The Delay Phenomenon: Is One Surgical Delay Technique Superior?

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Background: Surgical delay remains a common method for improving flap survival. However, the optimal surgical technique has not been determined. In this article, we compare flap perfusion, viable surface area, and flap contraction of 2 surgical delay techniques.

Methods: Male Sprague-Dawley rats were divided into 3 groups. In the incisional surgical delay group (n = 9), a 9×3 cm dorsal flap was incised on 3 sides without undermining, leaving a cranial pedicle. In the bipedicle surgical delay group (BSD, n = 9), a 9×3 cm dorsal flap was incised laterally and undermined, leaving cranial and caudal pedicles. Control group (n = 16) animals did not undergo a delay procedure. Ten days following surgical delay, all flaps for all groups were raised, leaving a cranial pedicle. A silicone sheet separated the flap and the wound bed. On postoperative day (POD) 7, viable surface area was determined clinically. Contraction compared to POD 0 was measured with ImageJ software. Perfusion was measured with Laser Doppler Imaging. The Kruskal-Wallis with Dunn's multiple comparisons test was performed for group comparisons.

Results: BSD preserved significantly more viable surface area on POD 7 (13.7 ± 4.5 cm^2) than Control (8.7 ± 1.8 cm^2; $P = 0.01$). BSD also showed significantly less contraction ($21.0\% \pm 13.5\%$) than Control ($45.9\% \pm 19.7\%$; $P = 0.0045$). BSD and incisional surgical delay showed significantly increased perfusion compared with Control on POD 0 ($P = 0.02$ and 0.049, respectively), which persisted on POD 3. This trend resolved by POD 7.

Conclusion: BSD showed improved early perfusion, increased viable surface area, and reduced contraction compared to control, suggesting that BSD is the superior flap design for preclinical modeling. *(Plast Reconstr Surg Glob Open 2017;5:e1519; doi: 10.1097/GOX.0000000000001519; Published online 23 October 20174.)*

INTRODUCTION

As millions of flap surgeries involving surgical flaps are performed each year,[1–3] surgical delay remains a valuable technique for increasing the viable surface area of pedicle, regional and random flaps.[4] With the advent of free tissue transfer, which can also undergo surgical delay, this procedure is not as commonly used as in the past. Nevertheless, surgical delay still provides a valuable and clinically used method to safely increase the amount of tissue transferred in patients that are too ill to undergo free tissue transfer or when more adjacent skin is required to provide a better color and contour match than can safely be elevated by a local flap such as in facial reconstruction. Delay entails a partial disruption of the blood supply to the flap 7–14 days before elevation.[5] In a pedicle flap, delay is commonly performed by either incising the 3 edges of the flap without undermining (incisional surgical delay, ISD), or incising the lateral edges of the flap and undermining (bipedicle surgical delay, BSD). By inducing transient tissue ischemia before elevation, skin flap blood flow from the dermal and subdermal plexus is generally improved. However, the mechanism behind the delay phenomenon is poorly understood. Current hypotheses include vascular dilation of choke vessels and

reorganization, sympathetic denervation, neovascularization through angiogenesis and vasculogenesis (although this short time frame does not allow for completion from the flap pedicle), metabolic adaptation to hypoxia and ischemia, and intracellular mediators.[6–14] Given the drawbacks of 2-stage surgical preconditioning, recent work has focused on alternative nonsurgical delay techniques such as thermoregulation[15] and preconditioning using pharmacologic agents such as adenoviral and acute vasodilatory vectors.[16,17] These other delay procedures are routinely compared with surgical delay as a gold standard, yet no study has performed a standardized comparison of the 2 most common types of surgical delay techniques.

Here, we utilize a novel modification of the murine McFarlane flap[18] to compare ISD and BSD. We then characterize these flaps using surface area and contraction measurements and laser Doppler perfusion analysis.

MATERIALS AND METHODS

Experimental Flap Creation

All animal protocols and husbandry were approved by the Institutional Animal Care and Use Committee of Stony Brook University. Eight- to 10-week-old male Sprague-Dawley rats (Charles River, Wilmington, Mass.) weighing 275–300 g were anesthetized using 3–5% isoflurane and secured in a prone position on a sterile field with their arms and legs fully extended. The dorsal hair in a 13 cm × 5 cm area centered 1 cm cranial to the scapulae and extending 1 cm caudal to the iliac crests, underwent trichotomy using an electric razor and Nair (Church & Dwight, Princeton, N.J.). Care was taken to avoid skin trauma or irritation with the clippers and to limit Nair exposure time.

Three treatment groups were established: control (n = 16), ISD (n = 9), and BSD (n = 9). To simulate surgical delay in the ISD group, a modification of the McFarlane flap described by Holzbach et al.[8] was utilized. In the ISD group, 10 days before flap elevation, a 3 × 9 cm cranially based pedicle flap was created by incising the 3 caudal flap margins without undermining the flap. This incision was closed with nylon sutures at 1 cm intervals. Animals within the BSD group, 10 days before flap elevation, received marginal longitudinal incisions to create a 3 × 9 cm cranially and caudally based bipedicle flap. This flap was undermined via blunt dissection. The resulting flap was closed with nylon sutures at 1 cm intervals (Fig. 1). Animals in the control group received no delay intervention before flap elevation. Following surgery, animals were allowed to recuperate normally with nonsteroidal anti-inflammatory drug pain relief postoperatively (Ibuprofen, 100 mg, ad libitum in drinking water and Ketorolac, 3 mg/kg, 3×/d via subcutaneous injection).

At the time of pedicle flap elevation, each animal was anesthetized with 3–5% isoflurane by face mask. A cranially based 3 × 9 cm modified McFarlane flap was created by incising through the panniculus carnosus, leaving a cranially based pedicle 1 cm caudal to the scapulae.

This area retains blood flow supplied by 1 or both branches of the thoracodorsal artery that runs at the pedicle base.[19] The flap was undermined using blunt dissection and elevated. A 3.5 × 9.5 × 0.0254 cm sterile silicone sheet (Technical Products of Georgia, Atlanta, Ga.) was placed between the wound bed, flap, and surrounding skin tissue to prevent vascular ingrowth from the subcutaneous and lateral margins. The flap was then reapproximated at 1 cm intervals with the silicone between the wound margins and excess silicone was trimmed away.

Euthanasia was performed 7 days post-flap via CO_2 asphyxiation followed by cervical dislocation.

SURFACE AREA AND CONTRACTION ASSESSMENT

Clinical assessments were performed on postoperative day (POD) 7. For our purposes, necrotic tissue was defined as dark and dusky skin lacking capillary refill and turgor compared with normal skin (Fig. 2). The total viable surface area was measured by a blinded single investigator (R.P.G., Fig. 3A). Contraction was measured using ImageJ (NIH, Bethesda, MD) software as the percentage difference between the measured surface area of the flap on POD 7 and the initial flap area on POD 0 (Fig. 3B). All values are expressed as mean ± SD.

LASER DOPPLER IMAGING ANALYSIS

Perfusion analysis focused on the middle/ischemic third of the flap, which commonly contained the interface between viable and necrotic tissue. One day before flap elevation, immediately after flap elevation, on POD 3, and POD 7, laser Doppler imaging (LDI) was performed using the Perimed PIM3™ (Perimed, Kings Park, N.Y.), with the scanning monitor head centered at mid-flap. Imaging at 255 × 555 pixels was performed over an area of 5 × 11 cm in triplicate, with aggregate scan times of 6 minutes per flap.

LDI analysis was performed using the Perimed™ analysis software (Perimed, Kings Park, N.Y.). Perfusion is measured as the average pixel return (perfusion values) of three 3 × 3 cm regions of interest: proximal/viable, middle/ischemic, and distal/necrotic. This technique is an accurate representation of previously described flap "choke vessel" anatomy.[19] Perfusion was reported in arbitrary perfusion units (APU) ± standard error, which correspond to signal return from erythrocytes in viable skin.[20]

Statistical Analysis

Statistical analysis was performed using Prism 7.0™ (GraphPad, LaJolla, Calif.). Following the establishment of normality with the Kolmogorov-Smirnov test, data sets were reviewed for outliers with the Grubbs Test for Outliers (alpha < 0.05). Statistical significance for clinical and perfusion measurements was determined using a nongaussian Kruskal-Wallis test with Dunn's post-hoc test for multiple comparisons between groups. A P value < 0.05 was considered significant in all cases.
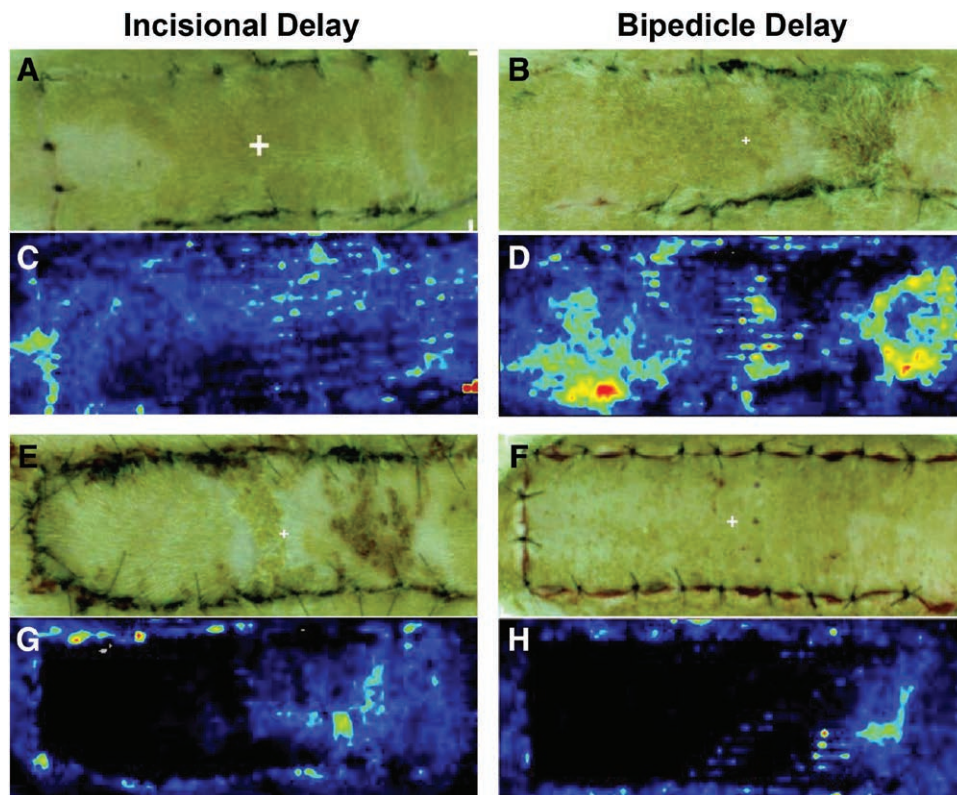
Incisional Delay
Bipedicle Delay

Fig. 1. Representative photographs and Doppler scans of ISD and BSD flaps. A, B, Photographs of representative animals that underwent ISD and BSD before flap elevation. C, D, Corresponding LDI scans of flap area pre-Elevation. E, F, Photographs of representative animals that underwent ISD and BSD immediately after flap elevation. G, H, Corresponding LDI Scans of flaps immediately after flap elevation. ISD shows moderate perfusion in anterior (Right) areas of the flap, denoted by green and yellow regions before flap elevation. Areas of heightened perfusion, denoted by yellow and red regions, can be seen both anteriorly and posteriorly (Left) in the BSD flaps before elevation. In both groups, perfusion only persists anteriorly immediately after flap elevation.

RESULTS

Viable Surface Area

BSD (13.7 ± 4.5 cm^2; $P = 0.01$) had a significantly larger viable surface area compared with control (8.7 ± 1.8 cm^2; Fig. 3A). No significant differences were observed when BSD was compared with ISD (12.4 ± 3.6 cm^2; $P = 0.60$) or when ISD was compared with Controls ($P = 0.30$).

Contraction Analysis

Total flap contraction on POD 7 was similar between ISD ($39.2\% \pm 11.0\%$) and Control ($45.9\% \pm 19.7\%$; $P > 0.99$), whereas BSD ($21.0\% \pm 13.5\%$; Fig. 3B) had significantly less contraction than Controls ($P = 0.0045$).

Perfusion Analysis

Pre-Elevation average perfusion, measured 10 days following the initial delay procedure, was significantly lower in the BSD group (142.9 ± 35.3 APU; $P = 0.0002$) compared with Control (266.6 ± 76.0 APU; Fig. 4A). ISD (207.4 ± 50.84 APU) was not significantly different than Control ($P = 0.22$) or BSD ($P = 0.17$). However, immediately after flap elevation, both ISD (63.3 ± 22.1 APU; $P = 0.049$) and BSD (66.7 ± 23.5 APU; $P = 0.02$) had signifi-

cantly greater perfusion levels than Controls (44.3 ± 9.2 APU; Fig. 4B). This significance persisted on POD 3 (BSD 61.1 ± 36.2 APU; $P = 0.002$; ISD 44.5 ± 22.32 APU; $P = 0.02$; versus Controls 20.4 ± 7.8 APU; Fig. 4C) but was lost on POD 7 (ISD 25.9 ± 20.4 APU; $P = 0.18$; BSD 23.1 ± 10.6 APU; $P = 0.10$; versus Controls 13.0 ± 9.2 APU; $P > 0.05$; Fig. 4D).

DISCUSSION

The characterization and comparison of current surgical delay techniques is difficult because of the inconsistency of previously described animal surgical delay models. Here, we sought to compare 2 commonly used surgical delay techniques utilized in a novel modification of the McFarlane oversized murine dorsal skin flap previously used by our group.[20] Animals that underwent BSD had significantly larger viable surface areas compared with Control ($P = 0.01$) while ISD showed a slight trend toward increased viable surface area but did not reach significance ($P = 0.3$). Both experimental groups had reduced perfusion before flap elevation in the middle third of the flap, a finding that was expected because both delay techniques entailed the surgical disruption of local blood
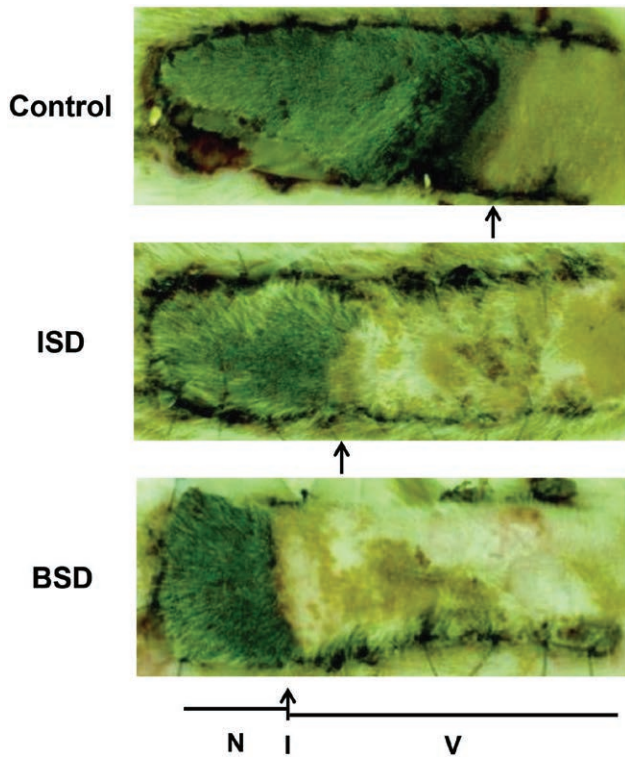
Fig. 2. Representative photographs of flap necrosis on POD 7 for each group. Black arrows denote mean interface (I) between viable (V) and necrotic (N) tissue.

supply.[19] However, both delay techniques then displayed greater middle third perfusion immediately after flap elevation, a finding that became insignificant within a week of surgery. BSD differed from ISD in 2 respects: (1) Pre-Elevation = perfusion levels in the middle third of the flap were significantly lower in BSD compared with ISD; and (2) contraction measured on POD 7 for BSD was significantly less than Control while ISD was not.

Each of the surgical delay methods tested in this study retained 2 independent blood supplies to the flap. After

ISD, the flap is perfused by the cranial pedicle as well as from the subcutaneous tissue, which is left intact. BSD maintains flap perfusion from the cranial and caudal pedicles. Although both methods result in similar increases in viable surface area 7 days after flap elevation, of particular interest is that BSD flaps had reduced contraction compared with Control, whereas ISD did not. This clinical significance outlasted the perfusion benefits of flap delay, which, while still a positive trend compared with Control, was not significant on POD 7. The favorable contraction outcome of BSD flaps is likely caused by the observable second peak perfusion area in the dorsal flap, a finding that was absent in ISD (Fig. 1D). We hypothesize that the increased perfusion of the caudal zone of the flap may mitigate the contraction that typically occurs in this region. Further characterization of these models will focus on validating and further describing this finding.

This work supports the use of BSD over incisional surgical delay as this technique demonstrated improved viable surface area with reduced contraction 1 week after flap elevation. To improve clinical outcomes, this work suggests that bipedicle delay should be utilized when a surgical delay procedure is planned. Future efforts to improve tissue viability and achieve maximal clinical benefits may include the combination of BSD with pharmacological intervention[21–24] and/or gene therapy intervention.[25–27]

Although our model appears to be a consistent representation of surgical delay, we note several limitations inherent to our study, and to the model itself. Although we are able to demonstrate increased clinical salvage and perfusion changes in delay-treated flaps, we do so with a small sample size. We therefore may not be powered well enough to identify all differences between ISD and BSD. Additionally, we cannot histologically evaluate the effects of 2-stage surgical delay compared with Control, particularly within common viable regions. Due to the pilot nature of this study, we did not characterize our surgical delay on a molecular level.
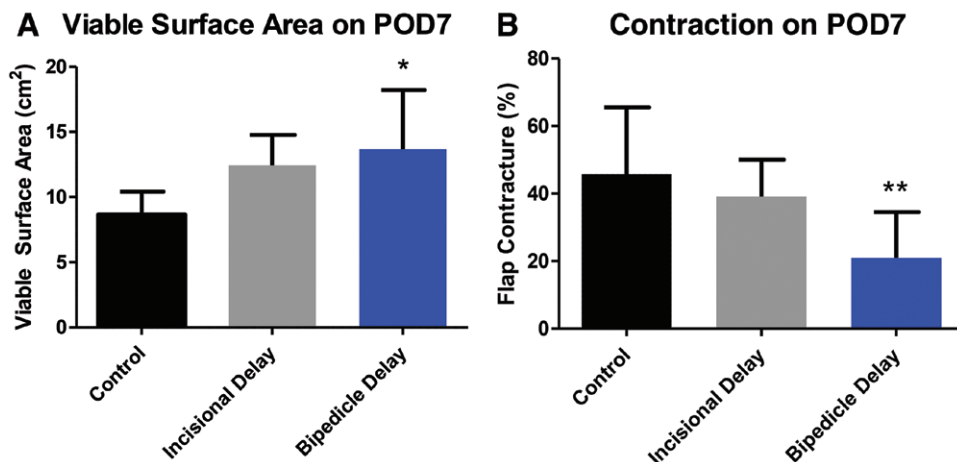

Fig. 3. Quantification of viable surface area and flap contracture. A, Viable surface area, defined by non-necrotic tissue, was determined by blinded observer for Control (n = 16), Incisional delay (n = 9) and bipedicle delay (n = 9) on POD 7. B, Contraction, defined as percentage reduction in flap surface area on POD 7 compared with POD 0 area. *$P < 0.05$, **$P < 0.01$ compared with Control.
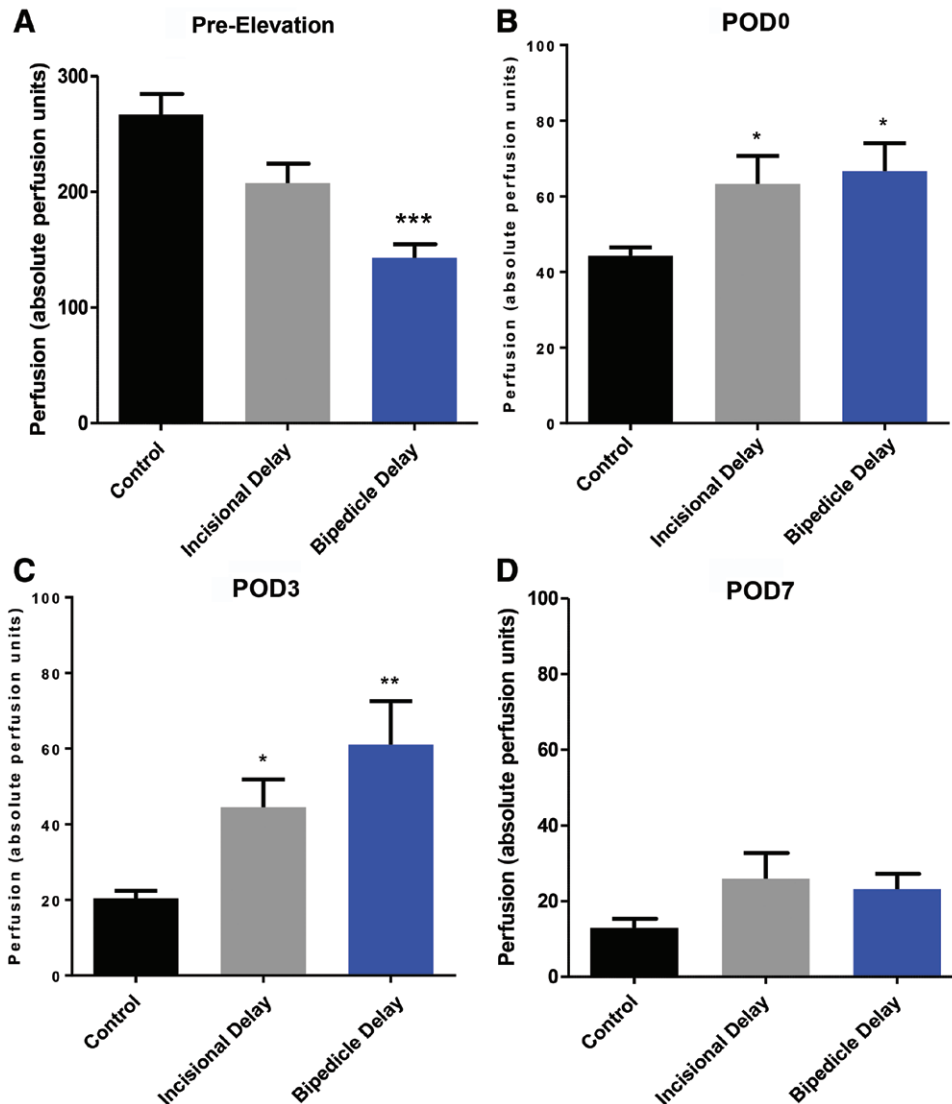
A Pre-Elevation

B POD0

C POD3

D POD7

Fig. 4. Quantification of perfusion levels in the middle/ischemic region of the skin flap throughout the time course. LDI perfusion levels were measured within a 3 × 3 cm region at the center of the skin flap. A, 1 Day preflap elevation/9 days postdelay procedure and on POD 0 (B), POD 3 (C), and POD 7 (D). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with Control.

Prior work suggests that increased nitric oxide expression may play a role in the acute period following the first stage of surgical delay.[21] Future work will attempt to validate this observation, as well as characterize late-stage expression of angiogenic and vasculogenic factors. Finally, rat models are nonoptimal representations of human anatomy and physiologic response. We intend to translate these techniques into porcine models, where the skin anatomy is more in line with that of humans.

CONCLUSIONS

In this article, we describe our initial findings as to the impact of surgical delay on the ultimate viability of modified McFarlane murine skin flaps. We are able to demonstrate that flap delay leads to significantly increased clinical salvage and perfusion compared with Control. Although this study serves as a purely clinical description of surgical delay, future work will focus on the histologic and molecular characterization of surgical delay, as well as the addition of other commonly utilized delay techniques. We hope that in the future this model may serve as a viable testing platform for the evaluation of new surgical delay techniques, as well as a more reasonable comparison of surgical and pharmacologic delay.

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