

Development of an Irradiated Rodent Model to Study Flap Revascularization

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Objective: To develop a reproducible free-flap animal model to study the effects of irradiation on flap revascularization.

Design: After institutional animal care and use committee review and approval, 16 Sprague-Dawley rats were subjected to either 23- or 40-Gy electron beam irradiation to their ventral abdominal wall. After a recovery period, the animals then underwent a ventral fasciocutaneous flap pedicled on the inferior epigastric vessels with subsequent pedicle ligation at 10 days. An additional 16 rats were subjected to 40 Gy of irradiation and underwent pedicle ligation at 8 or 14 days postoperatively to determine if time to pedicle ligation affected percentage of flap viability.

Results: Rats receiving 23 Gy of irradiation had the same viability as rats undergoing no radiation. Rats receiving

40 Gy of irradiation had a significantly lower average percentage of flap viability (56.9%) than animals receiving 23 Gy (90.9%) ($P < .001$). Furthermore, the longer duration until pedicle ligation after 40 Gy of irradiation led to significant increases in flap viability ($P < .001$ for analysis of variance).

Conclusions: This animal model establishes that external beam irradiation at a total dose of 40 Gy leads to significantly delayed flap revascularization over time compared with 23-Gy irradiation. This model will allow future investigators to study novel therapies to improve healing and flap revascularization.

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H EAD AND NECK CANCERS affect critical physiologic structures, and given the vital functions of these structures and the resultant morbidity of surgery, organ preservation treatment strategies have been devised. These treatments are based on primary irradiation or a combination of chemotherapy and radiation therapy. Unfortunately, salvage surgery after irradiation failure in advanced cancers may be fraught with complications. These complications may be due to the effect of irradiation on the host microvasculature, which ultimately affects wound healing. The effects of radiation therapy on the skin have been well described and are related to blood vessel changes, which can be observed clinically as pallor and telangiectasia and pathologically as a decrease in capillary density and diameter.¹

While free flaps or pedicled flaps may have adequate blood supply to survive, the irradiation effects on the host wound bed may delay flap incorporation and revascularization between the flap and host tis-

sue. Although free tissue transfer techniques offer a high success rate, wound complications often occur due to this delay in healing between the flap and its bed. Therefore, to study the effects of irradiation on host tissues, it is imperative that animal models of irradiated tissue(s) are established in a clinically relevant model to devise treatment strategies to improve wound healing in an irradiated field. Researchers have extensively investigated the rat ventral fasciocutaneous flap model.²⁻⁵ The blood supply to this fasciocutaneous flap is based on the inferior epigastric artery and vein as shown in **Figure 1**. The objective of this study was to use the rat ventral fasciocutaneous flap model to study the effects of irradiation on wound healing and flap revascularization.

METHODS

After institutional animal care and use committee review and approval, 16 male Sprague-Dawley rats were subjected to either 23- or 40-Gy (8 animals at each dose) electron beam irradiation to their ventral abdominal wall. (To

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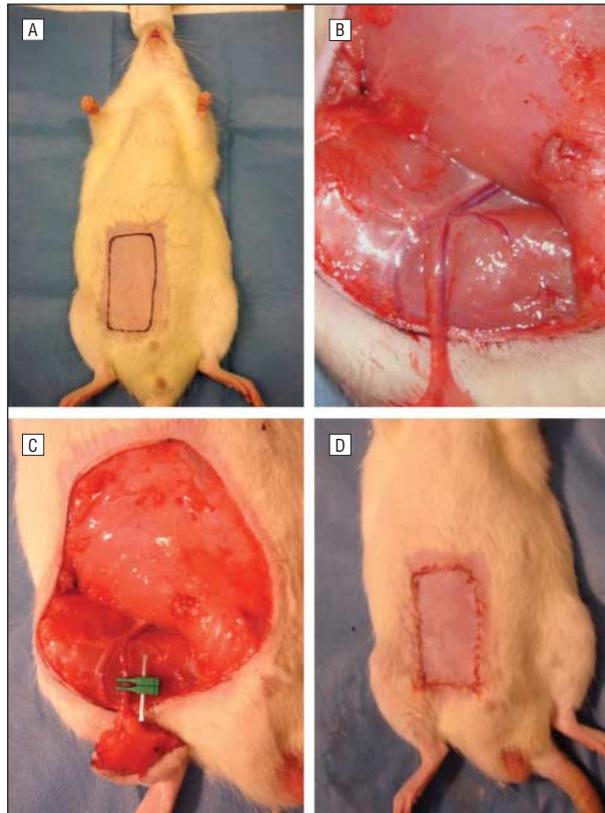


Figure 1. The rat ventral fasciocutaneous flap model, with blood supply based on the inferior epigastric artery and vein. A, A 3 × 6-cm ventral fasciocutaneous flap is outlined. B, Flap raised, demonstrating inferior epigastric pedicle. C, Vascular clip occluding inferior epigastric pedicle. D, flap inset and closure.

convert grays to rads, multiply by 100.) After a ventral fasciocutaneous flap procedure, pedicle ligation was performed 10 days postoperatively. Based on preliminary data, 10 days was believed to be an adequate time frame to capture the revascularization process. An additional 16 animals were subjected to 40 Gy of irradiation and underwent pedicle ligation at 8 or 14 days to determine if time to pedicle ligation affects percentage of flap viability (**Figure 2**).

IRRADIATION PROTOCOL

All animals underwent general anesthesia using isoflurane inhalation during irradiation. Once adequacy of anesthesia was confirmed, rats were placed in the supine position on the irradiation table. A lead shield was placed to isolate the templated flap region. A 6-MeV electron beam accelerator (Varian Clinac 2100EX; Varian Medical Systems Inc, Palo Alto, California) was used to irradiate the animals. A bolus material of 2 cm on top of the abdomen was used to improve radiation dose distribution. For animals receiving 23 Gy, irradiation was administered in 3 divided doses of 766 cGy over 5 days. For animals receiving 40 Gy, irradiation was administered in 5 divided doses of 800 cGy over 10 days.

VENTRAL FASCIOCUTANEOUS FLAP PROCEDURE

After a recovery period of 28 days post irradiation, the animals underwent a ventral fasciocutaneous flap procedure. A ventral 3 × 6-cm fasciocutaneous flap was raised, based on the in-

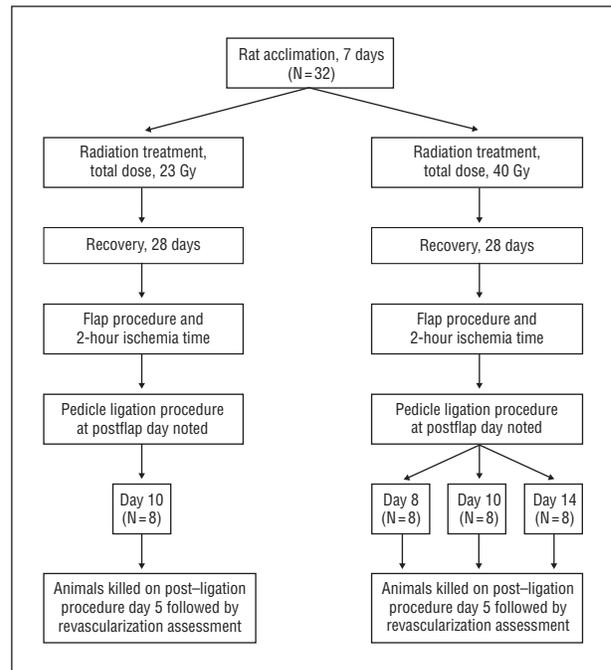


Figure 2. Study procedures overview.

ferior epigastric artery and vein (Figure 1B). Following elevation of the flap, a 20-g Heifitz clip was applied to the vascular pedicle for 2 hours to simulate ischemic time during free-tissue transfer (Figure 1C). During this ischemic time, the flap was inset, and the animals were awakened from anesthesia (Figure 1D). At the end of the 2-hour period of ischemia, the animals were briefly re-anesthetized, the corner of the flap elevated, the occluding clip removed, and the wound reapproximated. The animals were monitored daily for signs of pain and discomfort and treated with analgesics as needed. They then underwent ligation of the inferior epigastric vein and artery at the study intervals.

EVALUATION OF FLAP REVASCULARIZATION

Percentage of flap viability was evaluated on postligation procedure day 5 as a marker for flap revascularization. Viable flap area was characterized by warm, pink, hair-bearing skin. Nonviable flap area was characterized by dry, hard, hairless eschar. Animals were placed under general anesthesia, and standardized digital photographs of the ventral flap were taken. Three qualified blinded observers were used to delineate viable and nonviable areas by tracing template etchings. Then cutouts of viable tissue from the template were weighed to express a percentage of the total template weight, effectively giving the area percentage of viable flap tissue. Animals were excluded for evaluation if they developed a clinically significant hematoma, seroma, or infection.

STATISTICAL ANALYSIS

For establishing and reproducing the irradiated rat ventral flap fasciocutaneous model, a power analysis was performed using our preliminary data and indicated that a minimum of 7 animals for each group would be required to complete a meaningful statistical analysis of flap viability to obtain a *P* value of <.05 of significance using an analysis of variance. Analysis of variance with Tukey post hoc tests of significance for multiple

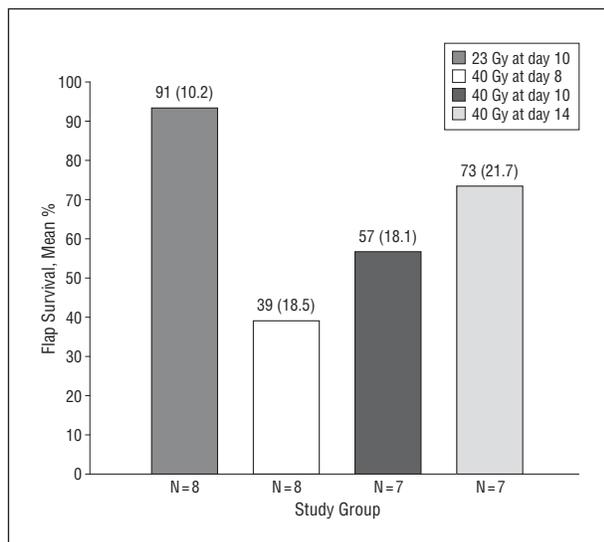


Figure 3. Mean (SD) percentages of flap viability by group. The numbers placed inside the tops of the bars are the standard deviations.

comparisons were used to analyze the difference in percentage of flap viability between groups.

RESULTS

Rats receiving a 40-Gy total dose of irradiation had a significantly lower average flap viability (56.9%) than animals receiving 23 Gy (90.9%) when pedicle ligation was performed 10 days postoperatively ($P < .001$) (**Figure 3**). However, we found that longer duration between pedicle ligation and 40-Gy total dose of irradiation led to significant increases in flap viability (**Figure 4**). The percentages of flap alive at 8, 10, and 14 days were 39.25%, 56.9%, and 73.1%, respectively ($P < .001$ for analysis of variance). Two animals had seromas and were excluded from analysis, each from the 40-Gy group, one at day 10, the other at day 14.

COMMENT

Reconstruction after salvage surgery for failed radiation or chemoradiation therapy is a difficult challenge with a high rate of postoperative complications. Long-term viability and healing of a reconstructive flap is not only dependent on the vascular pedicle but also on revascularization from the surrounding host tissue. It has been shown that previous radiation therapy leads to microvascular compromise in the host tissue manifesting as excessive fibrosis, endothelial cell damage, and reduced cellular turnover.^{6,7} Therefore, for a reconstructive flap to incorporate with the host tissue, revascularization must occur from the wound bed and surrounding tissue. This process must also occur from the flap tissue into the host tissue, effectively re-establishing improved microvasculature throughout the previously compromised wound bed.

The present project was undertaken to develop an animal model to study the effects of irradiation on wound healing and flap revascularization. In a previous study, animals receiving a total dose of 23 Gy of irradiation did

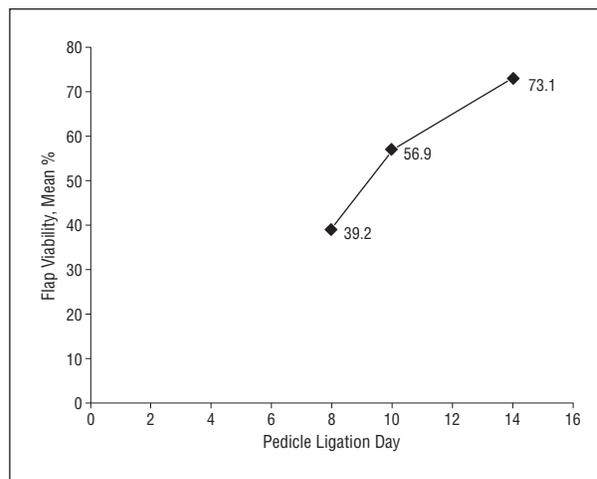


Figure 4. Percentage of flap viability with increasing time to pedicle ligation.

not have a significant difference in average flap revascularization compared with nonirradiated animals.⁸ To better mimic the clinical situation of radiation effects on host tissue, we evaluated an increased radiation dose to a total of 40 Gy. In the present study, we showed that external beam irradiation at a total dose of 40 Gy leads to significantly reduced revascularization compared with a dose of 23 Gy. This correlates with previous studies that have shown that irradiation to a total dose of 30 to 40 Gy was adequate to produce a compromised host bed mimicking the clinical situation.^{9,10}

Furthermore, we demonstrated that increasing the duration until pedicle ligation from 8 to 10 to 14 days after administration of 40-Gy irradiation led to significant increases in revascularization. We hypothesize that this observed phenomenon correlates with revascularization from the surrounding tissue. Tsur et al¹¹ studied neovascularization of axial skin flaps in rats and found that pedicle ligation beyond 6 days did not produce total flap necrosis. They found that adequate neovascularization for flap survival was demonstrated as arising from both the wound edges and the bed, although those from the bed appeared to be of greater importance. Previous studies of the effects of celecoxib by Jorgensen et al³ and Wax et al⁵ did not demonstrate a significant difference in flap viability prior to 8 days of pedicle ligation. However, in a study by Clarke et al¹² of delayed neovascularization in free skin flap transfer to irradiated beds in rats, significantly less tissue survived the loss of the complete vascular pedicle at the second to fourth days following flap creation in rats with an irradiated bed. Later survival was not different from controls.

In clinical correlation, there have been case reports describing survival of free tissue transfer grafts after pedicle ligation as early as 8 to 12 days.¹³⁻¹⁵ Enajat et al¹⁴ set out to answer the question of how long fasciocutaneous flaps are dependent on their vascular pedicles. The researchers reported a unique case in which the pedicle of a superficial inferior epigastric artery flap for breast reconstruction was avulsed 11 days postoperatively, with the flap surviving on its inferior wound edge alone. In a retrospective review, Salgado et al¹⁵ studied the effects of late loss of arterial inflow on free-flap survival. They concluded that the timing of late loss of arterial inflow does

not appear to be the primary determinant of free-tissue survival. The condition and quality of the recipient site plays a large role in survival of these flaps. Ischemic, irradiated, and scarred beds are inadequate to provide late flap neovascularization compared with healthy recipient sites.

The limitations of this study include the small number of animals, just above the threshold to be powered. Other limitations are centered on mimicking the clinical situation. The time between irradiation and surgery may vary in clinical practice and may be much longer than a month, which may have an effect on outcomes. Also it is rare that the graft and host tissue are both irradiated, as was this case in this model, which may affect the revascularization potential of the flap. Despite these limitations, we believe that this irradiated rat flap model is well suited to allow further study of novel therapies to improve wound healing, flap revascularization, and overall flap viability and survival with or without disruption of the vascular pedicle.

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