SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Examination of the viability of adipocutaneous flaps in an experimental ischemia-reperfusion surgical model

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen on 17 June, 2024 at 11:00 a.m.

1. INTRODUCTION

Plastic surgery is an extremely diverse science that performs surgeries on the entire body surface, regardless of age or gender. Anyone who would try to describe this science with a shorter definition faces a big challenge, because it is a profession that has become extremely differentiated in recent decades. It is not only difficult to define the specialty, but also to define the exact spectrum, as it includes many surgical activities that overlap with other specialties (e.g. otolaryngology, head and neck surgery, general surgery, oral surgery). Mathes defined the following, most approximate definition for a brief description of the profession: "Plastic surgery is a problem-solving field of surgery that treats soft tissue deficiencies without anatomical boundaries." Plastic surgery activities can basically be classified into three groups. We can talk about reconstructive surgery, regenerative surgery, and aesthetic surgery. All three are based on the same principles, such as precision, creativity and atraumatic surgical technique.

Flap reconstruction procedures play a major role in the reconstructive branch of plastic surgery. The vitality of these flaps and the success of the operation depend not only on good surgical technique, but also on preoperative planning, the establishment of appropriate indications and the correct postoperative treatment. It is important to emphasize that flap formation is a procedure where the blood supply of the mobilized tissue is inevitably damaged. As Professor János Zoltán descibes in his fundamental book of plastic surgery, the Cicatrix Optima: "Tissues that are already on the edge of their viability can be kept alive only by minimizing surgical trauma". A small error in surgical technique or planning, or even an unexpected factor from the patient's side, can lead to partial or complete flap failure. In the vast majority of cases, when complications occur, the surgeon uses his clinical experience and usually relies on his eyes. The symptoms that indicate flap-related complications are well known: signs of inflammation, such as swelling, redness, discharge, as well as the lividity or pale appearance of the flap, changes in temperature, changes in capillary refill, and tension of the flap. At the time of the appearance of these tangible, macroscopic signs, the underlying etiological factor(s) cannot be eliminated in all cases, partial or complete flap necrosis may occur despite all kinds of corrections and attempts.

The microcirculation and rheological factors related to flaps and the mechanisms of the wound healing process are now known in depth. Not only do we already know how the processes work at a molecular level, but several diagnostic tools have been developed in recent decades that are also suitable for measuring and quantifying microcirculation parameters bedside. Examples include Laser Doppler flowmetry, transcutaneous oxygen and carbon

dioxide tension measurement, fluorescent angiography, or the determination of micro-rheological parameters from a blood sample. However, the clinical experience is that during flap reconstruction procedures in Hungary, plastic surgeons rely only on their eyes, rather than on the use of the devices mentioned as examples. Explaining the reasons would go far beyond the scope of this dissertation, we have to accept that both professional and economic reasons play a role in the raised problem. However, the registration and monitoring of these parameters contains an important opportunity: it can indicate a possible complication even before the previously detailed (late) macroscopic phenomena and potentially irreversible changes occur. In this way, these methods can create the possibility of an early intervention for the surgeon. This is especially important in procedures where the flap reconstruction affects a large surface area, or in the case of flaps that are located under intact skin and therefore cannot be examined directly based on physical signs ("buried flaps"), and also in cases where additional operations or treatments depend on the reconstructive procedure (a particularly important example is the post-operative oncological care of malignant tumors).

This dissertation discusses the experimental and practical possibilities of using these examination methods in the light of the results of the adipocutaneous inguinal flap model conducted at the Department of Operative Techniques and Surgical Research, Faculty of Medicine, University of Debrecen.

2. AIM

1. We aimed to develop an adipocutaneous flap model that is suitable for examining the vitality of the flaps in the intra- and postoperative period and to model the behavior of the flaps used in the clinical practice, including ischemic damage and the resulting complications.

2. We aimed to examine a 1-hour ischemia-reperfusion of an adipocutaneous inguinal flap non-invasively during the intra- and postoperatively period, assuming that the microcirculatory BFU (Blood Flux Unit) values differ in different areas of the flaps and if they can predict the postoperative complications.

3. In addition to microcirculation, we also aimed to examine micro-rheological parameters. We hypothesized that micro-rheological studies can be informative in better understanding the pathophysiology of hypoperfusion and/or ischemia-reperfusion occurring during the preparation and reposition of flaps, also in connection with the wound healing process.

4. With the help of histological examinations, our goal was to compare the microscopic differences between ischemia-reperfusion and intact flaps, looking for the border of the tissue areas showing perfusion disorders.

3. METHODS AND MATERIALS

3.1. Experimental animals, surgical technique and experimental protocol

Our experiment (permit registration number: 20/2011/DEMÁB, Animal Welfare Committee of the University of Debrecen) was carried out in compliance with the current animal protection law (Act XVIII of 1998 on the protection and welfare of animals) and EU regulation Directive 2010/63).

Seventeen male Crl:Wl (Charles River Laboratories - Wistar han) rats were used in our experiment (Toxi-Coop Kft., Hungary), whose body weight was 399.5 ± 70.7 g. During the anesthesia, sodium thiopental was used (60 mg/kg dose, intraperitoneal administration).

Using a plastic template, we prepared elliptical adipocutaneous inguinal flaps on both sides along a preliminary marking, the area of the flaps were 8.24 cm^2. In addition to the excision lines, the measurement points for the registration of the temperature and microcirculation parameters were marked in the cranial, central and caudal parts of the flaps. In the control group (n=10), 1 hour after dissection, the flaps were repositioned and sutured tension-free by 32 knotted sutures (4/0 Dexon). In the ischemia-reperfusion (I/R) group (n=7), the supplying vessel - a. epigastrica inferior superficialis– was clamped with a microvascular clip. After 60 minutes of ischemia, the clips were removed, the flaps were repositioned and sutured using the same technique as in the control group.

Skin temperature and microcirculation parameters were recorded in the cranial, central and caudal parts of the flap (according to the marking points) before incision (baseline), after preparation, at the end of ischemia (I-60/R-0), 5 minutes after removing the clips (R-5), after suturing, and on the 1st, 3rd, 5th, 7th, and 14th postoperative days.

In addition to daily wound checks, Flunixin (2 mg/kg s.c.) was used for pain relief on the 1st, 3rd, and 5th postoperative days. A plastic collar was used to prevent autophagy during the first few postoperative days. In case of suture insufficiency, debridement and resuturing were performed under anesthesia. On the 14th postoperative day, the flaps were excised and sent for histological processing.

3.2. Skin temperature and microcirculation measurements

Skin temperature was measured by an infrared thermometer. To measure the microcirculation of the skin, we used a Laser Doppler (LD) flowmeter (LD-01 Laser Doppler Tissue Flowmeter, Experimetria Ft., Hungary) with a standard needle sensor (MNP100XP pencil probe, Oxford Optronix Ltd., UK). The device measures a relative microcirculation

parameter, the so-called blood flux unit (BFU), which integrates the displacement (velocity) of the amount of red blood cells in the bloodstream in a unit area (approx. 1 mm^3). After stabilizing the measuring needle we recorded measurement values of 10-20 seconds without artifacts and analyzed the average BFU values.

3.3. Histological examinations

After the 2-week follow-up period, the rats were over-anesthetized, and the flaps were excised for histological examination. The excised tissue included the flap, the suture line, and the surrounding 2 mm intact skin. The excised samples were placed in 10% formaldehyde and sent to the Institute of Pathology of the University of Debrecen, where 4 μm sections were prepared and embedded in paraffin. Hematoxylin and eosin staining (H&E) was performed during processing.

3.4. Hematological and micro-circulatory measurements

Before the operation, and on the 1st, 3rd, 5th, 7th and 14th postoperative days, blood samples were taken from the lateral tail vein to test the laboratory parameters (anticoagulant: K3-EDTA 1.5 mg/ml). Qualitative and quantitative hematological parameters were performed with a Sysmex F-800 cell counting machine (TOA Medical electronics Corp., Lt., Japan).
Red blood cell aggregation was measured by Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany). Myrenne MA-1 enables measurement based on light transmission. By applying a shear stress of 600 s^{-1}, the system is capable of completely dispersing the red blood cell aggregates. After that, for 5 or 10 s under stasis - M0 mode - it measures red blood cell aggregation using infrared light transmission, which is quantified by determining the aggregation index. In the case of the M1 operating mode, after the dispersion, a constant 3 s^{-1} shear stress is applied, similarly for a period of 5 or 10 s. Thus, the device can calculate a total of 4 aggregation index parameters: M 5s, M 10s, M1 5s, M1 10s. A higher index value indicates increased red blood cell aggregation. A 35 ml undiluted blood sample is required for the measurement.

Red blood cell deformability was measured using LoRRca MaxSis Osmoscan device (Mechatronics BS, The Netherlands). The device measures the deformability based on the diffraction of the emitted laser light. Its operating principle is that the device applies a specific shear force to the solution (so-called Couette-type system using two cylinders), while emitting continuous laser light and measuring its reflection. The reflection is recorded with a CCD video camera and the so-called elongation index (EI) can be calculated based on the resulting

diffractogram, which quantifies the extensibility of the cells. The elongation index can be calculated based on the length (A) and width (B) data of the diffractogram using the equation EI = (A-B / A+B). Higher EI values indicate adequate deformability of red blood cells, while a reduced EI value indicates deteriorating deformability. A 5 µl blood sample was used for the measurement, which was suspended with 1 ml isotonic polyvinyl-pyrrolidone (PVP) solution (360 kDa PVP, viscosity 27 mPas, osmolality = 290-300 mOsm/kg, pH ~7.3). The EI value was determined at shear stress values of 0.3 - 30 Pa. The elongation index can be plotted as a function of shear stress. The resulting curve provides important information about the deformability of red blood cells.

When analyzing this curve, we calculated the value of the maximum extensibility of red blood cells (EI_{max}) and the corresponding shear stress values ($SS_{½}$) according to the Lineweaver-Burke analysis: $1/EI = SS_{1/2} / EI_{max} \times 1/SS + 1/EI_{max}$. The $EI_{max} / SS_{1/2}$ ratio can also be calculated from the parameters obtained in this way, which enables the comparison of the curves.

3.5. Statistical analyisis

The Mead equation was used to determine the number of cases required for the experiments (Mead's source equation).

Data are presented as mean ± standard deviation (S.D.). One-way ANOVA (Bonferroni or Dunn method) tests were used to compare values between and within groups. For simple comparisons within the group (comparison of data measured at specific times), Student's t-test or Mann-Whitney non-parametric test was performed depending on the normality of the data distribution. A p value of less than 0.05 was accepted as statistically significant.

4. RESULTS

4.1. Skin temperature

Skin temperature values decreased moderately during the surgical period. Comparing the cranial, central and caudal regions of the flaps, no significant differences were found. After repositioning the flaps, the skin temperature values returned to the level of the initial (baseline) values. In the I/R group, local temperature values in the cranial region were significantly higher at the end of surgery after wound closure.

4.2. Microcirculation

A decrease in BFU values was observed at all flaps after preparation. The differences were more remarkable in the cranial part of the flaps. After ischemia, values remained low in the early stages of reperfusion. We observed similarly low values at the end of the operation, after wound closure. These values were significantly lower compared to baseline values for both groups, considering all flap regions.

In the early postoperative period, the BFU values in the I/R group were lower compared to the values of the control group. These differences were significant in the cranial region on the 3rd day ($p=0.015$), in the central region on the 7th and 14th postoperative days ($p=0.035$ and $p=0.045$), and the significance threshold was almost reached on the 5th postoperative day (central area, $p=0.061$).

4.3. Hematological parameters

The total white blood cell count (Fvs [G/l]) showed a moderate increase in the control group on the first postoperative day. After that, we saw a significant increase in both groups between the 5th and 14th postoperative days as follows. On the 5th postoperative day, $p<0.001$ vs. base in both groups; on the 7th postoperative day in the control group $p<0.002$ vs. baseline, in the I/R group $p<0.001$ vs. control; and on the 14th postoperative day in the control group $p=0.008$, in the I/R group $p<0.001$ vs. base values. The monocyte+granulocyte ratio (%) showed an increase between the 3th and 5th postoperative days (in the I/R group $p=0.007$ vs. base and $p=0.051$ vs. control). By the second week of the follow-up, the values were normalized (on day 7 in the I/R group $p=0.047$ vs. control).

The hematocrit values (Htc [%]) showed a decrease in the first postoperative week, which can be attributed to the regular blood sampling (day 1: in the I/R group $p<0.001$ vs. baseline and $p=0.031$ vs. control; 3rd, 5th and on 7th day: in the control group $p<0.001$ vs.

baseline). On the 14th postoperative day, the hematocrit values returned to the level of the base parameters without significant differences between the groups.

The platelet count (Thr [G/l]) was significantly lower in the I/R group on the first postoperative day ($p=0.033$ vs. baseline and $p=0.009$ vs. control), then a significant increase was observed by the second week of the observation period in both groups. On the 5th postoperative day, the degree of increase was similar in both groups (control: $p=0.006$; in the I/R group $p<0.001$ vs. baseline). On the 7th postoperative day, the values of the I/R group significantly exceeded the values of the control group ($p<0.001$ vs. baseline in both groups). On the 14th postoperative day, the values of the I/R group continued to increase ($p<0.001$ vs. base and $p=0.003$ vs. control), while the control values were similar to the data on day 7 ($p<0.001$ vs. base).

4.4. Red blood cell aggregation

In general, we saw increased index values on the 1st and 3rd postoperative days, followed by a decrease. On the first postoperative day, a greater increase was seen in the control group (M 5s: $p<0.001$, M1 5s: $p<0.011$, M 10s: $p<0.001$ and M1 10s: $p=0.006$ vs. baseline), compared to the moderate increase in the I/R group (M 5s: $p<0.001$ vs. base, M 10s: $p=0.011$ vs. base and $p<0.001$ vs. control, M1 10s: $p <0.001$ vs. control). On the 3rd postoperative day, the values of the I/R group showed an increase (M 5s: $p=0.002$ vs. base, M1 5s: $p<0.001$ vs. base and control, M 10s: $p=0.002$ vs. base and $p=0.001$ vs. control, M1 10s: $p=0.002$ vs. base and $p<0.001$ vs. control). The values of the I/R group were significantly higher on day 14 compared to the values of the control group (M 5s: $p=0.042$ vs. base and $p=0.012$ vs. control, M1 5s: $p<0.001$ vs. base and $p=0.033$ vs. control).

4.5. Red blood cell deformability

During the examination of the red blood cell deformability values, we found that the deformability significantly worsened between the 1st and 5th postoperative days, mainly in the I/R group. The decrease in the elongation value (at 3 Pa) was significant on the 3rd ($p=0.002$ vs. base), the 5th ($p=0.007$ vs. control), the 7th ($p<0.001$ vs. control), and on the 14th ($p=0.018$ vs. base) postoperative days. The calculated EI_{max} in the Control group showed an initial increase on the 1st postoperative day ($p=0.006$ vs. baseline) and a decrease on the 3rd postoperative day ($p<0.001$ vs. baseline). Meanwhile, the I/R group showed a decrease on postoperative day 1 ($p=0.008$ vs. control) and 3., however, the 5th and 7th postoperative EI_{max} values were higher compared to the values of the control group ($p=0.027$ and $p=0.007$). The

$SS_{1/2}$ values were generally stable in the control group, except for one stage showing a moderate increase (postoperative days 1-3). The values of the I/R group were significantly lower compared to the baseline values on the 3rd ($p=0.05$) and 5th ($p=0.009$) postoperative days. The quotient of the previous two parameters ($EI_{max}/SS_{1/2}$) did not show significant differences during the observation period.

4.6. Histological examination

During the histological examination, a normal healing process was observed with granulation tissue forming along the suture line. In some flaps of the I/R group, hypertrophised inguinal mammary glands were also observed in the subcutaneous layer.

4.7. A case with compication

In the case of one animal of the control group (serial number 13), unilateral lobe necrosis was observed on the right side. Already on the 1st postoperative day, lividity and edema of the flap were íevident. On the 3rd day, marginal necrosis was seen at the upper pole of the flap. On the 5th day, about 70% of the flap necrotized which progressed to a complete necrosis by the end of the first postoperative week. By the second week, the necros was detached, under which we saw the formation of granulation tissue.

In the case of this animal, we observed an increased white blood cell count compared to the other animals in the Control group. Hemoglobin and hematocrit values were also higher compared to animals belonging to the control group. Furthermore, this animal showed the highest platelet count of all experimental animals. Aggregation index values were elevated in the first postoperative week. The deformability values significantly deteriorated between the 5th and 14th postoperative day. In the case of the BFU values, we saw an initial increase compared to the values of the intact flap of the same animal, which was followed by a significant deterioration parallel to the development of necrosis. On the 5th postoperative day, there was no detectable flow in the necrotic flap. After that, with the appearance of granulation tissue, we saw normal BFU values again.

5. DISCUSSION

Rats are widely used in plastic and reconstructive surgery research. By their very nature, such experiments are necessary in surgical pathophysiology research, but their practical applicability is limited in many cases. The adipocutaneous inguinal flap used in rats is a particularly simple model that shows both similarities and differences with the human vasculature. In rats, the blood supply of the inguinal angiosome is provided by the superficial inferior epigastric artery. These vessels anastomse with branches of the thoracoepigastric and the inferior epigastric artery by many perforators. During the formation of the inguinal flap, these anastomoses are cut, so the inguinal flap is exclusively receives its blood supply from the superficial inferior epigastric artery.

The survival of the flaps is based on an adequate blood supply, which provides them adequate oxygenation and nutrient supply. Disruption of oxygen and nutrient supply leads to ischemia and cell death. During the period of ischemia, many changes can be observed that affect the flaps, such as endothelial damage, capillary thrombosis, among many other factors. All of the latter also affect wound healing and flap vitality. During the reperfusion process, in addition to cellular hypoxic damage, inflammatory reactions and free radical formation lead to further tissue damage, during which hemorheological differences can be observed among many other differences. The micro-rheological changes lead to further microcirculation disorders, which repeatedly result in the deterioration of tissue circulation.

During the first 4 weeks of wound healing, collagenogenesis is rapid, as a result of which the tensile strength of the wound increases rapidly. The two subsequent phases are maturation and remodeling during wound healing. Microcirculation plays an important role in the wound healing process in terms of the vitality of the flap. As Kusza and Siemionow mention in their article *"the knowledge of differences in microcirculatory responses presented by different tissue types should be of interest to microsurgeons and others specialists dealing with tissue ischemia and reperfusion injury to improve outcomes in patients exposed to lengthy procedures and unfavorable perioperative conditions".*

Several factors can lead to damage to the flaps, the most common is damage to the supplying vessels during surgery. Several methods are available to check the vitality of the flaps. This includes the use of fluorescent dye with a Wood lamp, tissue pH and transcutaneous oxygen pressure measurement, surface temperature measurement, Doppler Ultrasound or Laser Doppler Flowmetry (monitor, scan, confocal laser scan). Transit-time flowmetry, which is a non-Doppler US technology, can provide a great aid to the surgeon in decision-making during

microvascular free-flap procedures, as the system can detect higher flow in concomitant veins and identify anastomotic insufficiency. In addition, Laser Doppler Flowmetry and Intravital Videomicroscopy techniques can provide useful information about the blood circulation of the flaps in addition to contrast ultrasound techniques. Laser Doppler Flowmetry characterizes capillary flow by measuring the displacement of laser light reflected from red blood cells. Numerous clinical studies have proven its usefulness during flap reconstructions, which opens up the possibility of early intervention for the clinician to prevent possible failure of the flap. From this point of view, intra- and postoperative monitoring is of outstanding importance. Previous animal models have proven that this method is suitable for monitoring postoperative complications.

The skin tolerates the ischemic period very well. In our experiment, we chose a short ischemic period of 1 hour. Other studies have used significantly longer ischemic times of 4-8 hours. It is important to emphasize that the ischemia tolerance of different tissues is different. In the model we used, the endothelial layer of the vessel supplying the inguinal flap has a shorter tolerance to ischemia. During our experiment, we found discrepancies during the analysis of the Laser Doppler data, which indicates that even a 1-hour ischemia affects (deteriorates) the blood supply of the flap.

The differences found during the histological examination (hypertrophied inguinal mammary glands in the subcutaneous layer) point out the importance of time factor: shortening the ischemic period is vital in terms of reducing the chance of postoperative complications. We used male rats in our experiment. In contrast to female rats, the mammary glands of male rats are predominantly lobuloalveolar and have a lower number of ducts, but a larger number of alveoli, where ductal and alveolar epithelial apoptosis is more frequent. It is known that the effects of hypoxia in cells and tissues are regulated by hypoxia-inducible factor 1α (HIF-1α). In normoxia, the alpha unit of this heterodimeric transcription factor is present in reduced numbers, while hypoxia stabilizes HIF-1α. In mammary glands, HIF-1a stimulates glucose uptake through the GLUT1 mechanism, enhances anaerobic glycolysis, angiogenesis through increased expression of VEGF, and also enhances mammary gland development and lactation.

Several drugs have been tested under experimental conditions to prevent flap ischemia. Park et al. state in their work that botulinum toxin applied preoperatively can increase the blood flow (flow rate) in the blood vessels of the flap, thereby reducing vascular damage to the flap. Sen et al. used omeprazole in their experiment and found that increasing the concentration of gastrin during flap surgery improved the survival of the flaps. Wallmichrath et al. demonstrated that heparin and recombinant tissue plasminogen activator can be protective against flap

circulatory failure in adipocutaneous free flaps in rats. Uslu confirmed the beneficial effect of dipyridamole in terms of flap survival in an experimental model by it's antiaggregation effect, Ersan et al. reported the beneficial effect of taurine by it's antioxidating properties, which reduced the occurrence of flap necrosis.

In addition to pharmacological intervention, a number of additional techniques are available to the surgeon in the field of ischemia preconditioning, which have been shown in numerous studies. The essence of the preconditioning procedures is that artificially, often repetitively induced ischemia in the supplying vessels of the flap increases the tolerance to I/R damage through the adaptation of the tissues. The latter can be underpinned by a number of mechanisms (e.g. decreasing proinflammatory cytokine concentration, increasing number of vasodilatative agents, endothelial remodeling). In addition to minimizing the ischemic time and using the appropriate surgical technique, these additive preconditioning procedures and drug treatments can further reduce the complication rate of flap reconstruction.

In our rat model, in addition to microcirculation and surface temperature measurements, we also examined micro-rheological parameters. We hypothesized that micro-rheological studies could be informative to better understand the pathophysiology of flap hypoperfusion and/or ischemia-reperfusion injury. There are a large number of rat models suitable for both practical and experimental flap studies. In our experiment, we examined the adipocutaneous inguinal flap, which receive its blood supply from the superificial epigastric artery and vein.

Hematological and hemorheological signs of the acute phase reaction were observed in both groups, but their dynamics and extent were different. Anesthesia, immobilization, tissue preparation, and wound healing all contribute to the development of the acute phase reaction. Regarding inflammatory reactions, it is important to note that for each animal, the wound line with a total length of approx. 21-22 cm was still in the healing phase (two ellipsoidal flaps with a circumference of approx. 10.8 cm per side). The flaps affected by ischemia caused a greater inflammatory reaction, which was also reflected in hematological and micro-rheological changes; activated leukocytes, acute phase reaction, which are known to affect red blood cell deformability and red blood cell aggregation.

In a previous study in dogs, it was shown that 1 hour of ischemia applied to the latissimus dorsi myocutaneous flap significantly increased red blood cell aggregation and hematocrit in the first hour. These changes were not observed in blood samples taken from animals without flap ischemia. The results also showed that changes in TBARS and GSH concentrations were well correlated with oxidative damage during the reperfusion process (the former are degradation products of lipid peroxidation, the latter is one of the most important

low molecular weight antioxidants found in cells). By postoperative day 7, the I/R lobes were macroscopically indurated compared to the intact lobes of control animals.

Klarik et al. examined hemorheological (red blood cell deformability and aggregation) and microcirculatory (Laser Doppler Flowmetry) changes in the latissimus dorsi – cutan maximus musculocutaneous flap after two hours of ischemia. They found, among other non-specific acute phase-induced micro-rheological changes, that I/R resulted in deterioration of microcirculatory parameters. Monitoring the microcirculation parameters has proven to be vital in predicting postoperative complications, such as thrombosis and consequent flap failure.

These research results clearly show that ischemia-reperfusion affects different types of flaps differently. This can be explained primarily by the different tolerance of tissues to ischemia. Micro-rheological parameters also provide useful information during flap experiments. Laser Doppler measurement used intraoperatively can be informative in predicting postoperative complications, such as thrombosis or necrosis.

During our experiment, we also faced flap necrosis. In the case of experimental animal no. 13, the one-sided necrosis may have been caused by thrombosis, which may have occurred in the early postoperative period due to the torsion of the pedicle of the flap. Interestingly, this animal belonged to the control group, where we did not use a microvascular clip, and there was no induced ischemia. We assume that torsion or breakage of the pedicle may have occurred during the repositioning. The laboratory parameters changed parallel to the macroscopically observable process (inflammation, marginal and then extensive necrosis, repulsive necrotic tissue, and granulation). These results can be explained by inflammatory reactions, during which the resolution of necrosis was visible during the observation period.

Flap tissue replacement is one of the most frequently performed interventions in plastic reconstructive surgery. The viability of the flaps is determined by many factors. Considering that during flap formation, due to its nature, the circulation of the mobilized tissues deteriorates, the surgeon must do his best to achieve optimal flap survival and wound healing. This requires proper surgical planning, precise surgical technique, and adequate postoperative care.

Sufficient knowledge of anatomy plays an important role during flap formation. Flap design should take angiosomes and perforosomes into account for achieving successful flap reconstruction. It is known that perfusion in different areas of the flaps shows great variability. During our experiment, the microcirculation was measured in different areas of the flaps (proximal, central and distal). Depending on the distance to the supplying vessel, different BFU values were obtained. This clearly shows that the proper flap configuration (length-to-width

ratio, distance measured from the pedicle depending on the anatomical localization) and blood supply have a great role in flap planning and thus in the success of the operation.

During their experiment, Tushar and his colleagues concluded that by using fluorescent dye intraoperatively, the vitality of the flap can be determined more precisely than if only the clinical signs of vitality were examined (positive predictive value 99.03%, compared to the value of 95.7% of the vitality determined based on the clinical signs. This clearly shows, that additive testing methods can be of great use in the clinical practice.

In addition to instrumental examination methods, as can be seen from our study, parameters determined from blood sampling can also provide important information about the vitality of the flaps and the process of ischemia. Not only micro-rheological parameters, but also other biomarkers can indicate flap-related complications. Livaoglu et al. examined the correlation between ischemia and ischemia-modified albumin (IMA) levels in the case of muscle flaps in their research on rabbits. Examining a rectus abdominis muscle flap, they came to the conclusion that following the IMA levels as a biochemical marker is suitable for following ischemic processes during a muscle flap procedure. IMA is also an important biomarker in other ischemic diseases.

Regardless of the examination methods, it can be concluded that they provide the surgeon additional information regarding the viability of the flap. When choosing the method, the surgeon must take many factors into account: cost, measurement accuracy, interpreting the data, the invasiveness of the measurement, and the equipment and personal requirements of the measurement. During our experiments, we examined Laser Doppler Flowmetry, micro-macrocirculation and rheological parameters. We found that the LD device is suitable for determining intra- and postoperative vitality, and can possibly predict complications even before the appearance of clinical symptoms. The distinct advantage of the device is that its operation does not require the use of consumables, so it can be used without significant maintenance costs after a one-time investment. In the case of a properly placed sensor, qualified nursing staff can monitor the microcirculatory data, so circulatory disorders can be recognized in time in the case of hospitalized patients. Micro-rheological parameters are known to be good indicators of local and global circulation disorders. Because of this, they can provide important additional information for the surgeon in reconstructive surgery. The need for introducing the beforementioned methods into the clinical practice justifies the continuation of furher animal experiments.

6. Main results and conclusions

1. We developed an easy-to-implement, well-reproducible bilateral adipocutaneous flap model on rats, which models the behavior of flaps used in the clinical practice, and is suitable for the follow-up examination of damage caused by ischemia-reperfusion and other factors influencing wound healing.

2. The microcirculation parameters monitored by Laser Doppler tissue flow meter indicated the deterioration of tissue perfusion even after a relatively short, 1-hour ischemic period. The differences in wound healing between the control and the ischemia-reperfusion groups were easily monitored by examining the local skin temperature and microcirculation parameters. Laser Doppler tissue flow measurement sensitively indicated the onset of flap necrosis in the case of complication.

3. During the wound healing of the flaps in the early postoperative period, the micro-rheological parameters showed changes (deteriorating red blood cell deformability, increasing red blood cell aggregation). Ischemia-reperfusion reinforced these differences on the 3rd and 5th postoperative days. In the case of flap necrosis, the deterioration of these parameters was more prominent.

4. During the histological examinations, hypertrophied subcutaneous mammary glands were found in some cases of the I/R group, presumably due to the effect of transcription factors induced by hypoxia. These changes also draw attention to the importance of minimizing ischemic time.

7. Certified list of publications

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Registry number: DEENK/348/2023.PL
Subject: PhD Publication List

Candidate: Ábel Molnár
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. Magyar, Z., **Molnár, Á.**, Nachmias, B. D., Mann, D., Somogyi, V., Mester, A., Pető, K., Németh, N.: Impact of groin flap ischemia-reperfusion on red blood cell micro-rheological parameters in a follow-up study on rats.
   Clin. Hemorheol. Microcirc. 79 (2), 245-255, 2021.
   DOI: http://dx.doi.org/10.3233/CH-170277
   IF: 2.411
2. **Molnár, Á.**, Magyar, Z., Nachmias, B. D., Mann, D., Szabó, B., Tóth, L., Németh, N.: Effect of short-term ischemia on microcirculation and wound healing of adipocutaneous flaps in the rat.
   Acta Cir. Bras. 34 (12), 1-9, 2019.
   DOI: http://dx.doi.org/10.1590/s0102-865020190120000003
   IF: 0.974

List of other publications

3. Berhés, M., Németh, N., Pető, K., Deák, Á., Hajdu, E., **Molnár, Á.**, Árkosy, P., Szabó, J., Fülesdi, B.: Hemodynamic consequences of intravenously given E. coli suspension: observations in a fulminant sepsis model in pigs, a descriptive case-control study.
   Eur. J. Med. Res. 24 (1), 1-6, 2019.
   DOI: http://dx.doi.org/10.1186/s40001-019-0372-y
   IF: 1.826
4. Molnár, L., Németh, N., Berhés, M., Hajdu, E., Papp, L., **Molnár, Á.**, Szabó, J., Deák, Á., Fülesdi, B.: Assessment of cerebral circulation in a porcine model of intravenously given E. coli induced fulminant sepsis.
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   DOI: http://dx.doi.org/10.1186/s12871-017-0389-3DOI
   IF: 1.788

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5. Németh, N., Berhés, M., Kiss, F., Hajdu, E., Deák, Á., **Molnár, Á.**, Szabó, J., Fülesdi, B.: Early hemorheological changes in a porcine model of intravenously given E. coli induced fulminant sepsis.
   Clin. Hemorheol. Microcirc. 61 (3), 479-496, 2015.
   DOI: http://dx.doi.org/10.3233/CH-141914
   IF: 1.815

6. Kiss, F., Molnár, L., Hajdu, E., Deák, Á., **Molnár, Á.**, Berhés, M., Szabó, J., Németh, N., Fülesdi, B.: Skin microcirculatory changes reflect early the circulatory deterioration in a fulminant sepsis model in the pig.
   Acta Cir. Bras. 30 (7), 470-477, 2015.
   DOI: http://dx.doi.org/10.1590/S0102-865020150070000004
   IF: 0.58

Total IF of journals (all publications): 9,394
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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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