

The application value of early postoperative pain management (EPPM) combined with skin temperature monitoring (STM) after flap repair of soft tissue defects in the lower limbs: a non-randomized controlled trial

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Background: Flap repair is often required when repairing soft tissue defects in the lower limbs. Although early postoperative pain management (EPPM) can improve postoperative comfort and rest efficiency, and reduce the probability of complications, it cannot detect tissue blood circulation disorders in time. The purpose of this study was to explore the application value of EPPM combined with skin temperature monitoring (STM) after flap repair of soft tissue defects in the lower limbs.

Methods: We retrospectively collected the data of 101 patients who underwent lower limb soft tissue defect flap repair, which were divided into the EPPM group (n=50 cases) and the EPPM-STM group (n=51 cases). The EPPM group was given early postoperative pain management, and the EPPM-STM group was given additional skin temperature monitoring on the basis of the EPPM group. The clinical effect, reoperation rate, flap survival rate, pain score, postoperative quality of life and mental resilience, and complications were analyzed and compared.

Results: The average healing time of the EPPM-STM group was significantly lower compared with the EPPM group, and the serum interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and interleukin-10 (IL-10) levels were significantly lower after the intervention (t=7.418, 3.447, 7.472; P<0.001, =0.001, <0.001). Compared with the EPPM group, the reoperation rate and complication rate in the EPPM-STM group were significantly lower, and the flap survival rate was significantly lower than those of the EPPM group after intervention (At 3 days: t=4.723; P<0.001. At 5 days: t=5.261; P<0.001, <0.001). Logistic regression analysis showed that the postoperative management methods and the area of the flap defect were independent risk factors that affected the survival of the flap (t=7.358, 4.819; P=0.007, 0.028).

Conclusions: EPPM combined with STM can increase the speed of postoperative healing, increase the survival rate of skin flaps, reduce the rate of reoperation and complications, and improve the quality of life and mental resilience of patients who undergo flap repair.

Keywords: Early postoperative pain management (EPPM); skin temperature monitoring (STM); soft tissue defect in lower limbs; flap repair

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Introduction

With the acceleration of China's economic development, the incidence of traffic, sports, and construction injuries has increased significantly, and the incidence of soft tissue defects is on the rise (1). Soft tissue defects are common in sports people and mostly occur after limb trauma. The incidence of soft tissue defects in the lower limbs is the most frequent type of soft tissue injury. Defects in the soft tissues of the lower limbs expose the body's bones and blood vessels, and the wounds cannot be sutured directly, therefore flap repairs are often required when repairing the defects (2,3). The anterolateral thigh flap is easy to extract and has a long vascular pedicle, therefore, clinicians regard it as the first choice for the repair of tissue defects in lower limbs (4). Scientific and complete postoperative management is of great value because it can speed up the recovery of patients with lower limb soft tissue defects and increase the survival rate of skin flaps. Lower limb tissue defects are characterized by a slow recovery speed, a high disability rate, and a high postoperative complication rate (5), therefore, patients not only need to bear the surgical pain brought by the body after surgery, but also face the life pressure brought by the society and their family. In order to improve the treatment efficiency of patients and improve their postoperative quality of life, it is of great significance to find scientific and effective postoperative intervention methods. Although early postoperative pain management (EPPM) can improve postoperative comfort and rest efficiency, and reduce the probability of complications, it cannot detect tissue blood circulation disorders in time. Therefore, the purpose of this study was to explore the application value of EPPM combined with skin temperature monitoring (STM) after flap repair of soft tissue defects in the lower limbs. We present the following article in accordance with the STROBE reporting checklist (available at https://apm.amegroups.com/article/ view/10.21037/apm-22-161/rc).

Methods

Patient selection

The study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University (No. 2018006) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All participants provided informed consent. We retrospectively collected the data of 101 patients who underwent lower limb soft tissue defect flap repair in our hospital from January 2018 to August 2021. According to the intervention method after surgery, they were divided into the EPPM group (n=50 cases) and the EPPM-STM group (n=51 cases; *Figure 1*).

The inclusion criteria were as follows: (I) the patient met the criteria of "Diagnosis and Treatment of Soft Tissue Injury" and was diagnosed with soft tissue defects of the lower extremities after admission by the doctor's palpation and imaging results (6); (II) the patient had the characteristics of bone or blood vessel exposure, infection, and excessive secretion, which met the conditions for soft tissue defect flap repair; (III) the patient had a soft tissue defect area $\geq 2 \text{ cm} \times 3 \text{ cm}$; (IV) the patient was willing to undergo surgery; (V) the patient could communicate normally, fully understood the pros and cons of the treatment method, agreed to participate, and signed an informed letter. The exclusion criteria were as follows: (I) the patient had an autoimmune system disease or abnormal blood coagulation function, renal insufficiency; (II) the patient was pregnant or lactating; (III) the patient was allergic to the drugs given in this study, such as Caronine; (IV) the patient had lower limb trauma surgery in the past three months or the operation for this study was their second operation; and/or (V) the patient had incomplete case data.

Intervention method

This study was implemented by the same intervention team for both groups. The EPPM group was given early postoperative pain management. The specific measures were as follows: (I) intervention staff routinely gave postoperative health education to patients, including explanations about surgery-related knowledge, postoperative recovery procedures, and treatment of common complications; (II) intervention staff monitored the routine indicators of patients after surgery, such as blood pressure, blood sugar, heart rate, urine output, the affected side skin color, swelling, and capillaries; and (III) intervention staff gave EPPM to patients after operation. The specific method was normal saline (Qitaihe Pharmaceutical Factory, Heilongjiang Province, National Medicine Standard H20217079) 50 mL + Dezocine Injection (Northeast Pharmaceutical Group Shenyang First Pharmaceutical Co., Ltd., National Medicine Standard H21022246) 60 mg per hour with a 2.5 mL intravenous pump, and a papaverine hydrochloride intramuscular injection (Jiangsu Hengrui Pharmaceutical Co., Ltd., National Medicine



Figure 1 Patient selection flowchart.

Zhunzi H32020967), 6 hours per time. In the EPPM-STM group, skin temperature monitoring was added to the same treatments as the EPPM group. The specific method was to keep the room temperature at a constant temperature of 26 °C, to keep the skin flap warm with a light, and to keep the healthy area on the same side that was selected as the control area. The skin temperature of the flap area and the control area were monitored with a skin thermometer for 7 consecutive days after the operation.

Observation index

Clinical effect

The average healing time was recorded and the levels of serum interleukin 6 (IL-6), tumor necrosis factor- α (TNF- α), and interleukin 10 (IL-10) were measured using an enzyme-linked immunosorbent assay. Before and after the intervention, 4 mL of fasting blood was drawn from the patients in the morning and centrifuged for 20 minutes at 2,000 r/min. An automatic biochemical analyzer (Shenzhen Mindray Biomedical Electronics Co., Ltd., model BS-280) was used to measure the levels of IL-6, TNF- α , and IL-10 with its own kit and according to manufacturer instructions.

Reoperation rate and flap survival rate

The survival of the skin flap and the information of the patients who needed a second operation was recorded.

Pain scores

The Visual Analogue Scale (VAS) scale (7) was used to evaluate the pain of patients before and after intervention. The VAS scale score ranges from 0 to 10 points; the higher the score means the more obvious the pain (7).

Mental resilience

The Connor-Davidson resilience scale (CD-RISC) (8) was used to assess the psychological resilience of patients before and after intervention. CD-RISC contains three dimensions—tenacity, strength, and optimism—among which there are 13 items for tenacity, 8 items for strength, and 4 items for optimism. CD-RISC adopts the Likert 5-level scoring method; each item is scored from 0 to 4 points, and the total score is from 0 to 100 points. The degree of improvement in psychological resilience increases as the score increases (8).

Quality of life

The Generic Quality of Life Inventory-74 (GQOLI-74) (9)

Annals of Palliative Medicine, Vol 11, No 3 March 2022

| Group | Age (years) | Gender (male/female) | Course of disease (hours) | BMI (kg/m²) | Skin defect area (≤6 cm × 7 cm/>6 cm × 7 cm) |
|-----------------------|-------------|-------------------------|------------------------------|-------------|---|
| EPPM-STM group (n=51) | 36.10±3.02 | 31/20 | 2.39±0.67 | 25.07±2.76 | 22/29 |
| EPPM group (n=50) | 35.30±3.16 | 29/21 | 2.52±0.64 | 24.74±2.87 | 20/30 |
| t/χ^2 | 1.309 | 0.081 | 0.997 | 0.589 | 0.102 |
| Р | 0.193 | 0.776 | 0.321 | 0.557 | 0.749 |

Table 1 Comparison of the baseline data between the two groups

EPPM, early postoperative pain management; STM, skin temperature monitoring; BMI, body mass index.

was used to assess the quality of life of patients before and after intervention. GQOLI-74 includes 4 dimensions of society, body, material, and psychology, with a total score of 0 to 400 points. The improvement of the quality of life increases with the increase in the score (9).

Complications

Complications, such as wound infection, vascular crisis, spasm, edema, and bleeding, were observed and recorded.

Statistical analyses

The data of this study was processed and analyzed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Count data, such as reoperation rate and flap survival rate, were expressed in n (%), and the χ^2 test was adopted for comparison between groups. Measurement data, such as average healing time, inflammatory index, and pain score, were compared with $\bar{x}\pm s$; all continuous variables were tested for normality, and the *t*-test was used for comparison. Relevant risk factors, such as the influencing factors of flap survival rate, were analyzed by logistic regression. Two-sided P<0.05 indicated that the difference was statistically significant.

Results

General information

The EPPM group included 29 males and 21 females aged 24–75 years old with an average age of (35.30 ± 3.16) years old. Their body mass index (BMI) range was 17.91–32.58 kg/m², with an average of (24.74±2.87) kg/m². Their course of disease was 2–4 hours, with an average of (2.52±0.64) hours. The size of their skin defect area was $\leq 6 \text{ cm} \times 7 \text{ cm}$ in 20 cases, and $>6 \text{ cm} \times 7 \text{ cm}$ in 30 cases. The EPPM-STM group included 31 males and 20 females aged 23–73 years old, with an average age of (36.10±

3.02) years old. Their BMI range was $18.67-32.12 \text{ kg/m}^2$, with an average of $(25.07\pm2.76) \text{ kg/m}^2$. Their course of disease was 1-3 hours, with an average of (2.39 ± 0.67) hours. The size of their skin defect area was $\leq 6 \text{ cm} \times 7 \text{ cm}$ in 22 cases, and $>6 \text{ cm} \times 7 \text{ cm}$ in 29 cases. There were no significant differences between the two groups in gender, age, BMI, course of disease, and skin defect area, and they were comparable (P>0.05) (see *Table 1* for details).

Clinical effect

The average healing time of the EPPM-STM group of (4.02±1.14) days was significantly lower than that of the EPPM group, which was (4.98±1.24) days (t=4.059; P<0.001). Before the intervention, there were no significant differences in the levels of serum IL-6, TNF- α , and IL-10 between the two groups. After the intervention, the levels of IL-6, TNF- α , and IL-10 in the two groups were significantly reduced, and the levels of IL-6, TNF- α , and IL-10 in the EPPM-STM group were significantly lower than those in the EPPM group (t=7.418, 3.447, 7.472; P<0.001, =0.001, <0.001, respectively) (see *Table 2* for details).

Reoperation rate and flap survival rate

In the EPPM-STM group, 4 patients needed reoperation, which was significantly lower than that of the 12 patients in the EPPM group (χ^2 =6.966; P=0.008). In the EPPM-STM group, 49 cases of flaps survived, which was significantly higher than that of the 39 cases of flaps that survived in the EPPM group (χ^2 =7.358; P=0.007) (see *Table 3* for details).

Pain scores

Before intervention, there was no significant difference in

| | | IL-6 (μg/L) | | TNF-α (μ | µg/L) | IL-10 (μg/L) | |
|--------------------------|---------------------------------|---------------------|-----------------------|---------------------|--------------------|---------------------|-----------------------|
| Group | Average healing- time (days) | Before intervention | After intervention | Before intervention | After intervention | Before intervention | After intervention |
| EPPM group (n=50) | 4.98±1.24 | 20.13±3.69 | 12.37±2.62* | 145.49±8.26 | 100.26±16.54* | 22.65±4.31 | 12.69±2.94* |
| EPPM-STM group (n=51) | 4.02±1.14 | 20.69±3.70 | 9.34±1.27* | 144.51±8.07 | 89.29±15.43* | 23.01±4.34 | 8.67±2.45* |
| t | 4.059 | -0.762 | 7.418 | 0.603 | 3.447 | -0.418 | 7.472 |
| Р | <0.001 | 0.448 | <0.001 | 0.548 | 0.001 | 0.677 | <0.001 |

Table 2 Comparison of the clinical effects of the two groups $(n, \overline{x} \pm s)$

Compared with before the intervention, *P<0.05; EPPM, early postoperative pain management; STM, skin temperature monitoring; IL-6, interleukin 6; TNF-α, tumor necrosis factor-α; IL-10, interleukin 10.

 Table 3 Comparison of reoperation rate and flap survival rate between the two groups (n, %)

| Group – | Reo | Flap survival rate | | |
|-----------------------|-----------------------|--------------------------------|-----------|-----------|
| | Need a second surgery | No need for a second operation | Survived | Necrosis |
| EPPM group (n=50) | 12 (22.0) | 38 (78.0) | 39 (80.0) | 11 (20.0) |
| EPPM-STM group (n=51) | 4 (4.1) | 47 (95.9) | 49 (94.1) | 2 (5.9) |
| χ² | | 6.966 | | 358 |
| Р | | 0.0 | 007 | |

EPPM, early postoperative pain management; STM, skin temperature monitoring.

Table 4 Comparison of pain scores between the two groups ($\bar{x}\pm s$, points)

| Group | Before intervention | 3 days after intervention 5 | days after intervention | $F_{\text{time point}}$ | F _{between two groups} | F _{interactive} |
|-----------------------|---------------------|-----------------------------|-------------------------|-------------------------|---------------------------------|--------------------------|
| EPPM group (n=50) | 7.74±1.23 | 6.32±1.32 | 4.12±1.14 | 558.682 | 11.989 | 18.582 |
| EPPM-STM group (n=51) | 7.65±1.16 | 5.16±1.16 | 3.00±1.00 | | | |
| t | 0.391 | 4.723 | 5.261 | | | |
| Р | 0.697 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

EPPM, early postoperative pain management; STM, skin temperature monitoring.

pain scores between the two groups (t=0.391; P=0.697). After 3 days and 5 days of intervention, the pain scores of the two groups were lower, and the EPPM-STM group's score was lower than the EPPM group's score (At 3 days: t=4.723; P<0.001. At 5 days: t=5.261; P<0.001). Repeated measurement results showed that there were statistical differences in the effects of pain scores within and between subjects (P<0.05), the pain scores tended to change over time, and there were differences due to different intervention methods (see *Table 4* for details).

Mental resilience and quality of life

Before the intervention, there were no significant differences in the total scores of mental resilience (t=-0.459, P=0.647) and quality of life (t=-0.255, P=0.799) between the two groups . After the intervention, the total scores of mental resilience (t=-12.942, P<0.001) and quality of life (t=-9.975, P<0.001) of the two groups were higher than those before the intervention, and the EPPM-STM group was higher than that of the EPPM group (see *Table 5* for details).

Annals of Palliative Medicine, Vol 11, No 3 March 2022

| | Total scores of mental resilience | | | | Total scores of | | | |
|--------------------------|-----------------------------------|--------------------|---------|--------|---------------------|--------------------|---------|--------|
| Group Before interventio | Before intervention | After intervention | - t | Ρ | Before intervention | After intervention | - t | Р |
| EPPM group (n=50) | 30.36±7.22 | 56.50±8.90 | -26.013 | <0.001 | 146.22±15.35 | 177.60±19.30 | -13.454 | <0.001 |
| EPPM-STM group (n=51) | 31.02±7.23 | 79.59±9.02 | -44.849 | <0.001 | 147.00±15.35 | 216.78±20.16 | -28.633 | <0.001 |
| t | -0.459 | -12.942 | | | -0.255 | -9.975 | | |
| Р | 0.647 | <0.001 | | | 0.799 | <0.001 | | |

Table 5 Comparison of mental resilience and quality of life scores between the two groups (\bar{x} ±s, points)

EPPM, early postoperative pain management; STM, skin temperature monitoring.

| Table 6 Com | parison of the | occurrence o | of complicati | ons between t | the two group | os (n, %) |
|-------------|----------------|--------------|---------------|---------------|---------------|-----------|
| | | | | | | |

| 1 | 1 | | 0 1 (| | | |
|-----------------------|-----------------|-----------|---------|-----------------|----------|-------------------|
| Group | Vascular crisis | Vasospasm | Edema | Wound infection | Bleeding | Complication rate |
| EPPM group (n=50) | 2 (4.0) | 2 (4.0) | 3 (6.0) | 3 (6.0) | 3 (6.0) | 13 (26.0) |
| EPPM-STM group (n=51) | 1 (0.2) | 1 (0.2) | 1 (0.2) | 0 (0.0) | 1 (0.2) | 4 (7.8) |
| χ^2 | | | | | | 5.946 |
| Р | | | | | | 0.015 |
| EBBM I I II | | OTM IS I | | | | |

EPPM, early postoperative pain management; STM, skin temperature monitoring.

Complications

Complications occurred in 17 of 101 patients (16.83%). In the EPPM-STM group, there was 1 case of vascular crisis, 1 case of vasospasm, 1 case of edema, and 1 case of bleeding, and the complication rate was 7.8%. In the EPPM group, there were 2 cases of vascular crisis, 2 cases of vasospasm, 3 cases of edema, 3 cases of wound infection, and 3 cases of bleeding, and the complication rate was 26.0%. The incidence of complications in the EPPM-STM group was significantly lower than that in the EPPM group (χ^2 =5.946; P=0.015) (see *Table 6* for details).

Analysis of factors affecting the survival rate of skin flaps

The results of univariate analysis showed that the postoperative management methods (t=7.358, P=0.007) and the area of the flap defect (t=4.819, P=0.028) were statistically significant. The postoperative management methods and the area of the flap defect were the independent variables, and survival of the flap after intervention was the dependent variable. Logistic regression analysis showed the postoperative management methods [odds ratio (OR) =6.910; 95% confidence interval (CI):

1.446–33.024], the area of the flap defect (OR =5.022; 95% CI: 1.051–23.984) were independent factors that affected the survival of the flap (see *Tables* 7,8 for details).

Discussion

Analysis of accidents that result in lower limb trauma shows that a high-energy impact can manifest as different degrees of fractures, large-scale trauma, soft tissue defects, and necrosis, and severe cases require amputation (10). Soft tissue defects of the lower limbs frequently require surgical procedures, and surgeons usually use skin flaps to repair the defects of the lower limbs (11). Since it takes a long time for patients to recover after soft tissue injury, and the postoperative complications are prone to occur, patients often suffer from skin flap necrosis or require a second operation. Some scholars reported that not only does the surgical process affect the prognosis after the repair of the soft tissue defect of the lower limbs, but also the postoperative risk management and care of the affected side could affect the prognosis of patients (12,13). If the patient had severe pain and excessive expression of inflammatory factors after the operation, the survival rate of the flap would be significantly reduced (14). Therefore, successful

| | Table 7 Analysis of fa | ctors affecting the s | urvival rate of skin flaps |
|--|------------------------|-----------------------|----------------------------|
|--|------------------------|-----------------------|----------------------------|

| Baseline data | n | The survival rate of skin flaps, n (%) | χ^2 | Р |
|----------------------------------|----|--|----------|-------|
| Age (years) | | | 0.041 | 0.840 |
| >30 | 44 | 38 (86.4) | | |
| ≤30 | 57 | 50 (87.7) | | |
| Gender | | | 0.487 | 0.485 |
| Male | 61 | 52 (85.2) | | |
| Female | 40 | 36 (90.0) | | |
| Postoperative management methods | | | 7.358 | 0.007 |
| EPPM | 50 | 39 (78.0) | | |
| EPPM-STM | 51 | 49 (96.1) | | |
| Course of disease (hours) | | | 0.224 | 0.636 |
| >2 | 56 | 48 (85.7) | | |
| ≤2 | 45 | 40 (88.9) | | |
| Skin defect area (cm × cm) | | | 4.819 | 0.028 |
| >6×7 | 57 | 46 | | |
| ≤6×7 | 44 | 42 | | |

EPPM, early postoperative pain management; STM, skin temperature monitoring.

| Table 8 Logistic regression analysis affecting the | survival rate of skin flaps |
|--|-----------------------------|
|--|-----------------------------|

| 0 0 , | 0 | 1 | | | | |
|----------------------------------|--------|-------|--------|-------|-------|--------------|
| Factor | β | S.E. | Wald | Р | OR | 95% CI |
| Postoperative management methods | 1.933 | 0.798 | 5.866 | 0.015 | 6.910 | 1.446–33.024 |
| Skin defect area | 1.614 | 0.798 | 4.092 | 0.043 | 5.022 | 1.051–23.984 |
| Constant | -3.199 | 0.721 | 19.661 | 0.000 | - | - |

S.E., standard error; OR, odds ratio; 95% CI, 95% confidence interval.

postoperative rehabilitation management of patients is essential.

After repairing the soft tissue defect of the lower limbs, patients would have varying degrees of muscle and nerve damage, causing intense pain in the patient, resulting in increased body inflammatory response, sleep disturbance, and affecting the patient's postoperative recovery speed (15). Good postoperative pain management can significantly improve postoperative comfort and rest efficiency of patients and reduce the incidence of complications. Some scholars suggested that pain should be included in the scope of vital signs monitoring (16,17). Pain cannot only cause physical trauma to patients but can also induce psychological trauma for patients (16,17). The results of this study showed that the pain scores of the two groups of

patients decreased significantly, and the mental flexibility and quality of life scores increased significantly after the intervention. Results showed that EPPM combined with STM could reduce the pain of patients after the soft tissue defect flap repair of the lower limbs and effectively improve the mental state and quality of life of the patients.

After the repair of soft tissue defects of the lower limbs, medical staff need to regularly monitor the condition of the skin flaps. The traditional monitoring methods include observing the skin color, swelling, and capillaries of the affected side (18). Although the above method is simple and economical, it can be subjective and is not suitable for inexperienced nursing staff (19). In addition, when some patients first have tissue blood circulation disorders, the signs are not obvious, and when the signs appear, the

Annals of Palliative Medicine, Vol 11, No 3 March 2022

damage is irreversible (20). STM is a low price, simple operation that is easy to implement. It is not affected by the judgment of the operator's supervisor and can quickly analyze the condition of the patient's skin flap.

In this study, the average healing time of the EPPM-STM group was shorter than that of the EPPM group, and the serum inflammatory indexes decreased faster after the intervention. In addition, the reoperation rate in the EPPM-STM group was only 4.1%, the flap survival rate was as high as 94.1%, and only 4 complications occurred in 51 patients. The main reason was that the skin temperature monitoring method enabled medical staff to dynamically grasp the changes in the patient's body state in real time and deal with adverse phenomena in time. At the same time, the skin flap light improved the circulation of blood in the body tissues, accelerated the speed of microcirculation and lymphatic fluid return, and reduced the occurrence of vascular crises and spasms.

Logistic regression analysis showed the postoperative management methods (OR =6.910; 95% CI: 1.446–33.024), the area of flap defect (OR =5.022; 95% CI: 1.051–23.984) were independent factors that affected the survival of the flap. This result suggested that medical staff could improve the clinical efficacy and recovery rate of patients by improving postoperative management programs or targeted treatment of different degrees of flap damage.

In summary, the combination of EPPM and STM can reduce the incidence of reoperation and complications, improve the healing speed and flap survival rate of patients after surgery, and can also improve the mental state and quality of life of patients. It is worthy of clinical promotion.

But this study also has certain limitations. Due to the small sample size, long research time span, and retrospective analysis of this study, selection bias cannot be ruled out. However, because there is no difference in preoperative baseline data between the two groups of patients, the results are less likely to be misleading.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at https://apm.amegroups.com/article/view/10.21037/apm-22-161/rc

Data Sharing Statement: Available at https://apm.amegroups.com/article/view/10.21037/apm-22-161/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://apm. amegroups.com/article/view/10.21037/apm-22-161/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University (No. 2018006) and was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All participants provided informed consent.

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Shen et al. EPPM combined with STM after flap repair

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1076