Short thesis for the Degree of Doctor of Philosophy (PhD)

Complex blood flow studies of local, interpolated, and transferred free musculocutaneous flaps in an experimental surgical model

by Gergő Kincses, MD

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INTRODUCTION

Nowadays, thanks to the dynamic development of modern medicine, numerous options are available for replacing extensive skin and soft tissue defects of the human body. Technological progress and experiences gained through animal experiments can provide a way out even from situations previously considered incurable. In recent decades, the rapid advancement of microsurgery has brought revolutionary changes to the field of reconstructive surgery. The transplantation of various tissue flaps – whether skin, muscle, fasciocutaneous, or osteocutaneous flaps – is now a routinely applied procedure for numerous clinical indications, ranging from the reconstruction of traumatic, oncological, or burn-related defects to complex tissue replacements.

In order to study flap pathophysiology, optimize flap formation, and research preconditioning and preventive possibilities, numerous experimental models concerning various flaps (location, tissue composition) have been published.

The application of tissue flaps is one of the fundamental pillars of reconstructive surgery, the aim of which is the anatomical, functional, and aesthetic restoration of acquired or congenital tissue deficiencies. The use of flaps becomes necessary when primary wound closure or the coverage of a local tissue defect with a skin graft does not provide satisfactory results in terms of blood supply, tissue thickness, function, or stability. In contrast to grafts, flaps are tissue units possessing their own blood supply, which may contain various anatomical components (skin, subcutaneous tissue, muscle, fascia, bone, etc.), and thus can be suitable for replacing complex tissue deficiencies. With the development of microsurgery, reconstruction using tissues distant from the defect – the application of free flaps – has become possible. These flaps are completely independent of their original blood supply and can be transplanted to the recipient area via micro-anastomoses.

Ischemic damage to the flap can be effectively reduced by applying techniques personalized for individual patients. Preserving adequate blood supply is a fundamental criterion for successful transposition and transplantation. Perfusion disturbances delay healing, increase the risk of suppuration, abscess formation, and necrosis, and in severe cases can lead to flap loss. Although the major complications of flap surgery—such as necrosis, perfusion/microcirculatory disturbances, ischemia/hypoperfusion, venous congestion, suture insufficiency, and the attributes of infection—are well known, many questions remain unanswered. The perfusion threshold predicting flap survival and precise microcirculatory changes still need to be clarified. Furthermore, reliable early indicators of ischemia or venous congestion are lacking.

The tolerance of different flap types to ischemia and the molecular mechanisms underlying ischemia-reperfusion (I/R) injury require further investigation. Distinguishing between arterial and venous injuries is of paramount importance in clinical practice. Surgical technical aspects, including the biomechanical properties of suture materials and the stability of the anastomosis, continue to require great attention. The relationship between bacterial contamination, immune response, and wound healing disturbances is not yet fully clarified. A systematic evaluation is needed regarding the impact of flap design and geometry—particularly the pedicle-to-flap size ratio, orientation, and thickness—on perfusion and the development of necrosis.

Numerous studies are investigating the possibilities of reducing ischemia, both with pharmacological and surgical techniques. Vasopressors have been shown to be effective in free tissue transplantation, reducing the likelihood of graft necrosis. Clinical trials have investigated the positive effects of perioperative aspirin, heparin, and dextran. Meta-analyses have highlighted the importance of heparin and LMWH dosing. Postoperative use of dextran-40 reduced the risk of partial graft necrosis but may cause severe pulmonary edema. Graft

preconditioning is also a good method for reducing the incidence of graft ischemia. Negative pressure therapy, such as non-invasive vacuum therapy, promotes angiogenesis, has a positive effect on tissue perfusion, and improves the chances of graft survival. Electrical stimulation is an ideal method for improving the chances of graft survival prior to graft preparation. Hyperbaric oxygen conditioning can reduce the inflammatory response, increase perfusion, and reduce the rate of graft atrophy.

Various methods are available for the early detection of possible graft damage. These include skin discoloration observed during physical examination, changes in tissue temperature, prolonged capillary refill, and instrumental measurement of graft perfusion. However, their proper evaluation depends largely on the experience and judgment of the observer.

Deviations observed in laboratory parameters, such as red blood cell deformability and aggregation, are also of great importance. These parameters show changes in many pathophysiological conditions, so their examination is essential in surgical and microsurgical experiments. Thrombotic and ischemia-reperfusion complications can occur at any stage of reconstructive surgery: during preparation, flap transposition or rotation, and even during the healing period. However, their microrheological correlations are not yet fully understood. Furthermore, we found no examples in the literature of simultaneous examination of microrheology and microcirculation, nor any histological or biomechanical data comparing local and rotated flaps. Our hypothesis was that microrheological and microcirculatory parameters, as well as histological and biomechanical properties, could be informative in comparing different flap positions and monitoring the wound healing process in the early postoperative period.

AIMS

1. Development of an experimental musculocutaneous flap model in the rat that is highly reproducible, causes no significant functional deficit, and is suitable for the study of local, interpolated, and free flaps destined for transplantation.
2. Complex intra- and postoperative monitoring of flap perfusion, through the coordination of hemodynamic and microcirculatory methods.
3. Investigation of the changes in hematological and microrheological parameters (red blood cell deformability, red blood cell aggregation) that influence tissue microcirculation during the course of flap regeneration.
4. Comparative analysis of histological changes occurring during the early period of regeneration (the first two postoperative weeks) in flaps of different locations/types (local, interpolated, transplanted).
5. Biomechanical examinations of the flaps, with special regard to comparing areas at different distances from the supplying vessel and examining the effect of vascular pedicle tension.

MATERIALS AND METHODS

1.1 Experimental animals

The experiments were conducted with the appropriate permits (permit registration number: 19/2022/DEMÁB) in accordance with national regulations (Act XXVIII of 1998 on the protection and humane treatment of animals) and EU directives (2010/63/EU).

Rats were chosen as experimental animals due to their suitability for well-developed and reproducible experimental microsurgical models. Twenty-four adult male Wistar rats (Crl:WI, body weight: 382.99 ± 37.23 g, Toxi-Coop Zrt., Budapest, Hungary) were used in the study. The animals were kept in the department's conventional animal facility (standard cages:

Eurostandard IV, Tecniplast, Buguggiate, Italy; temperature: 22 ± 2 °C; humidity: 55 ± 10%; lighting: 12–12 hour light/dark cycle). They had free access to standard rat chow (SAFE® D132, Complete Care Competence, Augy, France) and water. The animals participated in the experiments after a 2-week acclimatization period.

1.2 Experimental groups

Based on computer-generated randomization, the animals were divided into three experimental groups:

- control group (control group, n = 8)
- group with local and interpolated flaps (flap group, n = 8)
- group with local and transplanted flaps (TransFlap group, n = 8).

In the *control* group, the elements of the protocol were as follows: anesthesia, depilation of the relevant areas, and fixation for the duration of the surgery (like the other groups).

In the *flap group*, we created muscle-skin flaps based on the musculus cutaneus maximus on both sides. One flap was sutured back to its original location (on the right side), while the other was interpolated to the anterior wall of the chest through a subcutaneous tunnel (on the left side).

In the *TransFlap* group, in addition to creating the local flap, we transferred the other flap to the left groin region (as a free flap), forming microvascular anastomoses.

1.3 Surgical protocol

For general anesthesia, we used ketamine hydrochloride (100 mg/kg body weight, i.p., CP ketamine hydrochloride 10%, Produlab Pharma BV, Raamsdonksveer, Netherlands) and xylazine hydrochloride (10 mg/kg body weight, i.p., CP xylazine hydrochloride, 2%; Produlab Pharma BV, Raamsdonksveer, Netherlands) for general anesthesia. The animals breathed

spontaneously. To monitor anesthesia, we observed respiratory rate and depth, mucous membrane color, and limb color. During surgery, body temperature was maintained with a heating pad (Bremed, Budapest, Hungary).

To create the musculocutaneous flap, the musculus cutaneous maximus (supplied by the lateralis thoracicus arteria), the covering fascia layer, and skin on both sides of the rat's flank were used. After hair removal and before flap preparation, the extent of the angiosome was determined using a CytoCam-IDF camera (Braedius Medical, Huizen, Netherlands). Based on the measurement results, an approximately bean-shaped plastic template (surface area = 7.13 cm^2) was used to mark the contour of the flap for proper skin incision and preparation. The convex part faced the animal's back, and its upper tip was located at the front of the armpit. The skin of the chest region is relatively thin, so the incision had to be made accurately and carefully.

The cutaneous maximus is a skin muscle, meaning that part of the skin's blood supply comes from the muscle. Therefore, we avoided completely separating the muscle from the skin to preserve the blood supply. We left a 5–6 mm wide strip of muscle around the flap. The vitality of the remaining muscle tissue was satisfactory thanks to collateral circulation. To preserve collateral circulation, it was important to find the appropriate avascular connective tissue layer during preparation. The blood vessels were covered by fatty tissue under the muscle layer. To avoid damaging the blood vessels, we separated this layer of fat and left it on the chest wall. The supplying vessels and the axillary region are also surrounded by fatty tissue. The strongest blood flow is found below the origin of the lateral branches, where we performed blood flow measurements. To maintain hydration, the dissected lobes were covered with moist gauze (soaked in physiological saline solution at body temperature) during the measurement.

In the case of local flaps (on the right side), after performing the necessary measurements, we sutured them back into their original anatomical position using tension-free, situational knot sutures and intracutaneous running sutures between the knots. The sutures were

performed with 4-0 polypropylene suture material and a reverse cutting needle (Pidelen; KOLLSUT, Hauppauge, NY, United States).

After preparing the interpolated flaps, we imitate an iatrogenic extensive skin and soft tissue defect. We made a circular 3x3 cm wound involving the skin and subcutaneous tissue on the chest wall above the sternum and abdominal muscles. The muscles are intact under the created defect. We carefully separated the surrounding subcutis from the muscle. To cover the flap, we created a "tunnel" under the skin bridge between the donor site and the defect, which was wide enough for tension-free flap interpolation without creating compression. We then carefully pulled the flap over to the recipient site. During the process, we had to avoid breaking, twisting, or overstretching the flap's vascular pedicle. The subcutaneous tunnel also served as a protective layer against external damage. We secured the muscle layer of the flap to the wound bed with galvanic sutures, thus helping the muscle to spread out and the flap to wrinkle. The skin was sutured in a similar manner to the local flaps. Subsequently, we performed the primary closure of the donor site.

We found the femoro-inguinal region to be suitable for free flap transplantation. The femoral vein and artery provide good accessibility for anastomosis. The relatively long pedicle of the flap provides a unique opportunity for free tissue transplantation. The supplying vessels are very small, with a diameter of less than 1 mm (artery = 0.53 ± 0.06; vein = 0.91 ± 0.11), therefore microsurgical techniques were required to create vascular anastomoses. We created iatrogenic skin and soft tissue defects in the inguinal region, where we transplanted the free flap. We made a 3x3 cm circular incision involving the skin and subcutis. The muscles were intact and not affected by the resection. The superficial epigastric artery and vein were carefully dissected and traced to their origin in the femoral vessels. The connective tissue surrounding the vessels was released and dissected longitudinally to the level of exclusion, and the structures were circumvented. Proximal clamping of the femoral artery was performed after the Murphy

branch, and distal clamping after the epigastric artery. To reduce the discrepancy in vessel diameter, the epigastric artery was elevated and its origin enlarged, making the femoral artery suitable for end-to-side anastomosis. After creating the anastomotic openings and flushing the lumen with diluted heparin-sodium solution (first the artery), we performed end-to-side anastomoses between the artery of the lobe pedicle and the femoral artery.

Prior to transplanting the lobes, we perfused them with heparin solution through the pedicle vessels. Vascular anastomoses were performed using 11-0 monofilament, non-absorbable polyamide-6 suture material and serosa (conical) needles (Daclon, SMI, Vith, Belgium) with knotted sutures. Following arterial anastomosis, circulation began on the venous side, providing feedback on the quality of perfusion. The anastomosis was completed in 20 minutes. We verified patency using a "milking" test, in which blood is expressed from the lumen of the vessel clamped proximally, and we then observe its reflux. Similar techniques were used for the vein. The skin was closed in a similar manner to other flap types.

A plastic collar was placed around the animals' necks to prevent autophagy. Follow-up lasted for two weeks, with daily checks of the wounds. Tramadol (15 mg/kg body weight/day) was administered for analgesia during the first three days after surgery. At the end of the experiment, the animals were euthanized, measurements were taken, and then they were exterminated with a high dose of anesthetic (50 mg/kg ketamine and 5 mg/kg xylazine) administered through a tail vein cannula.

1.4. Examination of flap viability

During the test, we measured the surface temperature of the skin (rodent NIBP infrared thermometer with LaserSight, ADInstruments, USA) of all flaps and intact abdominal skin areas, above the xiphoid process of the sternum, before flap preparation, immediately after skin closure, and on the 14th day after surgery.

Perfusion blood flow (ml/min) in the flap pedicle was measured using a Transonic T206 device (Transonic Microcirculation Flowprobe; Transonic Systems Inc., Ithaca, NY, USA). The ultrasonic probe of the device was carefully placed under the supplying pedicular artery. Measurements were taken after preparation of the flap, immediately before skin closure, and on the 14th day after surgery.

Skin microcirculation was monitored using a CytoCam-IDF camera (Braedius Medical, Huizen, Netherlands). The recordings were made before the operation (baseline), immediately after the operation, and on the 14th day after the operation. The method is based on incident dark field technology, which makes microcirculation visible in real time. The device software (CytoCamTools V3 Bedside Manager, Braedius Medical, Huizen, Netherlands) to analyze the high-resolution video recordings offline, determining the microvascular flow index (MFI [au]), the proportion of perfused vessels (PPV [%]), and the density of perfused vessels (PVD [mm/mm2]).

To standardize video recordings with the Cytocam-IDF camera, we selected fixed measurement locations and used precise focus settings (±2 μm) during recording. Short (~3-second) recordings were made with adequate lighting, stable positioning, and minimal pressure exertion. Three videos were recorded per measurement point, and after evaluating the focus, brightness, stability, and artifacts, poor-quality recordings were excluded from the evaluation. Suitable recordings were analyzed offline using special software and fixed algorithms. The software (Cytocam Tools 4.0.2) divided the acquired images into four quadrants for analysis. We kept the resolution and field of view constant (720 Å ~ 580 px, 0.94 Å ~ 0.75 mm). We determined the MFI by semi-quantitative evaluation of each quadrant on a scale of 0–3.

1.5. Laboratory measurement methods

Blood samples were taken from the lateral tail vein (0.4 ml/sample; 26 G Neoflon™ Pro IV cannula; K3-EDTA, Vacutainer®; both: Becton Dickinson GmbH, Franklin Lakes, NJ, USA) for the examination of haematological and micro-rheological parameters.

Haematological parameters were determined using a Sysmex K-4500 automatic analyser (TOA Medical Electronics Co., Ltd., Kobe, Japan). We analyzed white blood cell count (WBC [109/L]), red blood cell count (RBC [1012/L]), hematocrit (Hct [%]), platelet count (PLT [109/L]), hemoglobin concentration (Hbg [g/dL]), mean corpuscular volume (MCV [fL]), mean corpuscular hemoglobin (MCH [pg]), and mean corpuscular hemoglobin concentration (MCHC [g/dL]).

To examine the deformability of red blood cells, we used a LoRRca MaxSis Osmoscan ectacytometer (RR Mechatronics International B.V., Zwaag, Netherlands) was used to examine the deformability of red blood cells, which determines the elongation index (EI) as a function of shear stress (SS [Pa]) in the range of 0.3–30 Pa. For the examination, 10 µL of whole blood was mixed with 2 mL of polyvinylpyrrolidone (PVP) – PBS solution (PVP: 360 kDa, Sigma-Aldrich Co., St. Louis, MO, USA; viscosity of PVP-PBS solution = 30–35 mPas, osmolarity = 290–310 mOsmol/kg, pH = 7.5). Comparative data for the EI–SS curves were calculated, including EI values measured at 3 Pa, the maximum elongation index (EImax), and the shear stress corresponding to half of EImax (SS1/2, [Pa]) using the Lineweaver–Burk equation. The deterioration of red blood cell deformability is indicated by lower EI or EImax and higher SS1/2 values.

Red blood cell aggregation was examined using a Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Roetgen, Germany) with a light transmission method. For the examination, 20 µl of blood was used per measurement. The values were measured at 5 and 10 seconds of the aggregation process during stasis (velocity gradient: 0 s-1, M 5s and M 10s

indices) or at low velocity gradient (velocity gradient: 3 s-1, M1 5s and M1 10s indices). Higher index values indicate increased aggregation of red blood cells.

1.6. Histological analysis

On the 14th day after surgery, the animals were terminated, and standard-sized and -shaped samples were taken from both the control and rotated flaps for histological examination. These samples contained intact tissue, flap tissue, and the transition zone of tissue anastomosis. The samples were placed in formalin, then embedded using conventional methods and sectioned. The serial sections were stained with hematoxylin and eosin (H&E, Sigma-Aldrich, St. Louis, MO, USA) for morphological analysis and with orcein (Sigma-Aldrich, St. Louis, MO, USA) for elastin visualization.

All staining procedures followed the manufacturer's protocols. Microscopic images were captured using a DP74 camera (Olympus Corporation, Tokyo, Japan) mounted on an Olympus BÅ~53 microscope (Olympus Corporation, Tokyo, Japan). Picrosirius red staining (Sigma-Aldrich, St. Louis, MO, USA) was used to examine the orientation of collagen fibers in the blood vessels. The samples were analyzed under polarized light using an Olympus BÅ~53 polarizing microscope (Olympus Corporation, Tokyo, Japan), rotating the plane of light with λ/4 samples. To measure the thickness of the epidermis, we analyzed microscopic images of 20x magnification H&E-stained sections using ImageJ 1.40 g freeware. Twenty independent measurements were performed on each microscopic preparation, and six independent skin strips were examined per experimental group. In orcein-stained sections, the normal color was changed to green and black to improve pixel contrast. The number of green pixels was quantified using ImageJ freeware.

1.7. Tensile strength measurements

For the tensile strength tests, which were performed using a specially developed device, we used skin samples of equal width (5 mm) taken on the 14th day after surgery. These samples included both intact areas and surgical areas, as well as the healed suture line. The samples were placed in a tensile strength measuring device, where we applied a gradually increasing tensile force until they tore. The maximum load capacity of the samples just before breaking indicates the tensile strength of the tissue. The force required for rupture and elongation was continuously recorded, and the maximum stress, rupture point, and slope of the curve were determined using the stress-strain curve generated by the machine.

1.8. Statistical analysis

We used the Mead equation method to determine the number of elements required for the experiment. Statistical analyses were performed using SigmaStat Software 3.1.1.0 (Systat Software Inc., San Jose, CA, USA). Data are presented as mean ± standard deviation (SD). We used the t-test or Mann-Whitney rank sum test for comparisons between groups, and one-way analysis of variance (ANOVA) or the Kruskal-Wallis test for comparisons within groups, depending on the normality of the data distribution. A value of $p < 0.05$ was considered statistically significant.

RESULTS

2.1. General observations

All flaps remained viable, and we did not observe any serious complications that would have led to flap necrosis. We did not observe any thrombotic complications, swelling, or extensive flap necrosis.

Wound healing was adequate during the granulation phase. Of the 16 flaps, only 2 cases (1 local and 1 rotated flap) showed partial, thin marginal necrosis. These cases were not included in the final data analysis.

2.2 Body weight

The animals were weighed before surgery and during the first and second weeks after surgery. After surgery, we observed significant weight loss in most of the interpolated and transplanted flap individuals by the second week after surgery.

2.3 Skin temperature of the flaps

The skin temperature of the local and interpolated flaps did not change significantly immediately after surgery and on the 14th postoperative day. In the case of free flaps, the skin temperature decreased by an average of 4.77% immediately after surgery and was also lower at the end of the follow-up period (3.47% compared to the baseline value).

2.4 Flow values of the arteries supplying the flaps

Arterial blood flow (ml/min) in the flap pedicle did not show any significant differences after flap preparation. The highest blood flow values were observed in local flaps that remained in their original anatomical position.

We observed that arterial blood flow values were significantly lower in all flap types on the 14th day after surgery. The lowest values were observed in the transplanted flaps ($p = 0.039$).

2.5 Microcirculation studies

During the analysis of the videomicroscopic recordings, no microcirculatory or visible vascular abnormalities were observed before preparation. After the preparation of the lobes, we observed a temporary, moderate deterioration in microcirculation, with areas of hypoperfusion appearing. After closure of the skin wound, microcirculation normalized. On the 14th day after surgery, some areas of hypoperfusion, enlarged blood vessels, and red blood cell aggregates were visible, mainly in the rotated flaps.

The microvascular flow index (MFI) values were above 2 in all cases (2.72–2.84), so the video recordings could be easily analyzed for the safe evaluation of further parameters. The density of perfused vessels (PVD [mm/mm2]) remained unchanged in the control skin area in all groups. In the local, interpolated, and transplanted flaps, PVD values did not decrease significantly immediately after surgery, and values were also lower on the 14th postoperative day. However, the differences were not significant. The proportion of perfused vessels (PPV [%]) decreased significantly immediately after surgery in the interpolated ($p=0.001$ vs. baseline) and transplanted flaps ($p<0.001$ vs. baseline). On the 14th postoperative day, the values were the same as the baseline values.

When comparing non-transplanted flaps (local vs. interpolated), the most striking changes were seen in the change in the percentage of perfused vessels compared to baseline values. There was no change in the intact abdominal skin area, while a significant decrease was observed in both local and interpolated flaps immediately after surgery (interpolated flap: $p = 0.002$ vs. intact skin area), and the values increased on the 7th (interpolated flap: $p = 0.014$) and the 14th postoperative day (interpolated flap: $p = 0.004$).

The results of the post hoc analysis for the local flap were as follows: Control 14th p.o. day: 69.5%; Flap 7th p.o. day: 95.2%, 14th p.o. day: 99.7%, while for interpolated flap: Control 7th p.o. day: 59%, 14th p.o. day: 75.3%, Flap 7th p.o. day: 99.9%, 14th p.o. day: 99.6%.

2.5.1 Changes in haematological parameters

Haematological parameters during the two-week observation period reflected acute phase reactions occurring in the early granulation phase of inflammation and wound healing. The number of white blood cells and platelets increased in the first and second weeks after surgery, to a greater extent in the Flap group (Fvs on day 7: $p=0.013$ and on day 14: $p<0.001$; Thr on day 7: $p<0.001$ and on day 14: $p=0.002$ vs. baseline). The number of red blood cells, hemoglobin concentration, and hematocrit decreased to a greater extent in the Flap group (all: $p<0.001$).

2.5.2 Changes in red blood cell deformability

The elongation index of red blood cells measured at a shear stress of 3 Pa decreased on day 7 after surgery, along with the calculated EImax values ($p<0.001$) and a slight increase in SS1/2, reflecting the deformability of moderately damaged erythrocytes.

2.5.3 Changes in red blood cell aggregation

The M 5s value of the red blood cell aggregation index increased significantly on the 7th and 14th days after surgery (both: $p<0.001$, 7th day after surgery: 32.1% and 14th day after surgery: 31.2%) in the Flap group, while the values in the Control group did not show any significant change. Other index values, such as M1 5s, did not change significantly.

2.6 Histological changes

Samples taken from intact skin areas did not show any histological abnormalities. In the case of interpolated flaps, granulation tissue appeared in the surgical area as a normal part of the wound healing process. If the edges of the wound did not fit together properly, the formation

of scar tissue was more pronounced, which was accompanied by increased neovascularization and a larger number of fibroblasts and inflammatory cells.

Granulation tissue also appeared on the flap, filling the tissue gaps. Granulation tissue appeared around the flaps as well as in the incision area. The boundary between healthy and granulation tissue was clearly recognizable.

In some sections, foreign body-type giant cells (fusion of macrophages) also appeared due to the sutures.

2.7 Tensile strength

During tensile strength measurements, the interpolated groups showed tissue weakness. Elasticity and functionality were also reduced in this group. On the 14th day after surgery, the maximum tensile strength of intact skin was much higher than that of local or interpolated flaps (both: $p < 0.001$). When the flap was rotated, tissue strength decreased to a greater extent. At the same time, the slope of the force-elongation curve was significantly lower compared to the intact skin area (local flap: $p = 0.021$; rotated flap: $p = 0.002$).

KEY RESULTS AND CONCLUSIONS

1. We developed a well-standardized, reproducible experimental musculocutaneous flap model in the m. cutaneus maximus muscle region of rats, which did not cause significant functional impairment. Standard flaps can be studied in different types, such as local, interpolated position, or transplanted free flaps.
2. We performed complex intra- and postoperative follow-up examinations of flap perfusion. Monitoring of blood flow in the vessel supplying the flap, together with microcirculatory measurements, provided important data for optimizing intraoperative positioning and ensuring that the vessel was free of tension and kinks. Video microscopic examinations proved useful in more accurately monitoring the viability of the lobes in the early postoperative period, which is critical in terms of potential complications.
3. Acute phase reactions during the two-week follow-up period were reflected in general hematological and hemorheological abnormalities, which were typically detectable in the early postoperative period. Microcirculatory changes and blood micro-rheological abnormalities were correlated.
4. Histological examinations showed that the flaps did not suffer from significant healing disorders. The presence of granulation processes indicated the integration of the flap into its new position. Neovascularization along the suture line and a foreign body reaction around the sutures were observed.
5. We demonstrated that interpolated and transplanted flaps may be more vulnerable in terms of perfusion and biomechanics, which may manifest as impaired microcirculation, tissue weakness, and slower regeneration. All this underscores the importance of careful postoperative monitoring in clinical practice.

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Registry number: DEENK/4/2026.PL
Subject: PhD Publication List

Candidate: Gergő Kincses
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. **Kincses, G.**, Fazekas, L., Varga, Á., Mátrai, Á. A., Flaskó, A., Adorján, D. M., Molnár, Á., Deák, Á., Németh, N.: A refined experimental model for local, interpolated flap, and free tissue transfer studies using musculus cutaneus maximus-based musculocutaneous flap in the rat.
Acta Cir. Bras. 40, 1-11, 2025.
DOI: http://dx.doi.org/10.1590/acb408125
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2. **Kincses, G.**, Fazekas, L., Varga, Á., Mátrai, Á. A., Nguyen, X. L., Barabási, K., Flaskó, A., Juhász, T., Molnár, Á., Németh, N.: Following-Up Micro-Rheological and Microcirculatory Alterations During the Early Wound Healing Phase of Local and Rotated Musculocutaneous Flaps in Rats.
Life (Basel). 15, 1-14, 2025.
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List of other publications

3. Flaskó, A., Fazekas, L., **Kincses, G.**, Varga, Á., Mátrai, Á. A., Csóka, L. D., Záhorszki, S., Tóth, A. Z., Fillér, C., Juhász, T., Molnár, Á., Németh, N.: Tissue perfusion and its influencing factors in epigastrial adipocutanous flaps affected by ischemia-reperfusion in rats.
Acta Cir. Bras. 41, 1-13, 2026.
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