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Does Preconditioning with Transcutaneous Electrical Nerve Stimulation (TENS) Increase Transverse Rectus Abdominis Musculocutaneous (TRAM) Flap Viability? An Experimental Rat Model Study

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ABSTRACT

Objective: This study aimed to enhance transverse rectus abdominis musculocutaneous (TRAM) flap survival using non-invasive transcutaneous electrical nerve stimulation (TENS) in an experimental rat model.

Materials and Methods: Fifty male rats were divided into four groups. The Control group, consisting of 12 rats, underwent flap surgery only. The Surgical Delay group, also with 12 rats, received skin incisions and superior epigastric artery ligation seven days before flap surgery. The Sham group, comprising 12 rats, had electrodes attached for seven days without stimulation. The TENS group, which included 13 rats, underwent electrical stimulation for seven days before flap surgery. Post-surgery, perfusion was measured, vascular endothelial growth factor (VEGF) levels and vessel counts were assessed, and flap viability was evaluated after seven days.

Results: The TENS group demonstrated higher flap viability compared to the other groups ($p=0.097$), with viability rates of 17.4±19.80% in the Control group, 18.1±25.70% in the Surgical Delay group, 25.22±29.02% in the Sham group, and 40.44±30.92% in the TENS group. There were no significant differences in perfusion percentages among the groups. The Surgical Delay group exhibited a higher vessel count. VEGF levels were elevated in the TENS and Control groups compared to the Surgical Delay and Sham groups. Significant correlations were observed between perfusion percentages and flap viability.

Conclusion: TENS has been shown to effectively enhance flap viability when used as a preconditioning method in this experimental flap model with axial blood flow. It may be considered a simple preconditioning strategy for high-risk flap designs in clinical settings.

Keywords: Flap viability, preconditioning, transcutaneous electrical nerve stimulation (TENS), transverse rectus abdominis musculocutaneous flap (TRAM).

INTRODUCTION

The transverse rectus abdominis musculocutaneous (TRAM) flap is an autologous reconstruction method used to restore aesthetic appearance following breast tissue excision. Various preconditioning techniques, including surgical and chemical delay, have been developed to mitigate complications caused by inadequate blood flow to the flap.[1–3] Surgical delay is the most effective method but necessitates multiple operations, while chemical delay has limited clinical application due to systemic side effects.[2,3] Transcutaneous electrical nerve stimulation (TENS), commonly used to treat pain, itching, tinnitus, and chronic wounds, increases blood flow to ischemic tissues, enhances capillary density, promotes angiogenesis, and elevates vascular endothelial growth factor levels. Additionally, it exerts anti-inflammatory effects while reducing oxygen tension in tissues.[4] Research indicates that TENS improves the viability of ischemic random musculocutaneous flaps[5–7] and ischemic TRAM flaps.[8] As a preconditioning method, TENS has also been found to enhance the viability of flaps with a random blood supply.[7] This study hypothesizes that TENS preconditioning can improve the viability of an axial flap, such as the TRAM flap.

KEY MESSAGES

- This study is the first to demonstrate that TENS, when used as a preconditioning method, can enhance the viability of an axial flap, such as the TRAM flap.
- TENS is an easily applicable preconditioning method.
- TENS is effective in improving flap viability in both axial pedicled flaps and random pedicled flaps.

MATERIALS AND METHODS

Approval for this study was granted by the Experimental Animals Ethics Committee of Erciyes University Medical Faculty (Project No: TTU-2019-9400). The study was conducted at the Experimental Research and Applications Centre of Erciyes University.

Fifty male Wistar rats, with an average weight of 280 g, were used. Due to self-harm observed in preliminary studies, collars were applied post-surgery. The rats were divided into four groups: TENS (13 rats, 26%), Control (12 rats, 24%), Surgical Delay (12 rats, 24%), and Sham (12 rats, 24%). All rats were housed in cages at room temperature under a 12-hour light/dark cycle. Intraperitoneal anesthesia was administered using 10 mg/kg xylazine and 80 mg/kg ketamine. The study duration was 21 days.

Flap Model

A single-pedicle unilateral caudal rectus abdominis flap, perfused by the right inferior epigastric artery, was used as described by Ely et al.[8] The flap measured 5 cm by 3 cm, starting 1 cm below the xiphoid.

A silastic sheet was placed beneath the flap to prevent vascularization from the base, and the flap was then sutured. Tissue samples were collected from the necrosis-viable transition area, which was approximately determined by a preliminary study (zones 2 to 4) on the day the flaps were elevated. To minimize disruption of blood flow, the flap was extended by 1 x 0.5 cm cranially and caudally. Cranially oriented samples were used for enzyme-linked immunosorbent assay (ELISA), while caudally oriented samples were used for immunohistochemistry. Flap viability was assessed on day 7, after which all animals were sacrificed using a high-dose anesthetic.

Groups

Control Group: A TRAM flap with a right inferior pedicle was completely elevated on its pedicle, sutured back to its donor area, and monitored for seven days (Fig. 1).

Surgical Delay Group: The flap skin edges were incised, and the superior epigastric artery was cauterized and sutured. On day 7, the incisions were reopened, and the TRAM flap was created.

Sham Group: Anode electrodes were placed over the flap area, and cathode electrodes were positioned caudally for one hour daily over seven days without activation. On day 7, the TRAM flap was created.

TENS Group: Electrodes were positioned in the same manner as in the Sham group, and electrical stimulation at 20 mA and 80 Hz was applied for one hour daily over seven days using an ITOZ® TENS device. On the seventh day, the flap was created.

Histological Procedure

Blood Vessel Count with Immunohistochemical Assay

The avidin-biotin-peroxidase method was used with a CD34 antibody to assess blood vessel count. Primary antibodies were prepared using Labvision antibody diluent OP QUANTO (Thermo Scientific, TA-60-ADQ solution). Blood vessels were counted in ten randomly selected areas from each sample.

ELISA

Vascular endothelial cell growth factor A (VEGF) levels in skin tissue were measured using ELISA method with the Bioassay Technology Laboratory E0940Ra kit. Results were read at 450 nm.

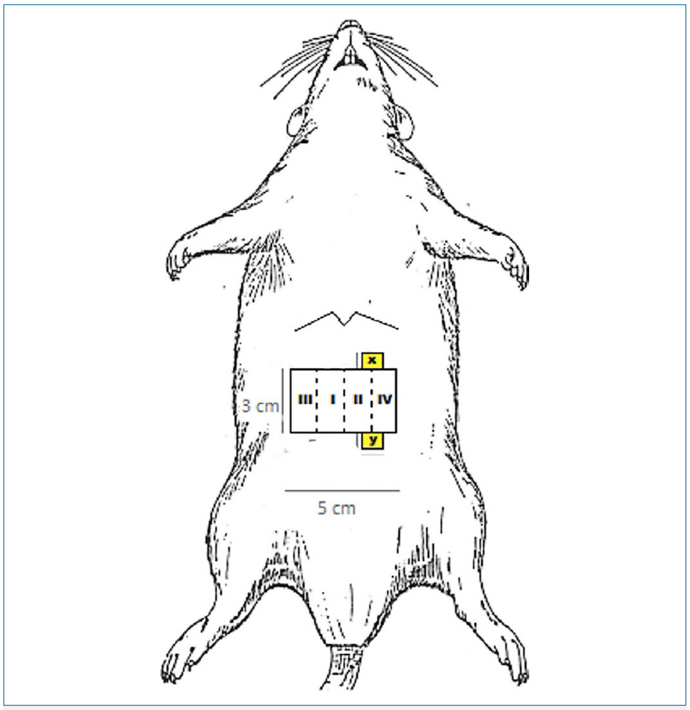

Figure 1. Transverse rectus abdominis musculocutaneous (TRAM) flap model and flap perfusion zones. x: Cranially oriented samples for enzyme-linked immunosorbent assays (ELISA). y: Caudally oriented samples for immunohistochemistry. Zone I: Overlying the muscle pedicle. Zone II: Positioned across the midline. Zone III: Lateral to Zone I. Zone IV: Lateral to Zone II on the contralateral side of the pedicle.

Flap Perfusion Evaluation

Fluorescence imaging of flap perfusion was performed using the SPY Elite device (model SP3055) with indocyanine green (ICG). Two hours after flap creation, 1 cc of ICG was administered intracardially. SPY measurements were obtained from zones 1 and 4 of the flap, relative to the reference area proximal to the flap pedicle (set at 100%). Preliminary studies indicated that intracardiac injection yielded more accurate SPY measurements compared to tail vein injection, leading to the selection of the intracardiac method for this study. None of the rats subjected to intracardiac injection died. Following the completion of flap procedures, the rats were re-anesthetized for SPY measurements.

Flap Viability Rates: Seven days after flap creation, the borders of necrotic and viable areas were outlined on clear acetate sheets, scanned, and uploaded to a computer. A simple program was used to calculate the ratio of viable tissue to the total flap area as a percentage.[9]

Statistical Analysis

Statistical analyses were performed using SPSS version 22.0 software (SPSS Inc., Chicago, IL, USA, Undergraduate: Hitit University). Descriptive statistics were reported as mean±standard deviation (SD) for normally distributed data and as median (Q1–Q3) for non-normally distributed data. Categorical variables were presented as numbers and percentages. Normality was assessed using the Shapiro-Wilk test, while variance homogeneity was evaluated with the Levene test. One-way analysis of variance (ANOVA) was used for comparisons of normally distributed data, and the Kruskal-Wallis test was applied for non-normally distributed data. Post-hoc analyses included the Tukey test following ANOVA and the Dunn-Bonferroni test following the Kruskal-Wallis test. Correlations were assessed using Pearson's coefficient. Given the small sample size, a p-value of 0.10 was considered statistically significant.

RESULTS

Eight Rats Were Lost During Follow-up

Two from the Control group, two from the Surgical Delay group, one from the Sham group, and three from the TENS group. Three of these losses (two in the TENS group and one in the Surgical Delay group) were attributed to additional anesthesia before ICG injection on day 7. Fluid collection beneath the flap was observed in all groups, likely due to the presence of the silastic layer. The use of collars successfully prevented cannibalism, and no losses related to cannibalistic behavior were recorded. The silicone layer remained intact, effectively preventing vascularization from the flap base.

Flap Perfusion Values

Flap perfusion was assessed using the SPY system. The perfusion ratio of flap zone 1 to normal skin was 61±27.16% in the Control group, 56.2±32.15% in the Surgical Delay group, 64.1±25.22% in the Sham group, and 81.9±14.52% in the TENS group. For zone 4, the perfusion ratios were 28.75±11.44% in the Control group, 27.9±15.73% in the Surgical Delay group, 29.1±14.18% in the Sham group, and 32.8±10.40% in the TENS group. No significant differences were observed between the groups for zone 1 or zone 4 perfusion percentages (p=0.141, p=0.840) (Fig. 2, Table 1).

Flap Viability Percentages

The mean flap viability rates were 17.4±19.80% in the Control group, 18.1±25.70% in the Surgical Delay group, 25.22±29.02% in the Sham group, and 40.44±30.92% in the TENS group. The difference was statistically significant at p<0.10 (p=0.097). The TENS group demonstrated significantly higher viability percentages than the other groups (p<0.10) (Table 2).

Figure 2. Flap perfusion evaluation using fluorescence imaging.

Vessel Counts

The vessel counts were determined to be 46.27±5.86 in the Control group, 56.18±12.31 in the Surgical Delay group, 40.27±5.55 in the Sham group, and 40.27±7.52 in the TENS group. The overall difference between the groups was statistically significant (p<0.001). Post-hoc test results indicated that the vessel count in the Surgical Delay group was significantly higher than in the Control, Sham, and TENS groups (p=0.037, p<0.001, and p<0.001, respectively). No significant differences were observed between the Control, Sham, and TENS groups in terms of vessel count (p>0.1) (Table 3).

VEGF Levels

The mean VEGF levels in the groups were 86.24±17.62 ng/L in the Control group, 68.95±12.06 ng/L in the Surgical Delay group, 73.29±19.14 ng/L in the Sham group, and 91.21±12.73 ng/L in the TENS group. The overall difference among the four groups was statistically significant (p=0.009). Post-hoc test results indicated that VEGF levels in the Control group were significantly higher than those in the Surgical Delay group at p<0.10 (p=0.081). Similarly, VEGF levels in the TENS group were significantly higher than those in the Sham group at p<0.10 (p=0.068). No significant differences were observed between the other groups (p>0.1) (Table 4).

Correlation Analyses

A statistically significant correlation was found between zone 1 and zone 4 perfusion percentages (p=0.002, r=0.461). Additionally, a significant correlation, approaching a moderate level, was observed between zone 1 perfusion percentages and flap viability percentages (p<0.001, r=0.594).

DISCUSSION

This experimental study is the first to demonstrate that the survival of an axial flap can be improved through preconditioning with TENS. The study design focused on the TRAM flap as an ischemic flap, aiming to ensure that the findings would be both clinically relevant and applicable to surgical practice.

Table 1. Comparison of Zone 1 and Zone 4 perfusion percentages

	Control group (n=12)	Surgical delay group (n=10)	Sham group (n=10)	TENS group (n=10)	p
Perfusion percentages in zone 1	61±27.16	56.2±32.15	64.1±25.22	81.90±14.52	0.141[a]
Perfusion percentages in zone 4	28.75±11.44	27.9±15.73	29.1±14.18	32.8±10.4	0.840[a]

a: One-way analysis of variance (ANOVA) with mean±standard deviation (SD).

Table 2. Comparison of flap viability percentages among groups

	Control group (n=10)	Surgical delay group (n=10)	Sham group (n=11)	TENS group (n=10)	p
Flap viability percentages	8.61 (0–43.09)	9.13 (0–31.19)	11.76 (3.43–39.61)	37.28 (15.48–68.58)	0.097

Table 3. Comparison of the number of vessels

	Control group (n=11)	Surgical delay group (n=11)	Sham group (n=11)	TENS group (n=11)	p	Post-hoc p values
Number of vessels	46.27±5.86	56.18±12.31	40.27±5.55	40.27±7.52	<0.001[a]	**1–2: 0.037** 1–3: 0.336 1–4: 0.336 **2–3: <0.001** **2–4: <0.001** 3–4: 1.000

a: One-way analysis of variance (ANOVA) with mean±standard deviation (SD) (following Tukey post-hoc test).

Table 4. Comparison of VEGF levels

	Control group (n=10)	Surgical delay group (n=10)	Sham group (n=10)	TENS group (n=10)	p	Post-hoc p values
VEGF levels	86.24±17.62	68.95±12.06	73.29±19.14	91.21±12.73	**0.009**[a]	1–2: 0.081 1–3: 0.269 1–4: 0.893 2–3: 0.922 **2–4: 0.016** 3–4: 0.068

VEGF: Vascular endothelial growth factor; a: One-way analysis of variance (ANOVA) with mean±standard deviation (SD) (following Tukey post-hoc test).

TRAM Flap

The TRAM flap is a preferred technique in breast reconstruction due to its ability to achieve a natural appearance and maintain symmetry with the contralateral breast.[1,2] It is particularly beneficial for elderly patients, individuals with a history of radiation therapy, smokers, those with diabetes or hypertension, and patients requiring a large volume of tissue, especially if they have a paramedian or midline abdominal scar. One of the most common complications associated with TRAM flap breast reconstruction is partial necrosis. To prevent this, surgical delay methods have been explored.[1,10,11] During surgical delay, vasoconstriction occurs due to reduced blood supply and physiological stress. The first 72 hours are critical, as vasospasm takes place, leading to permanent anatomical and histological changes that enhance flap vascularity and blood flow, lasting up to seven days.[12–14]

Transcutaneous Electrical Nerve Stimulation

In the search for a non-invasive and cost-effective approach to preventing distal flap complications, various alternative delay techniques have been investigated.[3,14–24] Among these, TENS has emerged as a promising option due to its low cost, non-invasiveness, ease of application, and absence of systemic side effects. TENS has demonstrated positive results in preventing ischemic complications in dorsal musculocutaneous and TRAM flaps in rat models.[4–7,24]

Transcutaneous electrical nerve stimulation has been demonstrated in experimental studies to enhance flap viability through various mechanisms. In an experimental rat study conducted by Doğan and Özyazgan,[6] TENS applied as a preconditioning method was shown to increase the survival rate of a random-pattern dorsal flap while also improving flap blood flow. In flaps with increased viability following TENS application, a more extensive but thinner vascular network was observed compared to other groups. Another experimental study demonstrated that preoperative TENS application increased flap blood flow in nicotine-treated rats.[7]

Furthermore, Patterson et al.[25] reported that electrical stimulation could enhance VEGF release by stimulating skeletal muscles, thereby promoting endothelial cell proliferation, migration, and reorganization.

These studies highlight the potential mechanisms by which TENS application may positively influence flap survival.

Niina et al.[24] created musculocutaneous flaps on rats' dorsal skin and applied either TENS or electroacupuncture postoperatively. TENS significantly improved flap survival in most subgroups, whereas electroacupuncture did not. Electrical stimulation at 80 Hz was found to be more effective than at 2 Hz for improving flap viability, although intensity had no effect. Conversely, Kjartansson found that increased blood flow was related to stimulation intensity.[5] Another study demonstrated that applying 80 Hz (high-frequency) and 20 mA (high-amplitude) pulsatile currents for three days postoperatively produced the best results, while preoperative TENS showed no benefit.[24]

In the study most similar to ours, Neves applied 100 Hz TENS postoperatively for two days to rats with inferior epigastric artery-based TRAM flaps and reported improved flap viability compared to the control group.[26] However, since TENS was applied postoperatively in this study, it should not be considered a delay or preconditioning procedure aimed at preventing potential flap ischemia.

In the study by Doğan and Özyazgan using a rat flap model, preoperative electrical stimulation at 20 mA and 80 Hz for seven days improved flap survival. Moreover, applying TENS at the same amplitude and frequency for one hour immediately after flap elevation further increased survival rates. Microangiographic imaging revealed more distinct vascular structures in the TENS-preconditioned group. Notably, that study was the first to demonstrate that TENS, as a preconditioning method, outperformed traditional surgical delay techniques in enhancing flap survival.[6]

In our study, TENS was applied at 20 mA and 80 Hz, as used by Doğan and Özyazgan[6] and identified by Kjartansson as the most effective settings.[27] Given the established success of seven-day classical delay methods, the current study also applied TENS for seven days before surgery. Although mean flap viability was higher in the TENS group compared to the other groups, statistical significance was not reached at $p<0.05$, though differences were significant at $p<0.1$. Preconditioning with TENS improved flap viability over the control and other groups, supporting the study hypothesis. The high standard deviations suggest that increasing the sample size in future studies may yield more statistically significant results.

Surgical Delay

In the present study, surgical delay did not improve flap viability, in contrast to previous studies where it was found to be beneficial.[19,22] Özgentaş et al.[22] reported that ligating of both the superficial inferior and deep superior epigastric arteries in a 10 x 3 cm bipedicle flap was the most effective approach. In contrast, the current study used superior epigastric artery ligation in a 5x3 cm single-pedicle flap. This methodological difference may explain the inconsistent results. Similarly, Cinpolat et al.[19] observed increased flap viability following a similar surgical delay procedure, despite minor differences in flap size. While a slight variation in flap size alone does not fully explain why surgical delay did not improve viability in the current study, the presence of a purulent collection at the flap base of the flap in all groups during follow-up may be a contributing factor. In the Surgical Delay group, the total duration from the delay procedure to the postoperative seventh day, when flap viability was assessed, was 14 days in total. Consequently, rats in this group were required to wear collars for seven days longer than those in the other groups. Although collars were used to prevent cannibalism, it was observed that the rats exhibited signs of stress, attempted to escape from the collars, and experienced difficulty feeding. These factors may have contributed to the lack of increased viability in the surgical delay group compared to the control group. Additionally, considering the earlier timing of the surgical delay procedure, the extended duration of potential exposure to infection should also be taken into account as a possible factor affecting flap viability.

SPY Perfusion Evaluation

In the SPY device perfusion assessment, the TENS group exhibited higher mean perfusion values for both Zone 1 and Zone 4 compared to the other groups; however, these differences did not reach statistical significance at $p<0.1$. Larger studies may be required to determine the significance of these findings. Although high flap perfusion values in the TENS group were not statistically significant, they correlated with the higher viability rates. The lack of a significant difference in SPY measurements among groups remains unexplained. Despite this, the increased viability in the TENS group suggests that factors beyond flap perfusion may contribute to the observed improvement.

Correlation analyses revealed a strong positive correlation between Zone 1 and Zone 4 perfusion values, indicating consistent SPY measurements. Increased blood flow in Zone 1, near the flap pedicle, also correlated with increased flow in Zone 4, further from the pedicle. Additionally, a strong positive correlation was observed between Zone 1 perfusion and flap viability, confirming that greater blood flow improves flap survival.

Vessel Density

Immunohistochemical examination with CD34, an angiogenesis marker, helps determine vessel density.[28] In this study, vessel counts were significantly greater in the Surgical

Delay group compared to the other groups. This supports the effectiveness of the surgical delay model in increasing vessel density.[29] However, as mentioned earlier, this did not translate into increased flap viability, possibly due to the reasons discussed.

VEGF

In this study, VEGF concentrations in the TENS group were similar to those in the Control group. However, previous studies have shown that TENS increases VEGF levels.[30] This discrepancy may be due to the use of TENS as a preconditioning method on healthy tissue in this study, whereas previous research applied TENS to ischemic tissues.

At first glance, the lower VEGF levels in the Surgical Delay group compared to the Control group may seem inconsistent with the higher number of vessels observed in the Surgical Delay group. However, literature indicates that VEGF levels peak within the first 72 hours after surgery.[30] In the current study, neovascularization may have increased VEGF levels within the first three days following the surgical delay procedure. However, since VEGF measurements were taken on day seven, levels may have declined by that time. The findings that there was no increase in the number of new vessels and no increase in VEGF levels in the TENS group appear to be consistent with each other. Although not statistically significant, the increase in flap viability in the TENS group suggests that rather than increasing the number of new vessels, TENS may act through another effective mechanism. Additionally, despite the higher vessel count in the Surgical Delay group, there was no corresponding increase in viability. One possible explanation is that, although new vessels were present, there may have been a dilatation mechanism affecting existing vessels. The increased perfusion measured by SPY in the TENS group, particularly in Zone 1, although not statistically significant, appears to support this speculation.

Planning of TENS Application

In this study, TENS electrodes were placed over the rectus muscle of the axial pattern flap. In contrast, Doğan's study positioned cathodal and anodal electrodes at the distal and proximal ends of the flap, respectively.[6] The amplitude and frequency of TENS in this study were based on the protocols used by Atalay and Kjartansson, who placed electrodes at the flap base.[5,31] Both studies suggested that TENS enhances flap viability through vasodilation. However, due to the size of the electrodes and the proximity of the flap ends, achieving optimal electrode placement was challenging in this study. We consider this difficulty to be a limitation of our experiment. Future research could utilize smaller electrodes applied separately to the rectus muscle or the TRAM flap skin island to better explore the detailed effects of TENS.

CONCLUSION

TENS is a promising preconditioning method for enhancing the viability of axial flaps, such as the TRAM flap. Following this study, the effects of TENS as a preconditioning technique for axial flaps can be further evaluated through clinical studies.

Ethics Committee Approval: The Experimental Animals Ethics Committee of Erciyes University Medical Faculty granted approval for this study (date: 15.07.2019, number: TTU-2019-9400).

Author Contributions: Concept – GA, İÖ, DK, TMÖ; Design – GA, İÖ, DK, TMÖ; Supervision – GA, İÖ, DK, TMÖ; Resource – GA; Materials – GA; Data Collection and/or Processing – GA; Analysis and/or Interpretation – GA, İÖ, DK, TMÖ; Literature Search – GA; Writing – GA;Critical Reviews – GA, İÖ, DK, TMÖ.

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