Development of surgical techniques for preparation of in vitro-isolated perfused porcine skin flaps for percutaneous absorption studies

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SUMMARY

We developed a single-pedicle, axial pattern tubed skin flap that could be transferred to an in vitro perfusion apparatus. On the basis of results of prosections, angiography, contact radiography, and surviving-length studies, it was concluded that a single-pedicle, axial pattern skin flap measuring 4 cm × 12 cm incorporating the caudal superficial epigastric artery would survive to its entire length. Subsequently, a surgical (stage 1) procedure was developed for the routine preparation of single-pedicle, axial pattern tubed skin flaps. Healing after the stage-1 procedure was evaluated by visual inspection and fluorescein angiography. Stage-1 procedures were performed successfully 136 of 144 (94%) times. A second surgical (stage 2) procedure was developed for routine cannulation of the caudal superficial epigastric artery and harvest of the tubed skin flap. Stage-2 procedures were performed successfully 136 of 144 (94%) times.

Successful in vitro perfusion of axial pattern porcine tubed skin flaps has been reported, along with their application for pharmacologic, toxicologic, metabolic, and physiologic studies of skin. This model uses viable epidermis and intact microcirculation, which allows percutaneous absorption studies to be conducted optimally. In addition, the use of the isolated perfused porcine skin flap (IPPSF) is an alternate surgical model that allows toxicity studies to be performed humanely.

Axial pattern skin flaps are supplied by discrete, direct cutaneous vasculature. Such skin flaps have been raised on pigs for experimental purposes, and single-pedicle, axial pattern tubed skin flaps have been used in reconstructive surgery in human beings and dogs. Pigs were selected for development of the IPPSF because the anatomy and physiologic features of pig skin is considered to be most similar to human skin, and data generated from IPPSF studies could be validated by comparison to in vivo percutaneous absorption studies on pigs.

The purpose of the study reported here was to develop a single-pedicle, axial pattern tubed skin flap that could be transferred to an in vitro perfusion apparatus. In this report, we describe the stepwise characterization of a suitable donor site and the surgical techniques used for routine preparation of the IPPSF.

Materials and Methods

Experiment 1 (prosection of cutaneous vasculature to determine donor site for single-pedicle, axial pattern tubed skin flap)—Two weanling female Yorkshire-crossbred pigs, weighing approximately 40 kg, were anesthetized with sodium pentobarbital administered iv to effect. Heparin (5,000 IU) was administered iv. The carotid artery in each pig was cannulated and exsanguination was performed. The jugular vein was cannulated, the pig was positioned in dorsal recumbency with the limbs extended, and embalming fluid was infused via the carotid artery under gravity flow. Embalming was completed when clear jugular effluent was obtained. Colored, injectable latex (approx 800 mL) was injected into the carotid artery (red) and the jugular vein (blue). The pellets were resected, pinned or stretched to original size, measured, and photographed to determine the cutaneous vascular pattern in the caudal abdominal region (Fig 1), and at 2 described skin flap donor sites, that is, the gluteal region and the lateral thoracic region.

Experiment 2 (in vivo angiography to confirm cutaneous vascular pattern in caudal abdominal region)—Two weanling female Yorkshire-crossbred pigs were given atropine sulfate (0.04 mg/kg of body weight, im) and xylazine hydrochloride (0.2 mg/kg, im). Anesthesia was induced with ketamine hydrochloride (11 mg/kg, im) and maintained with halothane delivered by endotracheal tube. The femoral and caudal superficial epigastric arteries (CSEA) were exposed. The arteries were cannulated sequentially to allow infusion of 60% iothalamate meglu-
Figure 1—Illustration of vascular anatomy of the caudal abdominal region in weanling female pigs. The caudal superficial epigastric artery has a distribution pattern consisting of an origin (a); caudomedial branches (b); and its cranial extension with lateral (c) and craniomedial (d) branches. The 2 venous systems consist of paired venae comitantes (arrow, inset); and the cranial superficial epigastric venous system (e), with an anastomotic branch to the deep circumflex iliac vein (f) and a caudal extent that terminates in the region of the caudal mammae (g).

mine for in vivo angiography of the cutaneous vasculature (Fig 2). After completion of these studies, 1 pig was heparinized and exsanguinated. The thoracic aorta was cannulated, and 2 to 3 L of micropulverized barium sulfate (30% w/v)5-gelatin (4% w/v)6 solution was injected until perfusate appeared on the cut surface of small incisions on the distal portions of the extremities. The cadaver was stored at 4 C to promote solidification of the gelatin; thereafter, the abdominal pelt was resected and contact-radiographed to determine the architecture of its cutaneous vasculature.6

Experiment 3 (determination of surviving lengths of skin flaps raised under various vascular conditions)—Six weanling female Yorkshire-crossbred pigs were anesthetized as described in experiment 2 and prepared for aseptic surgery. Random (n = 2) and axial (n = 10) pattern skin flaps that measured 12 cm x 32 cm (1 peninsular flap),4 cm x 32 cm (9 flaps), or 4 cm x 30 cm (2 flaps) were raised (Table 1). The base of each skin flap was located at the caudal teat, and its medial margin was approximately 1 cm lateral to the mammae. Axial pattern skin flaps incorporated the CSEA with (peninsular) or without (island) an intact skin bridge; random pattern skin flaps had an intact skin bridge, but the CSEA and associated venae comitantes were ligated. The skin flap was returned to its donor site and the wound was closed.

Legend for illustrations on facing page

Figure 2—Illustration of stage-1 procedure.

a—Skin incisions for an island tubed skin flap measuring 4 cm x 12 cm are outlined in the caudolateral epigastric region. Notice the superficial inguinal lymph node (large arrow) and the anastomotic branch of the deep circumflex iliac vein (small arrows), which are adjacent to the proposed skin flap. Points A, A', B, and B, are marked on the skin for reference during subsequent manipulations.

b—Using skin hooks, the wound margins of the medial incision have been retracted for direct visualization of the caudal superficial epigastric artery (arrow). Caudal medialized branches supplying the caudal mammae have been ligated and divided.

c—Scalpel dissection of the subcutaneous tissue and skin flap elevation are done. Division of the skin bridge and extensive dissection of the subcutaneous tissue are done to create an island skin flap (inset). Notice the direct cutaneous vasculature within the remaining subcutaneous tissue bridge (arrow).
Figure 2—Illustration of stage-1 procedure (continued).

d—The caudal skin incision is sutured in a simple continuous pattern. Modified 3-point sutures (inset) are used to anchor the corners.
e—The tubed skin flap is formed (A-A, and B-B₁ are joined) and closed in a simple continuous pattern.
f—Starting 2.5 cm cranial to the base of the tubed skin flap (B-B₁), the subcutaneous tissues are closed with vertically oriented, interrupted sutures. The superficial subcutaneous tissue is closed (inset).
g—A mattress-type suture is used to appose the incision where the wound margins of the tubed skin flap and donor site meet.
h—The remaining skin incisions are closed and the free end of the tubed skin flap is affixed to the abdomen with a simple interrupted suture.
primarily. Five milliliters of 10% fluorescein was infused. Using described photographic methods, fluorescein studies were evaluated at 12 and 20 minutes after its infusion for prediction of surviving skin flap length. The line demarcating perfused vs nonperfused portions (predicted surviving length) of the skin flaps was identified, and its distance from the base of the skin flap was measured. Two to 7 days later, each pig was anesthetized, fluorescein studies were repeated, and the surviving length of each skin flap was recorded. Thereafter, heparin was administered IV. The CSEA for axial pattern skin flaps was cannulated, the skin flap was resected, and barium sulfate-gelatin infusion under controlled constant pressure (approx 180 mm of Hg) was done until perfusate appeared at the cut edges of the skin flap. In pigs with random pattern skin flaps, barium sulfate-gelatin infusion was performed as described in experiment 2. The skin flaps were resected, pinned to original size, and contact-radiographed. Measurements obtained from the fluorescein, surviving length, and angiographic studies were compared.

**Experiment 4 (development of single-pedicle, axial pattern tubed skin flaps based on the CSEA, stage-1 procedure)—**Nine weanling female Yorkshire-crossbred pigs averaging < 35 kg in weight (range, 22 to 60 kg) were anesthetized and prepared for routine aseptic surgery. Three pigs were operated on twice; the second stage-1 procedure was performed approximately 2 to 3 weeks later. The proposed skin incisions, determined on the basis of results of experiments 1 through 3, were outlined in the caudalateral epigastric region, using a sterile marking pen (Fig 2a). Skin incisions were made and extended to the muscular fascia. Using skin hooks, the wound margins of the medial incision were retracted for direct visualization of the CSEA. Cranial medial branches of the CSEA to the caudal mammae were ligated and divided (Fig 2b). The subcutaneous tissue and skin flap were dissected, using a scalpel, without causing vascular damage (Fig 2c). In island stage-1 tubed skin flaps, the caudal incision was sutured with size 3-0 polypropylene, in a simple continuous pattern. Modified 3-point sutures were used to anchor the corners (Fig 2d). The tubed skin flap was formed and, if necessary, trimmed minimally of fat at its edges. Tubed skin flap edges were closed, using size 3-0 polypropylene in a simple continuous pattern (Fig 2e). Starting 2.5 cm cranial to the base of the tubed skin flap, deep subcutaneous tissues were apposed with interrupted sutures, using size 2-0 chromic gut. Superficial subcutaneous tissue was closed with size 2-0 chromic gut, in a simple continuous pattern (Fig 2f). The remaining skin incisions were closed, using size 2-0 nylon. The free end of the tubed skin flap was affixed to the abdomen with a single interrupted suture (Fig 2g and 2h).

Using described photographic methods, fluorescein studies were evaluated at 12 and 20 minutes after infusion of 5 ml of 10% fluorescein for prediction of surviving length of the tubed skin flaps (Fig 3). Thereafter, the surgical site and tubed skin flap were bandaged with a self-adherent wound dressing and affixed to the adjacent abdominal skin with simple interrupted sutures. After recovery from anesthesia, each pig was housed individually and observed daily for postoperative complications.

Healing of the single-pedicle, axial pattern tubed skin flaps was evaluated by visual and serial fluorescein studies. This experiment was considered to be successful when it was determined that a single-pedicle, axial pattern tubed flap could be raised and survive to its entire length for 5 to 29 days.

Subsequently, 148 stage-1 procedures were performed on 117 pigs (bilateral tubed skin flaps were raised on 31 pigs).

**Experiment 5 (cannulation of the CSEA and harvest of single-pedicle, axial pattern tubed skin flaps, stage-2 procedure)—**One hundred forty-four stage-1 tubed skin flaps were harvested from 118 pigs (bilateral tubed skin flaps were raised on 26 pigs). Each pig was anesthetized as described in experiment 2. Visual inspection and fluorescein studies were performed to ascertain that each single-pedicle, axial pattern tubed skin flap raised during stage-1 procedure had survived along its entire length. The caudal abdominal and inguinal regions of each pig were prepared for aseptic surgery. A 6-cm skin incision that extended caudally from the base of the tubed flap was made in the inguinal region (Fig 4a and 4b). Using blunt dissection, the incision was deepened to the superficial inguinal lymph node, thereby exposing the CSEA, which emerged from its deep surface. Each pig was given heparin (3,000 IU, iv), and the exposed vasculature was bathed with 1 to 2 ml of 2% lidocaine hydrochloride to minimize vasospasm during subsequent manipulations. Aided visually by 2.3 x microsurgical lenses, the surgeon isolated the CSEA between 2 stay sutures of size 3-0 polypropylene. An opening in the wall of the CSEA was established and extended, using delicate cardiovascular scissors. The CSEA was cannulated with size PE20 polyethylene tubing, which was placed deep to the major vasculature. The cannula was connected to the surgical site, and the anterior fat pad was apposed with interrupted simple interrupted sutures (Fig 4c and 4d). Subsequently, 148 stage-2 procedures were performed on 117 pigs (bilateral tubed skin flaps were raised on 31 pigs).

### Table 1—Comparison of fluorescein studies, surviving lengths, and angiography data in caudolateral epigastric skin flaps

<table>
<thead>
<tr>
<th>Flap type</th>
<th>Size (cm)</th>
<th>Fluorescein studies*</th>
<th>Surviving lengths (cm)</th>
<th>Vessels seen in radiographs*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penillar* (n = 4)</td>
<td>12 x 32</td>
<td>17.5</td>
<td>21.7</td>
<td>19.0</td>
</tr>
<tr>
<td>Penillar (n = 2)</td>
<td>4 x 32</td>
<td>8.8 (4.0 to 13.7)</td>
<td>13.2 (13.0 to 13.5)</td>
<td>12.8 (11.0 to 14.7)</td>
</tr>
<tr>
<td>Island (n = 4)</td>
<td>4 x 32</td>
<td>18.7 (17.2 to 19.7)</td>
<td>21.9 (19.7 to 23.5)</td>
<td>19.0 (19.7 to 26.5)</td>
</tr>
</tbody>
</table>

* Distance in cm (range of values) from base of flap. † One flap in this group measured 4 cm x 30 cm initially.

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1. Fluorescine injection, Alcon Laboratories Inc, Fort Worth, Tex.
2. Telfa adhesive dressing, 4 inch x 8 inch, catalog No. 5111, The Kendall Co, Boston, Mass.
4. Potts scissors, delicate, 45°, 7.0 inch, catalog No. 640212, Edward Week & Co, Research Triangle Park, NC.

was secured by the stay sutures (Fig 4c). The pig side of the pedicle containing the caudal superficial epigastric vasculature was cross-clamped, and the tubed skin flap was resected (Fig 4d). Heparinized, 0.9% normal saline (NaCl) solution (approx 20 ml) was infused via the arterial cannula to clear the tubed skin flap of blood and establish patency of the venae comitantes. The tubed skin flap with its cannula was transferred to the isolated organ perfusion laboratory. The vascular stump remaining with the pig was double-ligated with size 0 chromic gut. The wound was flushed and inspected to assure adequate hemostasis. Wounds were closed primarily or allowed to heal by granulation and contraction. One hundred twelve of 118 pigs recovered from anesthesia, were housed individually, and were observed daily for postoperative complications. Six pigs were euthanatized at the conclusion of their second stage-2 procedure.

Results

Experiment 1—The caudal 8 to 10 cm of skin flaps raised in the gluteal region were supplied by musculocutaneous perforators. Although the deep circumflex iliac artery provided a direct cutaneous arterial system to the cranial gluteal region, the vessels were difficult to isolate and cannulate without dissection of the medial aspect of the thigh. Muscle adhered to the skin in those regions with musculocutaneous vasculature. Inspection of the lateral thoracic region revealed variable distribution of feeder vessels from the cranial superficial epigastric arterial system and thick cutaneous trunci muscles. Because gluteal and lateral thoracic skin flaps were thick (4 to 14 mm), they were difficult to tube, and the donor site could not be closed primarily.

In the caudolateral abdominal region, the CSEA provided a direct cutaneous vascular system to the skin. Its
distribution pattern consisted of an origin, the caudomedial branches, and a cranial extension with branches supplying skin lateral to the line of mammae, and craniomedial branches supplying the caudal mammae and median skin. Two venous systems were observed in the caudal abdominal skin region: paired venae comitantes associated with the CSEA; and a cranial superficial epigastric (direct cutaneous) venous system with an anastomotic branch to the deep circumflex iliac vein and an ill-defined caudal extent that terminated in the region of the caudal mammae. Approximately 14 to 16 cm cranial to the caudal-most teat, that is, at the caudal extent of the cutaneous trunci muscle, the abdominal skin was supplied by musculocutaneous vasculature.

On the basis of these dissections and the results of another study, it was concluded that an axial pattern skin flap measuring 12 to 14 cm in length incorporating the skin perfused by the CSEA was feasible; experience indicated that 4-cm wide skin flaps could be tubed easily and the donor site closed primarily.

Experiment 2—Nonselective aortic angiography readily revealed the pudendoepigastric arterial trunk and its subsequent distribution, including the CSEA, to the paralumbar and abdominal skin. Using either nonselective aortic or selective CSEA angiography, it was possible to identify the CSEA and its lateral branches, which coursed the entire caudolateral abdominal region. Communications between the venae comitantes and cranial superficial epigastric venous system were observed on the contact radiograph.

Experiment 3—All caudolateral epigastric skin flaps survived to a length at least 12 cm cranial to the caudal-most teat (Table 1). The surviving lengths of island flaps ≥ peninsular flaps > random flaps, which have been seen in previous studies in which peninsular and island axial pattern skin flaps were raised under similar conditions.22-25 Two to 7 days after surgery, fluorescein studies correlated with the observed surviving length, which was observed in a previous study.25 In the angiography studies, vasculature was observed in the surviving portions of all skin flaps; however, filling of an axial vessel was seen in peninsular or island flaps only. Distribution of vasculature did not correlate as closely with surviving length, and in 2 flaps, filling of subcutaneous vasculature...
distal to the line of skin necrosis was observed. Contact radiography did not allow us to elucidate clearly the venous drainage of skin adjacent to the base of island vs peninsular skin flaps. On the basis of these studies, it was concluded that a single-pedicle, axial pattern (either island or peninsular), tubed skin flap measuring 4 cm × 12 cm would survive to its entire length.

Experiment 4—In the initial trial (9 pigs, n = 12 tubed skin flaps), it was determined that all stage-1 tubed skin flaps survived to their entire length for periods ranging from 5 to 29 days. Moderate edema of the tubed skin flap for 24 to 48 hours after stage-1 procedure did not affect its ability to survive to its entire length. Donor site incisions healed primarily and evidence of postoperative complications was not observed. In total, 149 of 160 consecutive stage-1 tubed skin flaps were prepared successfully. During one procedure, the CSEA was severed during questionable preparations developed distal tip necrosis; even distal fluorescence (Fig 3c and 3d). Three of 7 tubed skin flaps from 5 to 29 days. Moderate edema of the tubed skin flap for 24 to 48 hours after stage-1 procedure did not affect the flaps' ability to survive to its entire length. Postoperative complications in the combined series included infection in 4 preparations, distal tip necrosis (approx 3 cm) in 1 tubed skin flap, extensive blister formation in 1 tubed skin flap, and unexplained death of 3 pigs resulting in loss of 4 tubed skin flaps (1 of 3 pigs had bilateral tubed skin flaps raised simultaneously). Two tubed skin flaps had minor evidence of distal tip necrosis, but this did not affect their use in subsequent studies, and those preparations were not considered to be stage-1 procedure failures.

Fluorescein studies at the conclusion of 148 of 160 stage-1 procedures predicted survival of 139 of 148 tubed skin flaps as evidenced by bright or muted (patchy) yellow fluorescence, including 8 of 11 preparations destined for stage-1 procedure failure. Fluorescein studies did not predict infection or pig death, and were not performed on the tubed skin flap that developed extensive blister formation. Fluorescein studies confirmed loss of blood supply attributable to CSEA severance in 1 preparation, and possible distal flap necrosis in 8 others as evidenced by uneven distal fluorescence (Fig 3c and 3d). Three of 7 questionable preparations developed distal tip necrosis; the remaining tubed skin flap was harvested immediately after stage-1 procedure for another study. Differences were not observed visually between fluorescein studies obtained at 12 and 20 minutes after infusion during the first 28 consecutive studies.

Experiment 5—Stage-2 procedures were performed successfully in 136 of 144 consecutive axial pattern tubed skin flaps, which allowed either microangiographic or surgical studies (n = 18), or isolated skin perfusion studies including percutaneous absorption tests (n = 118), to be performed. In 4 of 8 tubed skin flaps in which the stage-2 procedure was performed unsuccessfully, the CSEA was too small to cannulate; in 4 others, technical errors resulted in undetected perforation of the CSEA, catheter slippage during subsequent handling, or clotting of vasculature. Stage-2 procedure wounds healed without complications.

Discussion

Prosection of latex-injected abdominal pelts identified the CSEA and its branches as providing the most suitable vasculature for single-pedicle, axial pattern tubed skin flaps. In vivo angiography clearly revealed the distribution of the CSEA to the flank fold laterally, and it provided information regarding venous drainage of the caudal-lateral abdominal skin. Both of these experiments allowed routine anatomic landmarks, that is, the superficial inguinal lymph node and the caudal-most teat, to be used for predictable identification of the location and course of the CSEA.

Experiment 3 determined the surviving length of skin flaps raised under various vascular conditions. On the basis of these results, and our estimates that at least 2 to 4 cm² of skin surface would be needed for optimal percutaneous absorption studies during in vitro perfusion, it was decided to create tubed skin flaps measuring 12 cm in length. Previous studies have demonstrated that flaps raised under similar vascular conditions will survive to the same length regardless of width. Therefore, the width of the skin flap designed for successful tubing could be selected on the basis of the surgeon's judgment. Examination of the pigs used in experiments 1 through 3 indicated that 4-cm wide skin flaps would be appropriate in pigs weighing < 35 kg.

Moderate edema of tubed skin flaps for 24 to 48 hours after the stage-1 procedure did not affect the flaps' ability to survive to their entire length or their suitability for use in percutaneous absorption studies. The formation of edema indicated variable degrees of venous compromise attributable to altered venous circulation, compression after tubing, and the dependent position of the tubed skin flap. The relative contributions of these factors to edema formation are not known; however, epidermal necrosis, succeeded by second intention healing, will develop if excessive subcutaneous tissue is included within the tubed skin flap. Our experience indicated that because their subcutaneous fat deposits make closure of the tubed skin flap difficult, even with maximal subcutaneous tissue debulking, pigs weighing > 35 kg are not suitable for preparation of single-pedicle, axial pattern tubed skin flaps.

All stages of development of the surgical procedures for preparation of IPPSF used fluorescein studies to assess viability. Previously reported rapid photographic methods have been used successfully in our protocols. Because no difference was observed between fluorescein studies obtained at 12 vs 20 minutes after infusion, we standardized our protocol by obtaining baseline and 12-minute postinfusion data only. Interestingly, subtle differences in skin fluorescence between individual preparations were observed when fluorescein studies were performed immediately after stage-1 procedure, for example, bright yellow vs muted (patchy) yellow vs uneven distal fluorescence (Fig 3). Our experience indicated that tubed skin flaps with bright or muted yellow fluorescence will survive along their entire length. In those preparations with uneven distal fluorescence, at least half will survive along their entire length. It is possible that uneven distal fluorescence in tubed skin flaps that survive along their entire length is the result of diminished perfusion in a random pattern vascular territory on the end of an axial pattern flap.

On the basis of our success rate (93%) with stage-1 procedures, fluorescein studies to assess viability immediately after stage-1 procedure may not be required; however, such studies provide objective confirmation of viability at the conclusion of surgery. Fluorescein studies at the time...
of stage-2 procedure are not needed because survival of the tubed skin flap is evident visually, tubed skin flap viability may be evaluated during in vitro isolated perfusion, and residual fluorescein in the tubed skin flap may confound percutaneous absorption studies.

Currently, a 2-day period between stage-1 and 2 procedures has been found to be optimal for percutaneous absorption studies. Selection of the 2-day period between surgery confers 2 technical advantages on the harvest of island tubed skin flaps. Because island tubed skin flaps are prepared by division of the skin bridge and extensive dissection of the subcutaneous pedicle, the stage-2 procedure proceeds quickly because the skin may be incised by finger dissection (Fig 4b), and there is minimal subcutaneous dissection to be done prior to vascular cannulation. Obviously, longer periods between preparation and harvest of tubed skin flaps, which may be important for other skin biology studies, would allow healing of the skin and subcutaneous tissue bridge, necessitating a separate skin incision and meticulous dissection during stage-2 procedures. The vascular dissection and cannulation are technically demanding procedures. Because the vessels to be cannulated are 0.5 to 2 mm in diameter, it is recommended that magnifying lenses be used to aid visualization of the operative field.

Initially, the stage-2 procedure was performed aseptically, which allowed the wound to be closed primarily. We now harvest tubed skin flaps after sterile water cleansing only, and allow the wound to remain open to heal (uneventfully) by granulation and contraction. Topical application of antiseptics during sterile preparation of the operative site may affect adversely the percutaneous absorption studies. Stage-1 procedure has been and will continue to be performed aseptically.

References