bed and flap undergo migration and mitosis toward angiogenic factors. The ingrowth of vessels and the development of interconnections between the flap and its wound bed decrease the dependency of the flap on its vascular pedicle. As these vascular connections proliferate, there will be a point when the transferred tissue is no longer dependent on its vascular pedicle to survive. One method of determining the course and extent of the revascularization process is to ligate the vascular pedicle at various times and measure how much of the flap survives. Belmont et al demonstrated that if the pedicle is transected at 5 days, roughly 30% of the flap will survive. At 7 days, more than 80% will survive. Our control data are similar to those previously reported, with 63.0% survival at 4 days, 46.2% survival at 5 days, 72.5% survival at 6 days, and 85.6% survival at 7 days.

Many factors may interfere with the revascularization process. The presence of an impermeable obstruction by a foreign body, such as a Silastic (Dow Corning Corporation, Midland, Mich) sheet, will completely inhibit revascularization via mechanical obstruction. One example of iatrogenic interference with flap revascularization is photodynamic therapy. Photodynamic therapy results in rapid vascular stasis, thrombosis, hemorrhage, and anoxia-induced tumor cell death. Damage to the vascular endothelium results in impaired release of nitric oxide–related endothelial-derived relaxing factor and subsequent arterial vasoconstriction. This endothelial destruction will lead to basement membrane exposure, which in turn causes the adhesion of leukocytes and platelets. This cascade of events potentiates a local tissue inflammatory response. Vascular shutdown ensues. In the pedicled flap model, photodynamic therapy decreases wound healing and leads to an increase in seroma formation and infectious complications. In a free flap model, photodynamic therapy markedly reduced revascularization at the periods measured. Belmont et al demonstrated that in their control group, there was a significant difference in flap survival if the pedicle was ligated on day 5 (29% survival in the control group vs 14% survival in the treated group). After 6 days, 60% of the control flaps survived compared with 23% of the treated flaps. Finally, when the pedicle was ligated on day 7, more than 80% of the control flaps survived compared with only 20% of the flaps in the treated group. Seroma and hematoma formation was increased in all treated groups and was believed to contribute to the decreased rate of revascularization.

Another intervention that may potentially affect the revascularization of fasciocutaneous flaps is the interposition of a foreign material between the flap and the wound bed. This material may interfere by inciting inflammation, with resultant seroma formation, or by mechanical blockage. There are various synthetic and processed agents that can be applied to the flap bed to promote hemostasis, decrease seroma formation, or stimulate wound healing. Thrombin-containing substances are finding increased applications in head and neck reconstruction. Their ability to promote hemostasis or form a fibrin clot, thus acting as a tissue glue, has many potential advantages. In a previous study, it was demonstrated that these agents do not have an adverse effect on the flap’s tolerance to ischemia or a detrimental effect on flap survival. There remained, however, concern that inserting a foreign body between the flap and its bed could have an adverse effect on revascularization of the flap. This, in fact, was not substantiated by our study. Revascularization of the flap was not significantly different between the control, FloSeal, and Tisseel groups on days 4, 5, 6, and 7. It would seem that these substances do not act as a mechanical barrier to the ingrowth of vascular endothelium. Additional studies that evaluate the histological features of these changes are in progress.

In conclusion, FloSeal and Tisseel did not demonstrate any short-term detrimental effect on flap survival in the rat fasciocutaneous free flap model. Furthermore,
FloSeal and Tisseel did not have any significant effect on flap revascularization compared with the no-treatment control group. The intraoperative use of these agents for hemostasis does not interfere with flap revascularization and survival.

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The Fusion matrix hemostatic sealant (FloSeal) used in this study was provided by Fusion Medical Technologies, Inc, Fremont, Calif; and the Tisseel 2-component fibrin sealant (Tisseel) used in this study was provided by Baxter International, Deerfield, Ill.

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REFERENCES


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Quotable

Results! Why, man, I have gotten a lot of results. I know several thousand things that won’t work.

Thomas Alva Edison (1847-1931)
US Scientist