Experimental

Basic Fibroblast Growth Factor Expression following Surgical Delay of Rat Transverse Rectus Abdominis Myocutaneous Flaps

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Partial transverse rectus abdominis myocutaneous (TRAM) flap loss in breast reconstruction can be a devastating complication for both patient and surgeon. Surgical delay of the TRAM flap has been shown to improve flap viability and has been advocated in "high-risk" patients seeking autogenous breast reconstruction. Despite extensive clinical evidence of the effectiveness of surgical delay of TRAM flaps, the mechanisms by which the delay phenomenon occurs remain poorly understood. To examine whether angiogenic growth factors such as basic fibroblast growth factor (bFGF) may play a role in the delay phenomenon, the authors studied the expression of bFGF in rat TRAM flaps subjected to surgical delay. Thirty-five female Sprague-Dawley rats were randomly assigned to one of four TRAM flap groups: no delay ($n = 6$), 7-day delay ($n = 12$), 14-day delay ($n = 10$), or 21-day delay ($n = 7$). Surgical delay consisted of incising skin around the perimeter of the planned 2.5 × 5.0-cm TRAM flap followed by ablation of both superior epigastric arteries and the left inferior epigastric artery, thus preserving the right inferior epigastric artery (the nondominant blood supply to the rectus abdominis muscle of the rat). TRAM flaps were then elevated after 7, 14, and 21 days of delay by raising zones II, III, and IV off the abdominal wall fascia. Once hemostasis was assured, the flaps were sutured back in place. All flaps were designed with the upper border of the flap 1 cm below the xiphoid tip. Three days after the TRAM procedure, postfluorescein planimetry was used to determine percent area viability of both superficial and deep portions of TRAM flaps. All rats were euthanized and full-thickness TRAM specimens were taken from zones I, II, III, and IV for enzyme-linked immunoabsorbent assay analysis of bFGF levels. Statistical testing was done by *t* test (percent viability) and two-way analysis of variance (bFGF levels). All delayed flaps had significantly higher bFGF levels when compared with all nondelayed control flaps ($p < 0.05$). The bFGF levels were not different in the rats that received TRAM flaps 7, 14, or 21 days after delay surgery. There was also no significant difference in bFGF levels among zones I through IV. Control rats had more peripheral zone necrosis compared with all delayed TRAM rats. All delayed flaps had a significantly higher area of flap viability superficially than nondelayed control flaps ($p < 0.05$). There was no difference in deep flap viability. Surgical delay of rat TRAM flaps is associated with improved flap viability and significantly elevated levels of bFGF over nondelayed TRAM flaps at postoperative day 3 after TRAM surgery. The increases in bFGF noted at this time point suggests that bFGF may play a role in the improved TRAM flap viability observed after delay surgery. Further investigation is needed to evaluate the role bFGF may play in the delay phenomenon. (*Plast. Reconstr. Surg.* 113: 2030, 2004.)

Since its introduction by Holmström[1] and Hartrampf et al.[2] approximately 20 years ago, the unipedicled transverse rectus abdominis myocutaneous (TRAM) flap has been a mainstay in the arsenal of the reconstructive breast surgeon. It has allowed for the complete autogenous reconstruction of a breast after mastectomy, unlike the pedicled latissimus dorsi flap that often needs an implant to match the volume of the contralateral breast. Over time, there have been several technical modifications made to this flap that include the use of

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both pedicles (bipedicled TRAM), vascular augmentation or "supercharging" of a unipedicled TRAM, free TRAM, and the deep inferior epigastric perforator (DIEP) flap. Bipedicle TRAM flaps suffer from the morbidity associated with the loss of both rectus muscles and added bulk of the pedicle in the region of the lower sternum. The DIEP flap is able to spare much of the substance of the donor rectus muscle; however, the procedure is much more technically demanding than the unipedicled TRAM. The addition of microsurgical techniques has been suggested to improve the vascularity of flaps, but not all patients are good candidates for microsurgery. In addition, in this era of cost containment, the time invested for microsurgical breast reconstruction needs to be balanced against the use of a shorter, less costly pedicled TRAM flap.

As an alternative to microsurgical techniques of improving the blood supply to the TRAM flap, the technique of surgical delay has been advocated.[3–6] By selectively dividing the blood supply to a flap, the remaining vasculature is able to dilate with improved perfusion to the flap on subsequent elevation. This observed delay phenomenon has been used for years in flaps all over the body. Despite this, the mechanisms by which this occurs are still poorly understood. To begin to understand this process, basic fibroblast growth factor (bFGF), a well-known potent angiogenic molecule[7–12] was measured in a rat TRAM model.

Materials and Methods

Thirty-five female retired breeder Sprague-Dawley rats (Charles River, Cambridge, Mass.) with an average weight of 385 ± 8 g (mean ± SEM; range, 282 to 558 g) were randomly assigned to one of four TRAM flap groups: no surgical delay ($n = 6$), 7-day delay ($n = 12$), 14-day delay ($n = 10$), or 21-day delay ($n = 7$). All animals were acclimatized to laboratory conditions for a minimum of 3 days in the Duke vivarium, where temperature and light-dark cycles were maintained in compliance with our animal protocols for small animals.

All procedures were performed under intraperitoneal pentobarbital anesthesia (Nembutal sodium solution, Abbott 13-1406-4RL; Abbott Laboratories, North Chicago, Ill.), using an initial dose of 50 mg/kg followed by supplemental doses as needed. Body temperature of the animals was maintained by a heating pad both intraoperatively and postoperatively. The abdomens of all animals were shaved using clippers (Conair HC100NCS clippers; Conair, East Windsor, N. J.) and depilated with hair removal lotion (Nair lotion hair remover with baby oil; Church & Dwight, Princeton, N.J.) 3 days before any surgical delay or TRAM flap procedure. Abdomens were surgically prepared with an iodine solution (Allegiance Caliber povidone-iodine swab stick, no. 40000-040; Cardinal Healthcare, Toronto, Ontario, Canada) before any incisions were made.

Surgical delay of the TRAM flap consisted of incising the skin around the planned TRAM flap and dividing both superior epigastric arteries and the left inferior epigastric artery, thus preserving the right inferior epigastric artery. The inferior epigastric system is the nondominant blood supply in the rat rectus abdominis muscle. TRAM flaps were then raised at 7 days, 14 days, and 21 days after the delay procedure by a elevating zone II, III, and IV full-thickness flap off the anterior abdominal wall fascia (Fig. 1). Nondelayed TRAM rats underwent the skin incision, division of the vessels, and elevation of the flaps all at the same surgery. Once hemostasis was assured with a hand-held eye cautery (Perfectemp cautery, no. 0233; Bovie Medical Corp., St. Petersburg, Fla.), the flaps were inset using 6-0 nylon (Monosef United States Surgical/Syneture, Norwalk, Conn.) in a running continuous fashion. The template flap measured 2.5 × 5.0 cm with its superior boarder placed 1 cm below the xiphoid tip.

Three days after the TRAM procedure, all rats were anesthetized with an intraperitoneal dose of pentobarbital. Sterile 10% sodium fluorescein (Sigma F-6377; Sigma Chemical Co., St. Louis, Mo.) in saline was then injected intraperitoneally and superficial flap perfusion was traced onto clear acetate sheets under ultraviolet light. The flap was then removed and a tracing of the deep surface was made. Full-thickness TRAM flap specimens were then harvested from zones I, II, III, and IV. Additional specimens from the superior abdomen outside of the TRAM flap were also harvested as a control. All fresh specimens were placed in Tissue Tek Uni-cassettes (Sakura Finetechnical, Torrance, Calif.) and immediately frozen in liquid nitrogen. Frozen specimens were then stored in a −80°C freezer until enzyme-linked immunoabsorbent assay analysis of bFGF levels.

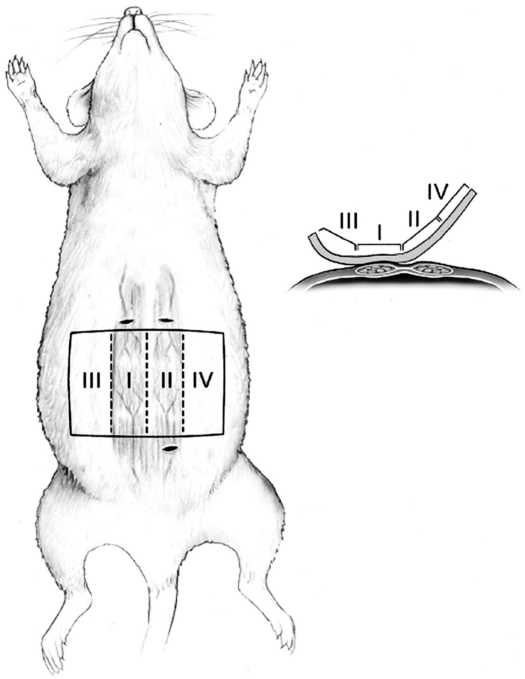

FIG. 1. Surgical delay of inferiorly based unipedicled rat transverse rectus abdominis myocutaneous (TRAM) flap. Surgical delay of the TRAM flap consisted of incising the skin around the planned 2.5 × 5.0-cm TRAM flap with its superior edge placed 1 cm below the xiphoid tip, dividing both superior epigastric arteries and the left inferior epigastric artery, thus preserving the right inferior epigastric artery (*left*). TRAM flaps were then raised at 7, 14, and 21 days after the delay procedure by elevating a zone II, III, and IV full-thickness flap off the anterior abdominal wall fascia (*right*). Nondelayed TRAM rats underwent the skin incision, division of the vessels, and raising of the flaps all during the same surgery.

bFGF Enzyme-Linked Immunoabsorbent Assay Analysis

Specimens were placed in 2-ml cryotubes. Lysis buffer plus protease inhibitors (5 ml at 4°C) were added. Each suspended sample was homogenized with an Omni International homogenizer (Omni International, Warrenton, Va.) for 1 minute at 13,000 rpm. The homogenate volume was transferred to a 1.7-ml microcentrifuge tube and centrifuged at 10,000 *g* (5 minutes at 4°C). The clear supernatant was then diluted with an equal amount of lysis buffer. Samples were run in duplicate wells according to the manufacturer instructions (200 μl/well) for bFGF enzyme-linked immunoabsorbent assay (Quantikine, R&D Systems Inc., Minneapolis, Minn.). Total protein content of the tissue lysate samples was determined using the Bradford assay.

Statistical Analysis

Statistical testing of bFGF levels was done by two-way analysis of variance and percentage of flap viability was analyzed using *t* test. All results are presented as the mean ± SEM. Probability values of less than 0.05 were considered statistically significant. No correction was made for multiple testing.

RESULTS

Delayed versus Nondelayed Flaps

All delayed flaps had significantly higher bFGF levels when compared with all flaps that were not delayed (Table I). In zone I, animals delayed 7, 14, and 21 days had bFGF levels of 93.2 ± 15.1 pg/mg, 93.8 ± 10.5 pg/mg, and 75.9 ± 11.2 pg/mg, respectively, whereas nondelayed flaps averaged 41.3 ± 16.2 pg/mg. Similarly, in zones III and IV, the delayed flaps had bFGF values that were significantly higher than all other nondelayed animals. The only exception to this was in zone II, where the nondelayed bFGF level (61.3 ± 19.4 pg/mg) had both a slightly higher mean and standard error compared with zones I, III, and IV. Combining this with the 21-day delay group having a slightly lower value of 66.0 ± 8.2 pg/mg compared with its other flap zones made this one comparison between zone II in the nondelayed TRAM versus the 21-day delay TRAM

TABLE I
Basic Fibroblast Growth Factor Levels*

Delay (days)	Zone				
	Control	I	II	III	IV
0	111.8 ± 5.0†	41.3 ± 16.2	61.3 ± 19.4	27.2 ± 11.3	24.1 ± 15.0
7	101.2 ± 18.9†	93.2 ± 15.1‡	88.2 ± 15.2‡	86.1 ± 14.5‡	94.0 ± 15.3‡
14	124.8 ± 13.2†	93.8 ± 10.5‡	94.5 ± 10.4‡	75.5 ± 5.9‡	77.5 ± 11.1‡
21	151.5 ± 13.3†	75.9 ± 11.2‡	66.0 ± 8.2	77.3 ± 8.7‡	79.2 ± 11.3‡

* All intragroup and intergroup comparisons revealed that all control tissue had significantly higher levels of bFGF than their accompanying flap zones. Delayed flaps had significantly higher bFGF levels compared with the corresponding nondelayed flap zone, except for zone II, where the nondelayed value was significantly higher than all other nondelayed TRAM flap zones I, III, and IV. The bFGF levels (pg/mg protein) are presented as the mean ± SEM.

† $p < 0.05$ versus zones I through IV at all delay times using analysis of variance.

‡ $p < 0.05$ versus nondelayed flaps from the same zone using analysis of variance.

not significantly different. The remaining comparisons within zone II (i.e., the 7- and 14-day delay rats) had significantly higher bFGF levels (88.2 ± 15.2 pg/mg and 94.5 ± 10.4 pg/mg, respectively) compared with the nondelayed animals (61.3 ± 19.4 pg/mg).

Length of Delays

The bFGF levels were not different in the rats that received TRAM flaps 7, 14, or 21 days after delay surgery. Nor was there a significant difference in bFGF levels among zones I through IV. In zone I, bFGF levels in 7-, 14-, and 21-day delays averaged 93.2 ± 15.1, 93.8 ± 10.5, and 75.9 ± 11.2 pg/mg, respectively. Zone III and IV values were similar at these three lengths of surgical delays. Again, zone II was the exception to this, as the 21-day delay bFGF level averaged 66.0 ± 8.2 pg/mg, significantly less than levels than 88.2 ± 15.2 pg/mg and 94.5 ± 8.2 pg/mg obtained for 7- and 14-day delays, respectively.

Flap Viability

All delayed flaps had significantly greater percentages of flap viability compared with all nondelayed TRAM flaps (Table II). The necrosis in these nondelayed rats occurred in the more peripheral zones (zones III and IV). In the early postoperative period, congestion of nondelayed flaps was frequently noted, whereas congestion was much less common in delayed TRAM flaps (Fig. 2). At the time of harvest, nondelayed TRAM flaps frequently exhibited necrosis of zone IV and to a lesser extent, zone III (Fig. 3). Delayed flaps demonstrated only marginal loss if any at all in zone IV (Fig. 4).

DISCUSSION

As increasing numbers of patients seek autogenous breast reconstruction, a delayed TRAM has been advocated as an alternative to microsurgical free TRAM flap transfer, particularly in "high-risk" patients.[13,14] Surgical delay of TRAM flaps is a simple, low-morbidity alternative to microsurgical vascular augmentation or "supercharging" and free TRAM flaps. At our institution, a review of 76 consecutive breast reconstructions using a unipedicled TRAM flap preceded by a routine delay procedure 2 weeks before flap transfer revealed a 6.5 percent partial flap (fat) necrosis rate without any occurrence of total flap loss.[15] These numbers compare favorably with total free TRAM flap loss rates between 0 and 10 percent and partial flap or fat necrosis rates after free TRAM flap transfer of up to 7 percent.[16–19]

TABLE II
Percentage of Flap Viability*

Delay (days)	Portion of TRAM	
	Superficial	Deep
0	55.0 ± 12.0	98.4 ± 0.8
7	88.8 ± 7.7†	98.7 ± 1.2
14	98.8 ± 1.2†	100.0 ± 0.0
21	98.5 ± 1.3†	100.0 ± 0.0

* All superficial portions of delayed flaps had significantly greater percentages of flap viability compared with all nondelayed TRAM flaps.
† $p < 0.05$ versus nondelayed flaps using t test.

The delay of TRAM flaps can be performed many different ways, including complete division of skin and ipsilateral or bilateral dominant pedicle ligation or division. With the advent of minimally invasive surgery, there has been the development of endoscopic,[20] percutaneous embolization,[21] and even chemical[22] techniques. Despite continued interest in the delay phenomenon, the mechanism by which this occurs remains poorly understood. As we begin to understand angiogenesis during embryogenesis, tumor growth, and wound healing,[7,23] angiogenic growth factors such as bFGF and vascular endothelial growth factor may provide new and appealing explanations for the increased vascularity seen following the surgical delay of the TRAM and other flaps.

bFGF, also known as fibroblast growth factor (FGF)-2, belongs to a group of 19 structurally related proteins.[24,25] It was initially purified from bovine pituitary extracts[26] and, like other FGF isoforms, mediates its biological effects by binding to specific transmembrane tyrosine kinase receptors (i.e., FGF receptors 1, 2, and 3),[27] although there may also be distinct FGF receptor-independent mechanisms of action as well.[28]

bFGF plays key roles in the development, remodeling, and disease states in almost every organ system.[29] It has been found to play a role in the processes of embryonic vasculogenesis and angiogenesis[7] and, along with vascular endothelial growth factor, is considered the most potent angiogenic growth factor currently known.[7,23] It is synthesized by all squamous carcinomas and is being investigated as to its role in the angiogenesis in squamous cell carcinoma of the tongue[8] and esophagus.[9] Elevated levels of bFGF are associated with neovascularization in prostatic adenocarcinoma,[10] and ad-

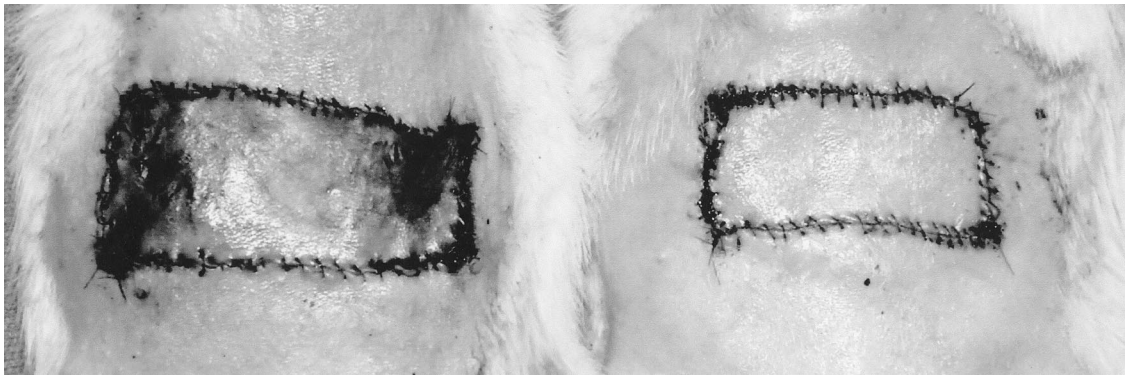

FIG. 2. Delayed versus nondelayed transverse rectus abdominis myocutaneous (TRAM) flaps in the immediate postoperative period. Notice the congestion in the nondelayed TRAM flap (*left*) not present in the delayed TRAM flap (*right*). Also note that the congestion in the TRAM flap is most evident in peripheral zones III and IV.

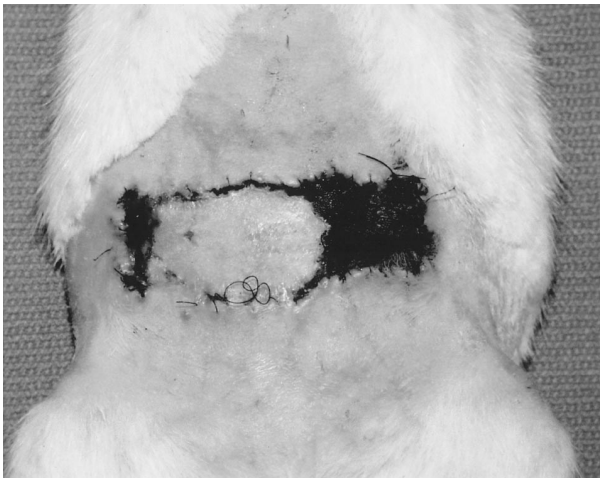

FIG. 3. Nondelayed transverse rectus abdominis myocutaneous (TRAM) flap before harvest. The entire zone IV is necrotic and replaced with eschar. There is also some mild evidence of ischemia at the periphery of zone III.

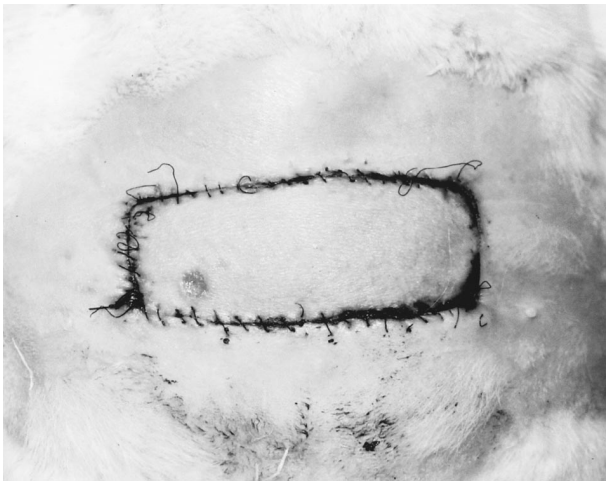

FIG. 4. Seven-day delay of transverse rectus abdominis myocutaneous (TRAM) flap before harvest. All zones of the flap are viable, with slight necrosis at the peripheral corner of zone IV.

ministration of soluble FGF receptors results in impaired pancreatic adenocarcinoma growth in mice, indicating its critical role in tumor anogiogenesis.[11] It also seems to play a role in angiogenesis and metastasis of melanoma.[12] Could it be possible that bFGF also plays a role in the improved vascularity after surgical delay?

In this preliminary study, we modified the rat TRAM flap as described by Özgentas et al.[6] to create a nondominant, unipedicled, right TRAM flap to study the role that surgical delay has on the expression of angiogenic growth factor bFGF. The bFGF levels were significantly elevated in association with the surgical delay of rat TRAM flaps. Delay times greater than 1 week did not result in any significant increase in bFGF levels, nor did they result in any significant changes in flap viability. In addition, we found that the surgical delay of TRAM flaps in our rodent model was associated with an improved vascularity to the flap as evidenced by less congestion seen in the early postoperative period and improved flap viability at 3 days after TRAM surgery. Restifo et al.[3] also showed improved blood flow and ultimately better flap survival in their rat TRAM model. These findings are also consistent with anatomic studies by Taylor et al.[5] that demonstrated a dilation of the arterial choke vessel system in the rectus abdominis muscle and venous valve incompetence that allowed venous drainage toward the nondominant epigastric system. Moreover, there seemed to be no further gains in vascularity or significantly less necrosis with surgical delays longer than 1 week. This has been observed by those performing TRAM breast reconstruction, and as a result, one of our senior authors routinely delays his TRAM flaps for no more than 2 weeks.[13]

This early investigation has shown that delay of a TRAM flap in this rodent model is associated with elevated bFGF levels compared with nondelayed TRAM flaps and greater flap viability 3 days after final TRAM surgery; however, there are still many unanswered questions. Most importantly, what is happening with bFGF levels after surgical delay before the TRAM surgery? Presumably, if bFGF is involved in stimulating angiogenesis in the delay of TRAM flaps, bFGF should be elevated at time points after delay surgery and the ischemic stimulus and should be elevated over baseline bFGF levels. Furthermore, what alterations in bFGF are occurring in each flap zone after the delay of TRAM surgery? The zone-specific alterations in bFGF after this surgical delay and before definitive TRAM surgery represent another area for further study.

CONCLUSIONS

Surgical delay of TRAM flaps is associated with improved flap viability and significantly elevated levels of bFGF over nondelayed rat TRAM flaps at postoperative day 3 after TRAM surgery. The increases in bFGF noted at this time point suggest that bFGF may play a role in the improved TRAM flap viability observed after delay surgery. Moreover, there is no significant additional benefit of surgical delay in the rat TRAM model beyond 1 week. Further study is warranted to explore the mechanisms by which the delay phenomenon occurs and to evaluate the role that bFGF may play in the delay phenomenon.

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