

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

**Study of the regeneration of fasciocutaneous and
adipocutaneous flaps in experimental models**

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INTRODUCTION

Flaps are often used in many fields of medicine to replace tissue defects caused by various factors. They are used for reconstruction in oncological surgery (eg. otorhinolaryngology and head and neck surgery, oral surgery, general surgery), traumatology, burn surgery and plastic surgery. When repairing a defect, it is not only a matter of simply „filling” the gap, but also of achieving various functional and aesthetic goals, thereby ensuring an appropriate quality of life for the patient. Flaps can be classified according to their tissue composition (e.g. cutaneous, fasciocutaneous, adipocutaneous, myocutaneous, osteomusculocutaneous), location (e.g. local, regional, distant), blood supply, configuration, and the method of transfer to the site of tissue deficiency.

It is important to note that the selection of the appropriate flap is critical to the success of the operation. The selection of the right flap type is based on several factors; we must take into account the location, size and depth of the tissue defect, the adjacent structures, the vasculature, Langer’s lines of the skin, aesthetic considerations, as well as any other comorbidities and influencing factors present in the patient (smoking, peripheral vascular disease, atherosclerosis, diabetes mellitus, steroid use, previous surgeries, etc.), and last but by no means least, the surgeon’s expertise.

It is generally accepted among surgeons that the best flap is one with which the surgeon has extensive experience, having used it frequently in their practice, and can therefore prepare and perform it with a high degree of confidence. However, simply selecting the appropriate flap is not enough, as numerous obstacles may still arise on the path to full recovery. This is precisely why adequate tissue perfusion of the flaps is crucial, a factor influenced, among other things,

by the morphological and functional state of the vascular system, microcirculation, haemodynamics, and macro- and micro-rheological parameters.

There are numerous methods for monitoring tissue perfusion and viability of flaps, which can be applied in both clinical practice and experimental studies. This is important because various complications frequently occur in flap surgery. Ischaemia, ischaemia-reperfusion injury (I/R), thrombosis and necrosis can develop for several reasons, including inadequate preparation, tissue damage, bending or torsion of the blood vessels supplying the flap, haematoma, oedema or infection.

Microcirculation, haemorheology and blood flow of the pedicle of the flap are all interrelated. If the pedicle is damaged and blood flow within it is reduced or ceases, the microcirculation of the flap deteriorates, leading to changes in the deformability and aggregation of red blood cells, as well as changes in the number of blood cells. Conversely, changes in these physical properties of the red blood cells themselves (aggregation and deformability) due to I/R injury or inflammation cause a further deterioration in tissue perfusion, leading to a 'vicious circle'. Previous research has demonstrated that in the early stages of ischaemia and reperfusion, red blood cell deformability deteriorates whilst their aggregation increases; for this reason, we believe that monitoring these parameters, alongside the assessment of microcirculation and peripheral circulation, may predict postoperative complications following the use of flaps.

The use of animal models is widespread; numerous models are known for studying the ischaemia-reperfusion and regeneration of flaps, involving flaps of various tissue compositions (fasciocutaneous, adipocutaneous, musculocutaneous, etc.) and different durations of ischaemia. We used fasciocutaneous and adipocutaneous groin flaps from rats; although their location and blood supply are identical, the fasciocutaneous flap consists solely of the skin, the subcutaneous tissue immediately beneath it and the fascia, whereas the adipocutaneous flap

also includes the abdominal fat pad. These flap types and their vascular pedicles are easy to prepare and, due to their suitable size and location, are also easy to examine instrumentally, e.g. Cytocam, and thus proved to be a suitable choice for our experiments, as they were appropriate for studying the pathophysiology of the flaps, the factors influencing wound healing and regenerative processes, as well as various prevention options and active substances.

In reconstructive surgery, there is a constant demand for active substances that improve wound healing; for this reason, we investigated the effects of two substances in our experiment. One was PACAP, an endogenous neuropeptide which increases cellular cAMP levels via the activation of adenylate cyclase; according to the international literature, it has been shown to have several protective roles, including anti-inflammatory and anti-apoptotic effects, as well as enhancing the production of extracellular matrix components. BGP-15, on the other hand, is a nicotinamide derivative developed by Hungarian researchers. It is capable of increasing heat shock protein induction, improves membrane fluidity, reduces the production of reactive oxygen species, and exhibits a protective effect in inflammatory and I/R processes.

AIMS

1. Our aim was to develop a rat model that allows the investigation of flaps with different tissue compositions, in which the ischemia/reperfusion induced changes as well as the alterations occurring during regeneration can be reliably monitored.
2. We aimed to examine how the morphology, histological characteristics, and biomechanical properties of fasciocutaneous and adipocutaneous flaps change during early regeneration.

3. Our objective was to determine the extent of I/R injury in adipocutaneous flaps by analyzing perfusion parameters, with particular regard to blood flow and microcirculation.
4. We aimed to investigate the microrheological and histomorphological effects of PACAP 1–38- and BGP-15 containing wound gels during the healing of I/R-exposed fasciocutaneous groin flaps.

MATERIALS AND METHODS

Our experiment was registered and officially approved by the University of Debrecen's Committee of Animal Welfare and the National Food Chain Safety Office (registration number 19/2022/UDCAW), in accordance with the national law (Act XXVIII of 1998 on the Protection and Humane Treatment of Animals) and European Union regulations (Directive 2010/63/EU).

1.1 Preparation and selection of experimental animals

A total of 31 male Wistar rats (source: Toxi-Coop Zrt., Budapest, Hungary) were used for the research. The rats were delivered to our Department's animal facility at six weeks of age and were acclimatised for three weeks prior to the start of the experiment in standard-sized cages (Eurostandard IV, Tecniplast, Buguggiate, Italy). A constant temperature (22 °C) and humidity (55±10%) were maintained in the animal house; the animals were provided with standard rat feed ad libitum and water via self-watering systems. A 12-hour light/dark cycle was maintained for lighting.

The selection of adult male rats was based on the need to create an animal model of suitable size and anatomy for the experimental surgical procedures, which required

appropriate age and sexual homogeneity. As the flaps were marked in the epigastric region, we also ruled out the use of female animals, which have six pairs of nipples.

Prior to surgery, the rats were weighed; based on this, 100 mg/kg body weight i.p. of ketamine (CP-ketamine hydrochloride 10%, Produlab Pharma BV, Raamsdonksveer, Netherlands), 10 mg/kg body weight i.p. xylazine hydrochloride (CP-xylazine-e hydrochloride, 2%; Produlab Pharma BV, Raamsdonksveer, Netherlands) and 0.05 mg/kg Atropine s.c. (Atropinum sulfuricum 0.1%, Egis Pharmaceuticals PLC, Budapest, Hungary) were used. During the operation, we monitored the animals' vital signs: respiratory rate, depth of breathing, and the colour of the mucous membranes and limbs. In addition, during the operations, the animals were placed on a heating pad to ensure optimal body temperature throughout the entire duration of the procedures.

There were differences in the surgical techniques used for the two surgical groups, but it can be stated that all animals received 2 ml of 0.9% NaCl subcutaneously postoperatively. Following the operations, the animals were kept in solitary confinement, and a plastic collar was placed around their necks to prevent autophagy. We did not use conventional NSAIDs for postoperative pain relief; instead, we administered tramadol (15 mg/kg/day, s.c.) to the animals for the first 3 postoperative days and the animals also received s.c. physiological saline solution to ensure adequate hydration.

1.1.1 Operative techniques, experimental and sampling protocol of rats'

fasciocutaneous groin flaps

Bilateral fasciocutaneous groin flaps were created in 21 male Wistar rats (322.79 ± 17.63 g). These animals were divided into 3 groups according to the wound gel used in the experiment: control group, PACAP group and BGP-15 group. The flaps were prepared

using a standard-sized oval template (area: 5.48 cm²), ensuring the same shape and size in all animals. The surgical site was depilated, disinfected and isolated, after which the skin incision was made in accordance with the markings on the template. The flaps were pedicled on the inferior superficial epigastric artery and vein.

The left-sided flap (hereafter the ischaemic side) was clamped with a microsurgical clamp for 120 minutes and placed in the wound bed to prevent it from drying out, whilst the right-sided flap (hereafter the control side) was also left in the wound bed following dissection. After the 120-minute interval had elapsed, 0.3–0.3 ml of hydroxypropyl methylcellulose gel (HPMC), specific to the respective group, was applied to the wound beds.

In the control group (n = 7), the gel contained no active ingredients. In the PACAP group (n = 7), 0.01% PACAP 1-38 was added to the gel, and in the BGP-15 group (n = 7), 10% BGP-15 was added. The specialised wound gels were prepared at the Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Debrecen. The bilateral flaps were then sutured back into their original position using 32 simple, knotted sutures (cutting needle, Silk, SMI, Steinerberg, Belgium). The wound gels corresponding to the groups were applied around the sutures and on the surface of the flaps for 5 days post-operatively, at a dose of 0.15-0.15 ml daily, alongside wound care.

Blood samples were taken from the lateral tail vein for laboratory tests prior to surgery (used as the baseline value) and on the 1st, 3rd and 7th postoperative days (0.4 ml per sample; K3-EDTA, Vacutainer®, Becton Dickinson GmbH, Franklin Lakes, NJ, USA).

On the 1st, 3rd and 7th postoperative days, photographs were taken of the flaps. The area of the flaps was determined using the free software ImageJ 1.40g, which was calibrated each time using a ruler placed on the animal.

In addition, skin temperature measurements were taken (Ri-thermo® N professional clinical thermometer, Rudolf Riester GmbH, Jungingen, Germany) on both flaps and the intact epigastric skin surface (control midline region) before surgery, during ischaemia, after surgery (following suturing of the flaps), and on days 1, 3 and 7 post-surgery.

On the 7th postoperative day, at the end of the experimental period, the animals were euthanised by intravenous injection of 300 mg/kg 10% ketamine hydrochloride and 30 mg/kg 2% xylazine hydrochloride via a lateral tail vein cannula, after which skin samples were taken for tensile strength and histological examinations. Skin strips measuring 0.5×3 cm were excised from the upper, lateral and lower parts of the flaps, including the skin area of the flap, the suture line and the adjacent intact skin for tensile strength measurements.

In addition, we also excised half-centimetre-wide skin strips from the central part of the flaps, together with the intact skin between them, for histological analysis.

1.1.2 Operative techniques, experimental and sampling protocol of rats'

adipocutaneous groin flaps

We created bilateral adipocutaneous groin flaps in ten male Wistar rats (body weight: 362.7 ± 17.08 g), which were also sized using a standard-sized oval template. After disinfecting and isolating the surgical site on the animals with depilated abdomens, we prepared the flaps on both sides (surface area: 400.76 ± 66.08 mm²), attaching the flaps to the superficial epigastric artery and vein.

On the left side, the flap pedicle was also clamped for 2 hours whilst the flaps were placed in the recipient site. Following the ischaemic period, the microvascular clamp was removed, the flaps were repositioned and sutured with 32–32 simple knotted sutures (4/0 Pidilen, Kollut USA), and the wound beds were treated with 0.3–0.3 ml of hydroxypropyl

methycellulose (HPMC) gel (Figure 13). Wound care was continued until the 5th postoperative day (0.15 ml/flap/day).

In two cases, wound debridement and/or re-suturing was required during follow-up. These procedures were performed under general anaesthesia.

Photographs of the flaps were taken postoperatively and on days 1, 3, 7 and 14 after surgery to monitor changes in their size (ImageJ 1.40 g freeware). We also performed skin temperature measurements: before surgery, during ischaemia, after surgery (following re-suturing of the flaps) and on the 7th postoperative day on the two flaps and the control epigastric skin surface (Rodent NIBP infrared thermometer LaserSight, AD Instruments, USA). Blood samples were taken from the lateral tail vein for laboratory analysis before surgery, and on days 1, 3, 7 and 14. On the final day, the animals were euthanised. Skin strips were then obtained for tensile strength and histological examinations.

1.2 Laboratory Analysis

1.2.1 Haematological parameters

The hematological parameters, like white blood cell count (WBC [G/L]), red blood cell count (RBC [T/L]), hematocrit (Hct [%]), and platelet count (Plt [G/L]) were measured by a Sysmex K-4500 microcell counter (TOA Medical Electronics Co., Ltd., Kobe, Japan).

1.2.2 Red blood cell deformability

Red blood cell deformability was determined using a LoRRca MaxSis Osmoscan ektacytometer (RR Mechatronics International B.V., Zwaag, Netherlands).

In the device, blood samples were subjected to shear stress, and red blood cell elongation was measured using a laser diffraction technique. The elongation index (EI) was determined as a function of shear stress (SS, Pa; range: 0.3–30 Pa). In the conventional deformability test, 10 μ L of anticoagulated whole blood sample was mixed with 2 mL of polyvinylpyrrolidone (PVP)-PBS solution (PVP: 360 kDa, Sigma-Aldrich Co., St. Louis, MO, USA; PVP-PBS solution viscosity = 31.6–33.6 mPas, osmolarity = 290–310 mOsmol/kg, pH = 7.5). All measurements were performed at 37 °C. Comparative data were calculated from the EI–SS curves, including EI values measured at 3 Pa.

To parameterise the individual EI–SS curves, we used the Lineweaver–Burk equation ($1/EI = SS_{1/2}/EI_{\max} \times 1/SS + 1/EI_{\max}$), which provided the maximum elongation index ($EI_{\max}$) and the shear stress value ($SS_{1/2}$ [Pa]) corresponding to half of the maximum elongation index

($EI_{\max}$). A deterioration in red blood cell deformability is indicated by lower EI or $EI_{\max}$ and higher $SS_{1/2}$ values.

1.2.3 Red blood cell aggregation

The aggregation index values of the blood samples were determined using a Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Roetgen, Germany).

This method is based on transmittance photometry and requires 20 μ l of blood. After achieving red blood cell disaggregation using a controlled shear system (shear rate: 600 s^{-1}), light transmission was measured for 5 or 10 seconds under static conditions (M values, shear rate: 0 s^{-1}) or at a low shear rate (M1 values, shear rate: 3 s^{-1}). Measurements were performed at room temperature (20–25 °C). Each index parameter was determined from four parallel measurements, and the average of these was used.

Higher index values (M 5 s, M1 5 s, M 10 s, M1 10 s) indicate increased aggregation of red blood cells.

1.3 Tensile Strength Measurement

For the tensile strength tests, following termination (on day 7 postoperatively for fasciocutaneous flaps and day 14 postoperatively for adipocutaneous flaps), skin specimens $\sim 5 \times 30$ mm in width were obtained from the upper, lateral and lower parts of both the ischaemic and non-ischaemic flaps. The excisions were taken in such a way that they included both the flapped areas and the intact skin, as well as the healed suture line separating the two.

The samples were then placed in a special tensile strength measuring device (developed by the Department of Information Technology in collaboration with the Department of Operative Techniques and Surgical Research) so that the suture line was centred.

During the test, we applied a continuously increasing tensile force (1.95 mm/s) until the samples broke, recording the force in grams; the data was saved in CSV files. We then converted the grams to newtons; from this data, the stress-strain curve generated by the machine enabled us to determine the most important parameters, such as the maximum stress, the breaking point and the slope of the curves. The tensile strength of the fabric is indicated by the maximum force that the fabrics could withstand just before breaking.

1.4 Histological analyses

The skin strips used for histological examination were obtained after the animals were euthanised, ensuring that they included sections from both flaps as well as the skin between them. The skin samples were mounted on dental wax and then stored in formalin for two days. The use of dental wax was necessary to prevent the samples from shrinking and wrinkling. Following fixation, the samples were washed three times in distilled water, then in alcohol of increasing concentration, and finally embedded in paraffin. A series of 7 µm thick sections was prepared using a microtome (Leica, Wetzlar, Germany). The sections were stained with dimethyl-methylene blue (DMMB, Sigma-Aldrich, MO, USA), haematoxylin and eosin (H&E, Sigma-Aldrich, MO, USA), picrosirius red (Sigma-Aldrich, MO, USA), and, in the case of the fasciocutaneous flaps, also with orcein (Sigma-Aldrich, MO, USA), in accordance with the manufacturers' instructions. The sections were then mounted with DPX (Sigma-Aldrich, MO, USA). The histological sections stained with DMMB, H&E and orcein were examined using a

BX53 Olympus (Olympus, Tokyo, Japan) light microscope with constant camera and exposure settings.

DMMB is a classic, rapid method that stains the granules found in mast cells metachromatically purple or violet due to their high heparin and histamine content, so that mast cells appear as purple-blue granules against a lighter background. This is possible because DMMB specifically stains the sulphated proteoglycans characteristic of mast cells. Following DMMB staining, we counted the mast cells within the selected 10× magnification field and recorded the total cell count.

The samples stained with picosirius red were examined using a polarising lens, where the plane of polarised light was rotated by $\lambda/4$, and a $\lambda/4$ compensator was used with an Olympus BX53 microscope (Olympus, Tokyo, Japan). Photographs were taken under normal light and then under polarised light, using constant camera settings. With picosirius red staining, thin collagen fibres appear green, whilst thick collagen fibres appear red. We used the ImageJ 1.40g freeware to analyse red-green pixel intensity, which provided semi-quantitative data that we compared with data from intact skin.

Following H&E staining of the tissue samples, we measured the thickness of the epidermis (ImageJ 1.40g freeware) and assessed the morphology of the tissues; where there were multiple groups (fasciocutaneous flaps), we compared the differences between the groups.

1.5 Blood flow test of flap pedicle

We measured blood flow in the superficial epigastric artery of the adipocutaneous flaps using the Transonic T206 device (Transonic Systems, Ithaca, USA). We performed measurements in the arteries of both flaps before and after the ischaemic period (prior to re-suturing) and on the 14th postoperative day.

The probe of the device was placed directly on the vessel wall, thus providing real-time flow measurements expressed in ml/min.

1.6 Microcirculatory investigation

A Cytocam-IDF (Braedius Medical B.V., Huizen, Netherlands) video microscope was used to assess microcirculation by illuminating the superficial tissue layers from above with incident light (at a depth of 2–3 mm), resulting in haemoglobin-containing red blood cells appearing as black or grey dots against the light tissue background, because in the Incident Dark-Field technology, the examined area is illuminated by scattered light reflected from the background, while direct reflections do not enter the optical system.

The microcirculation images were analysed offline using the following parameters: perfused vessel density (PVD [mm/mm^2]), proportion of perfused vessels (PPV [%]), and microvascular flow index (MFI [au])

1.7 Statistical analyses

To determine the sample size, i.e. the number of animals per group, we applied Mead's Source Equation.

For statistical analyses, we used SigmaStat Software 3.1.1.0. (Sys-tat Software Inc., San Jose, CA, USA). Data are presented as means $\pm$ standard deviation (SD). Where there were multiple groups (our fasciocutaneous flap experiment), we used the t-test or Mann–Whitney test for comparisons between groups, and we used one-way or repeated ANOVA test or the Kruskal–Wallis test for comparisons within groups (depending on the results of the normality test for the data distribution).

Furthermore, a p-value below 0.05 was considered statistically significant.

RESULTS

2.1 Experimental results of rats' fasciocutaneous groin flaps

2.1.1 General observation

During the postoperative observation period, we did not note any significant thrombotic complications or extensive flap necrosis.

By the first postoperative day, the area of the flaps had decreased significantly in all groups, a decrease that continued throughout the experimental period. There was no significant difference between the individual groups, but on the 7th postoperative day, the greatest reduction, compared with their own values on the first day, was observed on the ischaemic side (control: 24.27%; PACAP: 32.6%; BGP-15: 30.6%), whilst a smaller reduction was observed on the control side (control: 25.2%; PACAP: 15%; BGP-15: 13.4%). Furthermore, although there was a similar reduction in size in both flaps in the control group, a smaller reduction was observed on the non-ischaemic side in the groups treated with the active substance, and in the PACAP group, an increase in size was even observed on the 7th day post-surgery.

2.1.2 Skin temperature

The skin surface temperature of the flaps decreased significantly on both sides during the ischaemic period, as the surgical procedure itself displaced all flaps from their original anatomical position. The temperature of the ischaemic flaps was slightly lower than that of the non-ischaemic (control-side) flaps. After repositioning and reattaching the flaps, their temperature returned to normal. In the postoperative days, there was no significant difference between the non-ischaemic and ischaemic flaps, although the values for the ischaemic flaps were slightly lower.

2.1.3 Hematological parameters

White blood cell counts decreased mainly in the control group on the first postoperative day. By the 7th postoperative day, the values had risen to a level close to the baseline data. In the BGP-15 group, lower white blood cell counts were found on the 3rd postoperative day compared to the other groups. The red blood cell count showed a slight decrease during the observation period, despite the haemoconcentration observed in the PACAP and BGP-15 groups on the first postoperative day. Haematocrit values changed accordingly. Platelet counts rose steadily, with the highest values observed in the BGP-15 group.

2.1.4 Micro-rheological parameters

As red blood cell deformability deteriorates, the elongation index (EI) decreases. In all groups, a slight decrease was observed in EI values at a shear stress of 3 Pa and in maximum elongation index values ($EI_{\max}$) during the postoperative days. The $EI_{\max}/SS_{1/2}$ ratio increased significantly only in the BGP-15 group.

The aggregation index values (M 5 s, M 10 s) increased significantly by the 1st and 3rd postoperative days in the control and PACAP groups, whilst the increase observed in the BGP-15 group was much smaller and not significant.

2.1.5 Tensile strength measurements

The tensile strength of the flaps (i.e. the suture line) decreased by more than 70% compared to intact skin (intact skin strip: 17.01 ± 3.65 N; skin strip torn after suturing: 10.68 ± 2.31 N). The suture lines of the lateral (i.e. horizontally oriented) skin strips were weaker than those of the vertically oriented ones (lower and upper). Compared to the control group, a significant ($p=0.0025$) reduction in tensile strength was observed on the ischaemic side in the PACAP group. The slope of the curves increased significantly in the PACAP-treated control flap for the lateral skin strip ($p=0.0116$ vs. BGP-15), whilst it decreased significantly for the lateral skin strip taken from the ischaemic flap ($p=0.0077$ vs. control; $p = 0.0241$ vs. BGP-15). In the BGP-15 group, only the slope of the curve of the lower vertical skin sections showed a significant ($p=0.0171$) decrease compared to the control.

2.1.6 Histological analyses

H&E staining revealed sterile acute inflammatory processes with dominant lymphocytic infiltration. Nucleus-free necrotic foam cells were visible around the sutures, mainly in the ischaemic flaps. Fibrocytes and fibroblasts were visible in the granulation tissue, consistent with physiological wound healing. Tissue degradation was observed in the ischaemic flaps. In the PACAP group, an increased number of macrophages was observed, alongside a large

number of fibroblasts. No tissue degradation was detectable, but we observed ongoing, vigorous tissue remodelling. In the BGP-15 group, macrophages and fibroblasts were observed in the ischaemic flaps. Although tissue remodelling was not strongly evident, no tissue degradation was visible in these groups. The thickness of the epidermis in the granulation tissue over the sutures increased significantly in the flaps, particularly in the ischaemic ones. The increase was smaller in the PACAP group and greater in the BGP-15 group.

In sections stained with DMMB, the number of mast cells in the ischaemic flaps of the PACAP group was significantly reduced compared to the control group. On the other hand, PACAP also reduced the number of mast cells in the control-side flap group. Significantly fewer mast cells were observed in the ischaemic flaps of the BGP-15 group. In contrast, there were more mast cells in the control-side flap of the BGP-15 group than in the control group.

Picrosirius staining displays thick collagen fibres as red without specificity, whilst thin collagen fibres appear green under polarised light (with a $\lambda/4$ rotation of the light plane). In intact skin, the dermis showed a dense network of collagen fibres, corresponding to the physiological, dense, irregular connective tissue. In the ischaemic flap group, a more diffuse and thinner fibre network was observed, corresponding to the loosening of the dense connective tissue during wound healing. Following treatment with PACAP and BGP-15, the diffuse orientation of the collagen fibres was restored, giving the dermis a thicker, dense connective tissue-like appearance. In the BGP-15 group, thinner, green-coloured fibres were also observed.

Orcein staining indicates elastic fibres in brown. In normal, intact skin, brown-coloured fibres can be observed in the dermis within the physiologically dense, irregular connective tissue. These fibres varied in length and overlapped the collagen fibres, thereby ensuring the skin's elasticity. On the control side, the number of these fibres, stained with orcein, was lower in the PACAP-treated group. In the PACAP group, significantly more thin, elastic fibres were observed in the developing scar tissue than in the control group on the ischaemic side.

Following BGP-15 treatment, the number of orcein-stained fibres on the control flap side was similar to that of the control group, whilst on the ischaemic side it was higher, and thicker elastic fibres were observed here.

2.2 Experimental results of rats' adipocutaneous groin flaps

2.2.1 General observations

During the observation period, we observed no thrombotic complications or visible flap necrosis. The size of the flaps, in terms of both area and circumference, decreased; the flaps on the ischaemic side showed a greater degree of shrinkage (on postoperative day 1: area: $p=0.007$; circumference: $p=0.024$).

2.2.2 Body temperature

Skin temperature decreased during the ischaemic period, as the excised flaps were displaced from their physiological position, although we repositioned them into the flap grooves within 120 minutes.

After re-suturing them to their original position, the values normalised and showed no significant change thereafter.

2.2.3 Hematological parameters

The white blood cell count decreased slightly on the first postoperative day ($p=0.022$ vs. baseline), then began to rise during the observation period (day 14: $p=0.017$ vs. baseline).

Red blood cell count, haemoglobin and haematocrit levels continued to decrease during the first week after surgery (day 7: $p=0.003$, $p=0.007$ and $p=0.003$ compared to baseline) and then normalised by po. day 14.

MCV, MCH and MCHC values showed no significant change. Platelet count increased by the 3rd ($p=0.027$ vs. baseline) and 14th postoperative days ($p<0.001$ vs. baseline)

2.2.4 Micro-rheological parameters

Among red blood cell deformability parameters, the elongation index measured at 3 Pa decreased by the 7th postoperative day ($p=0.003$ compared to baseline) and then normalised by the end of the observation period.

EI_{max} values decreased moderately, mainly on the 1st and 3rd postoperative days, and then normalised by the 14th day. $SS_{1/2}$ values did not change significantly, although they rose slightly on the 7th and 14th days.

Significant changes were observed in the red blood cell aggregation index values. By the first postoperative day, all four index values had increased (M 5 s: $p<0.001$, M1 5 s: $p<0.001$, M10 s: $p=0.005$, M110 s: $p=0.014$ vs. baseline) and remained at an elevated level, then showed a further increase by the end of the observation period (M110 s: $p=0.022$ vs. baseline).

2.2.5 Tensile strength measurements

Compared to intact skin, tensile strength values were significantly lower along the suture line of the flaps in all flap sections and directions ($p < 0.001$).

Tensile strength values were higher in the horizontal direction than in the vertical direction (tensile strength of intact skin: $p = 0.008$, slope of the curve: $p = 0.005$).

2.2.6 Histological analyses

Histological analysis using H&E staining revealed inflammatory and healing processes in the ischaemic flaps. No significant morphological changes were observed in any of the skin samples. The thickness of the epithelium and the granulation tissue showed no pathological abnormalities. The integrity of the dermis also remained unchanged.

Mast cells were visualised using dimethyl-methylene blue staining, which renders them metachromatic with a purple colour using this specialised technique. The number of mast cells in the ischaemic flaps was reduced compared to intact skin and the contralateral control side.

Tissue collagen fibres are made visible by picrosirius red staining. When the plane of polarised light is rotated by $\lambda/4$, the arrangement of the collagen fibres becomes apparent: the thicker ones appear bright red, whilst the thinner ones appear green. Our analysis showed that both light intensities - that is, red (thick fibres) and green (thin fibres) - were significantly reduced on the ischaemic side compared to the intact skin and the contralateral side. Red light intensity showed a significant reduction ($p = 0.004$) compared to the contralateral side.

2.2.7 Blood flow of the pedicles and microcirculatory changes of the flaps

Arterial blood flow in the arteries of the flap pedicles on the ischaemic side was lower (0.77 ± 0.24 ml/min) than on the control side (0.90 ± 0.26 ml/min) immediately after ischaemia. A further decrease was observed by the 14th postoperative day, particularly in the ischaemic flaps (control-side flap: 0.64 ± 0.25 ml/min; ischaemic-side flap: 0.60 ± 0.17 ml/min), although the changes were not significant. Following flap dissection, the vascular density according to De Backer decreased compared to the intact skin. On the 7th postoperative day, perfusion values increased in the ischaemic flap and then decreased by the 14th day. Microcirculation values showed an increase in the ischaemic flaps during the first week and then decreased by the end of the observation period.

KEY RESULTS AND CONCLUSIONS

1. We have successfully established a well-reproducible and standardisable bilateral fasciocutaneous and adipocutaneous groin flap model in rats. Although the two types of flaps originate from the same site and are prepared in a similar manner, their tissue composition differs, thus allowing the modelling of the regeneration of flaps with varying compositions required to replace tissue defects of different magnitudes. By clamping the pedicle, I/R injury can be induced in a controlled manner for related research objectives.
2. Macroscopically, the adipocutaneous flaps on the ischaemic side showed greater shrinkage. Histological examination revealed a reduction in the number of mast cells, as well as changes in the quantity and arrangement of collagen fibres in the adipocutaneous flaps on the ischaemic side.
3. Blood flow in the capillaries of the adipocutaneous flaps on the ischaemic side was reduced, although not significantly. We observed that the microcirculatory parameters of the ischaemic adipocutaneous flaps remained lower than those on the non-ischaemic side even two weeks later. The Cytocam-IDF method was suitable for monitoring the intra- and postoperative viability of the flaps. The micro-rheological damage caused by ischaemia-reperfusion manifested as impaired red blood cell deformability and increased red blood cell aggregation.
4. The active substances used in fasciocutaneous flaps alleviated tissue damage to the flaps to varying degrees. Our findings regarding anti-inflammatory effects (PACAP 1-38, BGP-15) and effects on fibre composition (PACAP 1-38) encourage further studies with a longer follow-up period. In fasciocutaneous flaps subjected to ischaemia-reperfusion, tensile strength increased in areas with improved perfusion as a result of the accumulation of fibrotic matrix.



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Candidate: Anna Flaskó

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List of publications related to the dissertation

1. **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 epigastric adipocutaneous flaps affected by ischemia-reperfusion in rats.
Acta Cir. Bras. 41, 1-13, 2026.
DOI: <http://dx.doi.org/10.1590/acb410226>
IF: 1.3 (2024)
2. **Flaskó, A.**, Fazekas, L., Kincses, G., Varga, Á., Mátrai, Á. A., Cziráj, I., Dodity, N., Bácskay, I., Pető, Á., Reglődi, D., Fillér, C., Juhász, T., Németh, N.: Impacts of PACAP 1-38 and BGP-15 on the Healing of Fasciocutaneous Groin Flaps Affected by Ischemia-Reperfusion in Rats.
Biomedicines. 13 (9), 1-18, 2025.
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List of other publications

3. 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.
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IF: 1.3 (2024)
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Érbetegségek. 23 (3), 47-54, 2016.

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