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

Morphological, biomechanical and hemodynamic alterations of microvascular anastomoses

Dr. Balazs Szabo

Supervisor: Prof. Dr. Norbert Nemeth

University of Debrecen
Doctoral School of Clinical Medicine

Debrecen, 2022

MICROVASCULAR ANASTOMOSES MORPHOLOGICAL, BIOMECHANICAL AND FLOW DYNAMIC ALTERATIONS

By Balazs Szabo, MD

Supervisor: Prof. Norbert Nemeth, MD, PhD, DSc

Doctoral School of Clinical Medicine, Uversity of Debrecen

Head of the Defense Committee:

Prof. Arpad Illes, MD, PhD, DSc

Reviewers:

Dr. Gabor Jancso, MD, PhD

Dr. Dezso Toth, MD, PhD

Members of the Defense Committee:

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Dr. Andrea Frenecz, MD, PhD

The PhD Defense takes place at the lecture hall of the Urology Clinic, Faculty of Medicine, University of Debrecen, 2022. december 5., 13:00

INTRODUCTION

Nowadays, numberless vascular sutures are made as a routine procedure, during different surgical procedures. However just as with every medical intervention, here too we have to count with complications. One of the most fundamental problems are that after the surgery the vessel constantly changes and the initial situation can drastically differ from the postsurgical conditions. This can lead to the occlusion or even the rupture of the suture, also abnormal dilatation or the pathological maturation of the graft can occur. These problems are similarly present during microvascular procedures. Vascular surgery has gone through substantial development, in terms of materials and techniques, however this couldn't solve all complications completely. Therefore, the modification of these pathological processes is an important filed of research.

There are several different parameters that can be used to evaluate the quality of vascular sutures, that is why the researches that are focusing on these topics are so versatile. The postoperative pathological changes can be categorized in to early and late groups. The early complications can ben thrombosis suture failure, which can be provoked by several different causes. For example, one of the stiches can tear out, a leakage can occur between the stiches, or even the whole suture can fail. The incorrect surgical technique can cause tissue damage which can lead to thrombosis. The late complications are the narrowing or the severe dilatation of the vessel which is also known as aneurysm. The latter can rupture and cause severe bleeding, which can lead to the patient's death in seconds. During every procedure regardless of the utmost care, the geometry of the vessel will somewhat change the changed flow dynamics in the vessel can initiate different histopathological processes, like intima enlargement which can eventually lead to the occlusion of the vessel. Avoiding these are still a big challenge today. With the appearance of the ultrastructural, immunohistochemical, flow simulation and several other testing method currently unknown pathological causes become observable.

One of the rapidly developing method is the 3D flow simulations, which is widely used in the studies of the cardiovascular system. The basis of these simulation is the Navier-Stokes equation, which describes the flow of viscus fluids. This method allows us the examine certain vascular location's flow pattern, which has well known histological alterations. With this we can conclude important conclusion, because the vessels' histological alterations beside the suture technique, are also greatly influenced by the circulating blood flow properties. Using these methods even an operation before the surgery can be planed, to achieve the most physiological circulatory and flow dynamic environment.

The pathological flow conditions can also damage the circulating red blood cells, which in turn can impair the microcirculation in the long run, therefore decreasing the tissue perfusion. Because of the previously described effect, it is important to also monitor these changes. These are measured and examined by the science of hemorheology.

We must not forget about the materials and surgical methods as they are an integral part of the surgical work. When a vessel is sutured we have to take it in consideration whether the thread and the suture technique can withstand the tension of the tissue, the blood pressure and the constant pulsation of the human body. In addition, depending anatomical location the anastomoses can be subjected to external forces, and due to the body's movement too bending and torsion forces. It is common that the vessels are reconstructed after traumatic injury, resulting from this the sutures can be under increased tension due to the tissue loss. This can decrease the circulation of the supplied area and it can also increase the suture failure complications.

Because of all the previously mentioned factors, the biomechanical examination of vascular sutures in the expected surgical environment is an important filed of research, so that we are aware of the structural integrity of the specific suture technique. A few types of these testing methods are for example the tensile-strength and pressure resistance measurements of the suture materials and vessels. During the writing of this thesis, on the well know website PubMed, searching the key words „arterial anastomosis" and „tensile-strength" only 22 result are found from the last ten years. This shows how under researched is this scientific field.

These testing methods can provide useful feedback for the participant during microsurgical teaching courses.

AIMS

1. Using biopreparates and *ex vivo* vessel we aimed to examine the biomechanical properties of different anastomosis suturing techniques (simple interrupted, continuous and the modified "Lauritzen"-type sleeve technique) measuring the required time, the stitch count, pressure resistance and tensile-strength.
2. We aimed to develop a suitable model for the examination of vessel graft regeneration and complex functional and morphological evaluation of the arterio-venous fistula (AVF) and a loop shaped venous graft interposit in the rat.
3. In the *in vivo* model, we wished to compare the pattern of the present flow profile, wall shear-stress, and the pressure and other flow parameters, with the histological alteration arising in the vessel wall, using 3D flow simulations performed on special corrosion vessel moldings. For this we choose a five-week follow-up period.
4. We wished to examine how the newly emerged flow conditions effect the hematological, hemorheological and microcirculatory parameters in the presence of an AVF and a loop shaped vein graft interposit.

MATERIAL AND METHODS

Biomechanical examinations of the selected anastomoses

Biomodels, experimental animals, and groups

During the experiment we used chicken thigh (n= 60). These were bought fresh without any previous freezing and the surgeries were performed on the specimens at the same day. Every thigh was from female animals, and they were kept in conditions regulated by the 32/1999. (III. 31.) FVM law.

Six group were made with ten specimens in each group. The pressure resistance were measured in three group examining the simple interrupted the continuous and the ”Lauritzen”-type suture. In the other three groups we compared the tensile-strength of the simple interrupted suture using eight and twelve stitches and the continuous using twelve stitches.

The eight stitch simple interrupted sutures were also performed and examined on rat abdominal aortas (n=10). The animal experiments were conducted according to the year of 1998. XXVIII. law (permission registration number: 25/2016/UDCAW, University of Debrecen, Committee of Animal Welfare). During the surgeries we used standard microsurgical tools, every suture was performed using 8/0 monofilament polyamide with serosa needle (Salon, Chirmax®, Czech Republic). The microsurgical interventions were carried out on an operating microscope (Leica Wild M650 operating microscope, Leica Ltd., Germany). For the anastomoses performed on the chicken arteries ten times magnification was used, during the rat surgeries 16 times magnification was used.

Surgical protocol for pressure resistance measurements

In total three group were created for the pressure resistance measurements. These were the simple interrupted, the continuous and the ”Lauritzen”-type sutures. Ten anastomosis were performed in each group.

Preparation of the vessels: A five-centimeter-long incision was made on the chicken thigh above the vessels adjacent to the femoral bone. Then the femoral artery was gently dissected and all the side branches were ligated, and the distal end was clamped down. In these groups the average vessel diameter was 3.25±0.38 mm. The proximal end of the artery was cannulated with a 20 G cannula. The cannula was fixed to the surrounding tissue and to the vessel with holding

stitches, and the distal end of the vessel was clamped down. An infusion bag was connected to the cannula and the vessel was filled with a mixture of saline solution and Betadine. A pressure test was performed at 200 mmHg pressure to test a ligatures, if there were no leakages we continued with the surgery. The pressure was initially set for 120 mmHg, to try to imitate the physiological conditions.

Simple interrupted suture: An approximator was placed on the vessel and the artery was cut between the approximator clamps. The loose adventitia was removed 1-2 mm from the vessel ends. After this two corner stitches were placed in the opposite end of the vessel. This divided the vessel to an anterior and a posterior wall. These were sutured together with individual stitches. The posterior wall was made visible by the 180º degree flip of the approximator. The anastomoses were tested at 120 mmHg pressure by the opening of the approximator clips. In case of severe leakage additional sutures were placed in the vessel. If more than 2 extra stitches were needed the anastomosis was discarded and replaced.

Continuous suture: Until the adventitia removal all steps were the same in this group too. The two corner stitches were made differently, because the threads weren't cut off from the corner stitches. These stitches were used to suture the anterior and posterior walls. Just like before, the approximator was rotated 180º degree to suture the posterior wall. The thread was knotted with the short thread of the opposite corner stitch. In this group we also performed a 120 mmHg pressure test, if it was necessary maximum of two stitches were placed in the vessel just like in the previous group.

Modified "Lauritzen"-type suture: The essence of this technique is that the proximal end of the vessel is inserted in to the distal end of the vessel. For the sleeve-technique we had to modify the original Lauritzen's method because of the vessel diameter. The original technique requires only two stitches in a 1-mm thick vessel. In case of a chicken femoral artery we needed to use four stitches, and additional ones in case of leakage. Instead of corner stitches two pulling stitches were made 180º degree apart. The pulling stitches were placed in to the distal end of the artery as far from the cut as wide the vessels were under pressure, which is 4-5 mm in these vessels. These sutures were stitched through the edge of the proximal end of the artery. Then it was stitched back from to lumen next to the starting point of this suture. By tying the pulling stitches, the proximal end of the vessel was pulled into the lumen of the distal end of the artery. Two additional sutures were placed between the pulling stitches on the front and the back wall. These were superficial stitches connecting only the edge of the distal and the adventitia of the

proximal ends. Just like in the other group a 120 mmHg pressure test was performed, and if needed maximum of two additional stitches were placed in the vessel.

Pressure resitance measurement

The pressure measuring device consisted of a blood pressure monitor, an infusion bag and an infusion set. The measuring cuff was wrapped around the infusion bag to measure and control the pressure. The bag was filled with 1000 ml of saline solution mixed with 20 ml of Betadine. The Betadine was used to make the leakage in the anastomoses visible.

The measurement started by elevating the pressure in the infusion bag to 280 mmHg, and then the infusion tube was opened. The vessels were under water therefore the leakage was visible due to the Betadine content. The pressure drop was continuously measured for five minutes. The pressure resistance was calculated from the pressure drop that occurred during five minutes of observation.

Surgical protocol for the tensile-strength measurements

For this part of the experiment we established four groups, three on the chicken thigh model and one on the rat abdominal aorta model. These were the eight and twelve stich simple interrupted suture, and the twelve stitch continuous suture on the chicken model, and eight stitch simple interrupted on the rat model (2.21± 0.26 mm). In each group ten anastomoses were made. The sleeve-technique, because of the highly different suture count, was not comparable with the other suture techniques, in this regard.

Simple interrupted: The anastomoses were performed the same way as it was described in the previous study part The only difference was that the suture count was standardized, to achieve this the vessel diameter was strictly controlled (chicken: 2.96±0.3 mm; rat: 2.21± 0.26 mm)). In the twelve stitch group five stitches were placed between the corner stitches and in the eight stitch group this was three.

Continuous suture: Just like in the other group we followed the previously set surgical steps. The anterior and the posterior wall was connected with five continuous stitches as evenly as possible, which meant twelve stitches in total including the corner stitches.

Tensile-strength measurements

Biomodel: The measuring device was custom made with the collaboration of the Department of Information Technology, Faculty of Informatics, and the Department of Operative Techniques and Surgical Research, Faculty of Medicine, University of Debrecen, Hungary. The device is consisted of a CNC servo motor, a torsion sensor, and an "Arduino" microcontroller. The device measured the tensile-strength in Newton (N). Each second it made 11 steps, and each step equals 1.8º degree of rotation, which means 0.079 mm puling distance at each step. The specimens were fixed between the jaws of the instrument which were 1 cm apart at the beginning of each measurement. It pulled the vessels in a distance of 2 cm. Each vessel was tested twice, the anastomosis itself and 1 cm proximally to test the intact vessel tensile strength as a control. The equipment measured the puling force in Newton and draw a stress-strain curve. In this case, stress means the force, which acts upon the vessel and strain the distance which it has been pulled. Then the curves were analyzed. Using the given data, the maximal tensile-strength, the elongation and the elasticity was calculated. For the representation of the elastica Young's modulus was used. Video and photos were taken from each measurement for further analysis.

Thread model: The suture material was also examined in different scenarios to better understand the biomechanics of anastomoses. We measured: a single thread, a thread line with a knot in the middle, single stitch, incorrectly tied knot, and stitch with a flattened (damaged) area in the middle. Each knot was made out of three half knots. A thread model was also set, consisting of two 2/0 polyester braided thread loops (Tervalon) which were sutured together with the same anastomosis techniques as mentioned before, to make an anastomosis analog. By this way the tissue variable was excluded, and only the suturing technique and the suture material could be investigated.

Examination fo the arterio-venous fistula, and the loop shaped venous graft

This part of the experiment was also conducted according to the 1998th year XXVIII. law with the permission of the Committee of Animal Welfare at the University of Debrecen (permission registration number: 25/2016/UDCAW). During the experiment 36 male Wistar (Crl:WI) rat were used, the average age was between 8-10 weeks and the average weight was 349.7 ± 13.76 grams. These were randomly divided in to three groups, as sham-operated (n=12), fistula (n=12) and loop group (n=12). The rats were kept in 22-24 ˚C temperature under 12-hour light cycle. After the surgeries the animals were kept in individual standard cages and fed ad libitum with commercially available food and water. During the surgeries standard microsurgical instrument were used, and the sutures were performed with 10/0-s monofilament polyamide with serosa needle (Silon, Chirmax®, Czech Republic). The operations were done under 16x-26x magnification using an operating microscope (Leica Wild M650 microscope, Leica Ltd., Germany).

Surgical Protocol

We started with the weighing of the animal, according to their weight the rat received the anesthetics. The anesthesia was done using a mixture of ketamine (100 mg/kg), xylazin (10 mg/kg) and atropine (0.05 mg/kg), administered intraperitoneally. For thrombosis prophylaxis heparin was administered i.v. (80 IU/kg). After the anesthesia the lower abdominal wall and both inner thighs were shaved and disinfected using Betadine solution. A 26-gauge cannula was inserted into the lateral tail vein for blood sampling and fluid therapy. The surgical site was carefully isolated with gauze and an incision was made above the right inguinal ligament, and then the femoral vessels were isolated. The superficial inferior epigastric vein (SIEV) was also isolated, which is the second side branch of the femoral vein on the medial side from the inguinal ligament. The previously mentioned steps were the same in each group. In case of the sham-operated group after the dissection the rat stayed under anesthesia for an additional 90 minutes, which was the duration of the vascular intervention in the other groups. Then the skin was closed with a continuous suture.

Fistula: The fistula was performed after the dissection using the SIEV. The vein was mobilized, but a significant amount of connective tissue was left intentionally on the vessel wall. This was useful in a later part of the surgery. The distal end of the SIEV was ligated, cut and finally flushed with heparin solution (50 IU/ml). After that the vessel was positioned by bending the SIEV 180º degree in the shape of a U.

A side cut was made on the femoral artery forming approximately the same diameter orifice as the SIEV, and it was also washed with heparin solution. The vein was sutured to the orifice on the femoral artery using simple interrupted stitches, so creating and end-to-side anastomosis.

We checked the flow of the fistula by closing the SIEV with a microvascular clip. By releasing the clip arterial blood entered the femoral vein and changed the color of the vessel bright red. Two anchoring sutures were used to secure the U-shape of the fistula. The stitches were placed into the connecting tissue around the vessel and into the gracilis muscle. The skin was sutured in the same fashion as in the sham-operated group.

Loop: In case of the loop instead of the side-to-end anastomosis two end-to-end anastomoses were performed using simple interrupted stitches. First the SIEV was mobilized and washed just like in the fistula group, then the femoral artery was cut in half and each vessel end was flushed with heparin solution.

The start of the loop making process was to connect the distal end of the SIEV to the proximal end of the artery with and end-to-end anastomosis using simple interruptes stitches. After the anastomosis was made it was tested for leakage and flow. Since the proximal end of the SIEV was still connected to the femoral vein, at this point it was acting as an AVF, therefore it was tested in that manner.

After the completion the previous steps, the proximal end of the vein was ligated and cut at the junction of the SIEV and the femoral vein. The distal end of the femoral artery and the proximal end of the SIEV was also sutured forming an end-to-end. All of the microvascular clips were removed, and the arterio-arterial graft was tested for patency and leakage. The patency was tested using the double occlusion test (“milking” test). At this stage the venous graft was twisted so that it would be shaped like a loop. This was fixed with two anchoring stitches just like in the case of the fistula. The skin was sutured as it was mentioned previously. During the postoperative weeks flunixin was used for pain management (10 mg/kg, s.c.). At the 5th postoperative week

under general anesthesia the vessels were dissected again for visual inspection and for patency testing.

After the surgery and at the postoperative fifth week (PO5) video and images were taken of the vessel. Using these images, we measured the vessels outer diameter at marked locations and inside the area of the fistula and a loop the longest horizontal and vertical axel was measured. The change in the ratio of these axles showed the deformation of the graft. We also recorded the course of the vessels. At the first postoperative week (PO1) the skin sutures were removed under general anesthesia. The animals were observed for five weeks, at the PO5 week the animals were terminated and the vessels were removed for histological evaluation. However, before the termination and explorative laparotomy was performed to measure and record the possible distant alterations. The abdominal vessels were dissected between the inguinal ligament and renal arteries and the diameter was measured of the femoral artery and vein, external and common iliac vessels, abdominal aorta and the caudal vena cava. During the exploration we observed the blood flow in both the AVF and the loop.

Imaging techniques: MRI, SPECT-CT

All the imaging's were done at the 2nd postoperative week, to monitor the patency of the vessels and the tissue perfusion. The patency was examined with a Gadolinium-based MRI angiography (nanoScan® PET/MRI, Mediso Kft, Budapest, Hungary). The contras material was injected through the lateral tail vein of the rat, and the lower half of the animal was scanned during the arterial phase. The patency of the vessels was clearly visible.

In case of the tissue perfusion a Technetium^{99m}-mibi-based SPECT-CT was used (nanoScan® SPECT/CT/PET, Mediso Kft, Budapest, Hungary). The Technetium was also injected through the lateral tail vein. High activity on the image showed good tissue perfusion. When thrombosis, or partial occlusion happened the low activity in the limb muscles showed the disfunction. If the vessels thrombosed or measurably occluded, the rats were excluded and replaced.

Vessel molding technique and 3D flow simulation

In our study a new technique was used for highly accurate simulation measurements which were done with the cooperation of the Department of Surgical Research and Techniques, at the University of Pécs. The vessel shape and diameter were recorded during the physiological circulation with a single-lens reflex camera with a resolution equivalent of 4K (Canon EOS 1100d).

The 3D CFD method was based on protocol of lumen 3D scanning and application of standardized in silico examination of vessel-specimens (ME3D-Graft, Hungary). Briefly a 22-gauge canula was inserted into the common iliac artery and an ultra-low viscosity polymer was is injected in to the vessels. Using the previously taken images we inflated the vessel to the same shape and size as it was when the animal was still alive. It took 30 second for the initial hardening and an additional 10 minutes to fully cure. Then the tissue was removed from the hardened castings. Then the lumen castings are scanned in high resolution for further applications for the simulation software with Ansys 20 R1 background (CARAT dental scanner, Kulzer, Japan, ME3D-Graft, Hungary). The scanner has a resolution up to 10 μm, which is smaller than a vascular endothelial cell. This provides a standardized, scalable method for examination of microvascular structures.

A standard, transient fluid dynamic simulation was applied with pre-set, standardized boundaries on the in and outflow of vessel structure. The endings were elongated with at least three times of the diameter, after the simulation the elongated parts of the models were not taken into consideration. For boundary conditions of the average and normal rat vessel aortic velocity profile, distal aortic resistance and resistance of vena cava inferior was applied, these were pre-recorded in 100 timesteps per cardiac circle from invasive arterial pressure measurements.

More than 30 parameters are routinely measured, including spatial and time profile of velocity, pressure, vorticity, helicity, Reynold's number, wall shear stress (WSS) and further derivates of WSS like oscillating shear index etc.

In this study we were focusing on the pressure and wall shear stress. For visualization and measuring the co-appearance of pressure and WSS parameter values with different cut-off values, Ansys 20 R1 CFD-Post software was used (Ansys, Inc; Canonsburg, Pennsylvania, USA). Frozen frames from the simulation were used for the histological comparison.

In every group two animals were used to make the casting right after the surgery, after that four from each group was used for the matured vessels. This way we were able to compare the

effect of the maturation on the morphological and geometrical changes. At definitive locations the WSS and the pressure values were collected from the simulation model for numerical comparison and statistical analysis.

Electron microscopy

The surface of the vessel castings was examined with scanning electron microscopy (JSM-IT500HR, Jeol Ltd, Akashima, Japan). Each plastic casting was cleaned with absolute ethanol and then it was coated with three layers gold of atoms which was 5-10 nm. This provided a good reflective surface, for the imaging. We used the images to compare the unoperated vessels with the matured ones.

Histological examination

The histological examinations were carried out by the Institute of Pathology, University of Debrecen. Hematoxylin&eosin stain was used for the morphological examinations. The animals were terminated at the PO5 week and an explorative laparotomy was carried out to observe any possible distlal alterations due to the vascular interventions. That is why we also removed the hearts of each animal, and measured the inner diameter of the common iliac artery and vein and also the femoral artery and vein. From every group six animals were used for histological examination. The samples were removed from the animals under an operating microscope, we noted different reference point in order to later identify these locations on the histological slides. These were sutures and anatomical structures like know side branches. During the comparison of the flow simulation and the histological result these points were used to identify the same locations. The vessels were removed with the surrounding muscle so that the original shape won't change during the preparation processes.

The removed specimens were fixed in a 4% solution of formaldehyde, after 24 hours these were dehydrated with xylol. Then the tissue samples were imbedded in paraffin wax and with a microtome 4 μm thick slices were cut. The paraffin blocks were checked under a microscope to see whether we are at the right anatomical plane. The finished slides were checked for artefact like bubbles under microscope (Leica DM 200, Leica Microsystems Ltd., Germany) That is how we inspected the slides if they were suitable for further analysis. In case the samples have passed

the inspection, they were digitalized with a histological slide scanner (Pannoramic MIDI Scanner, 3DHistech, Budapest, Hungary). The virtual slides were viewed and analyzed with a histological analysis software (Caseviewer 2.2, 3DHistech, Budapest, Hungary). We measured the thickness of different tissue layers (tunica intima, tunica media) and inner vessel diameter. The diameter of the collapsed vein was measured in a different manner. The uneven border of the lumen was traced and with the software the length of that was what we identified as the circumference of the round vein.

After the tissue samples were fixed in formaldehyde, they were cut in specific planes. Using an operating microscope, the location of the cutting planes was placed with the accuracy of tenth of a millimeter.

In the fistula group we defined 5 cutting planed. One was in the middle of the arterio-venous anastomosis parallel with the SIEV. The other four was evenly distributed 1 mm apart, two proximal and two distal to the anastomosis parallel to the first cutting plain. At the site of the SIEV between the SIEV-femoral vein junction and the anastomosis the vessel evenly divided to 3 planes perpendicular to the vessel. The SIEV-femoral vein junction was cut in 5 planes in the same manner is it was described at the arterio-venous anastomosis. The tissue segments were imbedded in paraffin wax regarding the cutting planes.

In the loop group the SIEV graft was cut across the loop in three radial planes between the anastomoses. In case of the loop arteriovenous anastomoses those were cut parallel to the anastomoses, and two additional planes were made 2 mm away from these anastomoses perpendicular to the artery. The exact locations were 2 mm distal to the distal anastomosis and 2 mm proximal to the proximal anastomosis.

The described cutting planes were found easily and with high accuracy, this enabled us to accurately compare the flow simulations with the histological slides.

Blood sampling protocol

Blood samples were taken from the tail cannula before/after the surgery and at the 1st 3rd and 5th postoperative week (PO1, PO3, PO5 week). The blood was stored in vacutainer tubes (BD Vacutainer® tubes, 5.4 g K_3-EDTA, 3 ml). Each time 300 µl of blood was taken for the hematological and hemorheological measurements, plus an additional 90 µl was taken before and after the surgery and also at the PO5 week for blood-gas and blood electrolyte analysis. At the postoperative weeks the rats were anesthetized with the same anesthetic mixture, and the lateral

tail vein was cannulated again. At the PO5 week blood was also taken from the inferior caval vein in the fistula group to test the composition of the mixed (arterio-venous) blood.

Haematolgical measurements

All the measurements were performed by a Sysmex K-4500 automate (TOA Medicor Electronics Co., Ltd., Japan). For every measurement 70 µl of blood was needed. The measurements were performed before and after the surgery and also at a the PO1, PO3,PO5 week. In this study we analyzed the red blood cell count (RBC [10^{12}/L]), the white blood cell count (WBC [10^{9}/L]), the hematocrit (Hct [%]), the hemoglobin concentration (Hgb [g/L]), the mean corpuscular volume (MCV [fL]), and the platelet number (Plt [10^{9}/L]).

Blood-gass analysis

For the blood gas and electrolyte measurements 90 µl non-anticoagulated blood was used. These measurements were repeated before and after the surgery and also at the PO5 week. We measured the partial oxygen and partial carbon dioxide pressure (pO_2, pCO_2 [mmHg]), the oxygen saturation (sO_2 [%]), the pH and the bicarbonate (HCO_3- [mmol/l]), glucose (Glu [mmol/L]), lactate (Lac [mmol/L]) concentrations. In addition, the following electrolyte concentrations were also recorded: sodium (Na^+ [mmol/L]), potassium (K^+ [mmol/L]), calcium (Ca^{2+} [mmol/L]) and chloride (Cl^- [mmol/L]). For these measurements we used an EPOC® Blood Analysis System (Epocal Inc., Canada). For every test a disposable card was inserted in to the device, and after the calibration the blood sample was injected.

Hemorheolgical measurements: Red blood cell deformability and red blood cell aggregation

The erythrocyte deformability was examined using the LoRRca MaxSis Osmoscan ektacytometer (Mechatronics BV, The Netherlands). Ten µl of blood was diluted in 2 ml of polyvinyl-pyrrolidone (PVP) and phosphate buffered saline (PBS) solution (viscosity: 27 mPas, osmolarity: 300 mOsm/kg, pH: ~7.3). The deformability of the red blood cells was tested by determining the elongation of the cell under increasing shear stress (SS [Pa]) based on laser-diffractometry.

The elongation of the cells was recorded between 0.3 to 30 Pa. The range of SS could be set between 0.3-75 Pa, but the upper range is not physiological which could show unrealistic values. A laser diffraction pattern was provided by shining a beam on the red blood cell suspension. The laser beam is scattered on the surface of the red blood cells. The device analyses the pattern and the software calculates and presents the data as an elongation index (EI) – shear stress curves (SS). The values of the elongation index are proportional to the erythrocyte deformability. Using the Lineweaver-Burke analysis the maximal EI (EI_{max}) and the shear stress at half-maximal elongation ($SS_{1/2}$ [Pa]), and their ratio ($EI_{max}/SS_{1/2}$) were also calculated.

Erythrocyte aggregation was measured using a Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany), based on light-transmittance method. Approximately 20 µl of blood was required for each test. Aggregation indices as M 5 sec and M 10 sec were tested at 0 s-1 shear rate, and M1 5 sec and M1 10 sec at 3 s-1 shear-rate.

Hemodinamicaland microcirculatory measurements

Since the fistula creates a connection between the arterial and the venous system, thus to keeping the physiolgical arterial pressure the heart needs to do excesses amount of work. Therefoe, besides the histological observations we decided to also record invasive blood pressure measurements to see the cardiovascular effect of the AVF. To measure this Haemosys invasive blood pressure measurement system was used (Haemosys, Experimetria Kft.). A blood pressure was recorded in the right common carotid artery at the PO5 week, which was recorded as a continuous pressure wave. While we recorded the blood pressure the AVF was closed and opened 3 times 10 minutes apart, this way we were able to see the hemodynamic effect of the graft.

The microcirculation of the tissue was monitored using a Laser Doppler (LD) fluxmeter (LD-01, Experimetria Ltd., Hungary) and a standard pencil probe (NP-100 Standard Pencil Probe, Oxford Optronix Ltd., UK). The device expresses the blood flux unit (BFU), which is a dimensionless number as an integral over the number and velocity of red blood cells in the given region (~1 mm^3). The device uses short impulses of laser light this the wave length of 780±10 nm. The frequency of the light is 10-19 Hz, and between the pulses the device detects the reflection. The reflected light is dependent on the speed and number of the red blood cells. These parameters which are defining the BFU.

This measurement was taken before, 10 minutes after ischemia and 10 minutes after reperfusion, and also at the 1st, 3rd and 5th postoperative weeks. Six points were tested on each

side of the animals: lower abdominal wall, the inner thigh, and the second metatarsal foot pad. Each location was measure for 10 seconds, the average value of this measurement what gave us the recorded BFU.

Although it is important to note that lot of other parameters can alter the final data like: skin color, probe angle, probe surface pressure etc. That is why to eliminate most of the variables one person did all them measurements, following the one hand rule. For the data analysis S.P.E.L. Advanced Kymograph software (Experimetria Ltd., Hungary) was used. Ten seconds of Laser Doppler recordings were recorded after the stabilization of the waves, averaging the values

The surface temperature of both hind limbs' foot (°C) was also monitored at the PO5 week, using an infrared thermometer at the 5th postoperative week (Braun IRT 6030 Thermoscan, Braun® Kronberg, Germany).

Statisztical analysis

The statistical analyses were performed using the GraphPad Prism 8 software. The significance level was set to $p \leq 0.05$. All data distribution was checked for normality, and accordingly, Student t-test, or Wilcoxon, or Mann-Whitney non-parametric tests, as well as two-way ANOVA tests were used.

RESULTS

Anastomoses' biomechanical examination

Technical observations

Leakage: Az we described it in the Material and Method chapter photos and videos were taken from every measurement. The analysis of the images and videos showed the in case of the simple interrupted and continuous the leakage never came from the space between the stitches. It was always from the suture hole, where the thread passes through the vessel wall. In most cases the leakage was notably bigger in the upper suturing hole where the needle passed through the arterial wall from the outside to the inside. The corner stitches showed the greatest leakage.

Vessel torsion: While in case of the simple interrupted anastomoses narrowing of the vessel was never observed, during the making of continuous suture it was visible that it is prone to stenosis. This type of suture is very easy to over tighten, by pulling the suture line just a millimeter more. However, this was only visible when the vessel is under pressure, tha is why we can only find out if the suture is over tightened when the anastomosis is finished. I would like to note that the over tightened sutures showed significantly less leakage.

Usability of the sleeve technique: The week point of this technique is the principal of the method, because when the vessel is inserted into itself the vessel becomes significantly shorter. If we want this method to properly work we have to insert the proximal end of the artery, one and a half times of its diameter, deep. In this case we have to be taken into account the vessel diameter under pressure. This work well for a 1 mm wide vessel, however in our study we used arteries with the diameter of 3 mm, which means 5 mm of insertion depth. In normal circumstances this amount of vessel length is not available. The holding suture, which hold the distal vessel end to the proximal vessel's wall, requires special attention. It is not enough to simply place in the stitch, because the flexibility of the adventitia allows the proximal vessel end to slip out a little bit while under pressure. That would result in leakage because the two adjacent vessel wall would separate and won't seal. This had to be taken into account when placing the stitch.

Vessel rupture: The ruptured vessels were examined under the microscope to see if we can find any currently unknown correlations. In case of the simple interrupted stitch we found two main rupture patterns. The first was when after the test all the stitches stayed in on end of the vessel, in the other group both vessel and contained stitches after the test. On the video recordins we were able to see that when all the suture line got tight at the same time the rupture pattern was

group one, there for the stitches were evenly paced. Thus, the vessel tore apart not at the middle but adjacent to the suture line. Contrary to that if one stitch held more tissue than the others, the tearing started at that point and the anastomosis rupture pattern became uneven. The properly performed continuous suture always tore in a characteristic ring pattern.

Thread rupture: In the literature it is a well-known fact the weakest point of the thread or a rope is the knot. This was the same in our experiments, because the undamaged thread always broke at the knot. On the other hand, the thread damaged by the forceps always broke at the flattened area

Pressure resistance

Between the groups there were no significant differences in terms of vessel diameter (interrupted vs. continuous vs. sleeve: 3.25±0.35 mm vs. 2.95±0.28 mm vs. 3.15±0.47 mm). The sleeve-techniques leaked the most because, the pressure drop in case of the simple interrupted suture was 40.2±12.8 mmHg; at the continuous suture it was 39.6±9.8 mmHg; and the modified Lauritzen's method resulted in leaked at 56.0±16.7 mmHg ($p=0.029$ vs. interrupted, and $p=0.016$ vs. continuous). The least amount of sutures was required by the sleeve suture as well. The suture count of the simple interrupted suture was 13.1±2.4 stitches, and for the continuous suture 15.1±1.4 stitches were used, the sleeve-technique needed 6.4±1.1 sutures ($p<0.0001$ vs. interrupted, and $p=0.0001$ vs. continuous). And the sleeve technique turned out to be the quickest. Even though the continuous required more stitches it was faster then the simple interrupted suture.

The correlation was calculated between the number of stitches used to the amount of pressure drop in each group. In each group the correlation coefficient wasn't notable, and there was no difference between the groups.

Tensile-strength

Testing of the tensile strength revealed that the shape of the stress-strain curve can give information about the technical mistakes that happen while the anastomosis was made. If a stitch was incorrectly placed and it was holding more tissue than the other stitches, it broke first which could be seen on the curve.

In case of the continuous sometimes a notable indentation was visible in the curve, that happened when one sides of the anastomosis broke separately due to the damage of the knot, or

the thread. A stair-like patter was visible when the sutures were unevenly placed and the anastomosis was torn apart stitch by stitch.

The tensile strength of the vessels was significantly decreased after the anastomoses were performed in every group. There were no differences between the 12-stitch simple interrupted and the 12-stitch continuous suture, but the 8-stitch anastomoses were significantly weaker than the ones made with other two suturing techniques (12-stitch: 4.31±0.64 N; 8-stitch: vs. 3.47±0.32 N). The 12-stitch interrupted and the 12-stitch continuous suture tensile strength on the thread model was notably higher than the anastomoses or the intact vessels. Significant difference couldn't be found between the 8-stitch interrupted thread model anastomoses and the intact vessels. Which suggests that this amount of stitches on a 3 mm vessel is not enough, because instead of the vessel, the thread itself was the weaker link. Every anastomosis was inspected after the test, and we found that in case of the 8-stitch anastomoses not the vessels were torn but the stitches were broken.

The elongation of the vessels showed that the 8-stitch anastomoses on the vessel was the shortest 6.82±2.02 mm. And all the intact vessel groups could elongate significantly more than the other anastomoses groups. The elongation of anastomosis performed on thread model didn't differ significantly from their matching anastomosis group.

The elasticity was calculated by the ratio of the elongation and the tensile strength on the high strain region of the strain/stress curve, higher values show that de material deforms less under tension, therefore the higher the value the stiffer the material. In case of the intact vessels the elasticity was significantly changed by the anastomoses in all groups. Each type of anastomoses technique made the vessels more elastic. At the thread model each group showed drastically higher values which meant that the threads on their own, are much more rigid then the anastomoses performed on the vessels.

The rat aorta behaved the same as the chicken femoral arteries. The basic tensile strength was smaller because the vessels were significantly thinner (2.21±0.26 mm vs. 3.25±0.38 mm; rat vs. chicken; $p<0.0001$). The main difference was that the decrease of elongation, tensile strength, and elasticity caused by the anastomosis was greater than what occurred at the chicken femoral artery groups. This enlarged reduction was significantly bigger in case of the elongation, and the elasticity parameter.

When testing the threads themselves, they broke at 0.71±0 N which shows how well the suture material is made and how accurate the custom-made device is. The knot decreased the

tensile strength by an average of 0.23 N. One stitch could withstand no more than 1.29±0.03 N, but a flattened area weakened it to 1.06±0.06 N. The elongation of the thread wasn't changed by the knot, and we got the same result at the flattened area group as well. The elasticity of the thread was decreased by the knot almost 30% (0.68±0.11 vs. 0.42±0.06; thread vs. knot on a thread; p=0.0006), the flattened area also significantly decreased the elasticity values (3.00±0.49 vs. 2.1±0.10; one stitch vs. one stitch with flattened area). The correlation between the intact vessel tensile strength and the anastomosis tensile strength was also calculated. The continuous suture, and the 8-stitch simple interrupted showed small p values, but the 12-stitch simple interrupted anastomosis p value showed a strong correlation.

The lowest tensile-strength and elasticity values was shown by the incorrectly tied knot. These were significantly lower than the undamaged knot and the damaged knot as well (knot vs. incorectly tied knot, 2.18±0.1 N vs. 0.33±0.09 N, p=0.0007).

Arterio-venous fistula and the loop shaped graft interposit

General observations

The animals were removed and examined every second day, we examined the surgical site and the state of the operated legs. In the SHAM operated group, we haven't found any alterations during the follow-up period.

The patency of the anastomoses was measured at the PO2 week on every animal using a SPECT-CT and an angio-MRI machine. In total only two animal showed occlusion, on in the fistula and on in the loop group. These animals were excluded from the study and replaced.

All of the fistulas matured and all of the loop shaped graft arterialized during the follow up period. As the graft in the loop group arterialized it provided sufficient blood flow to the limb. All operated hind limbs remained functional, and had no morphological alterations compared to the non-operated limb.

Macroscopic morphological examination

At the PO5 week the animals underwent anesthesia and an explorative laparotomy. We measured the outer diameter of the operated vessel and the common iliac vessels. The right common iliac artery in the fistula group which goes to the operated leg was significantly larger than the left (1.46±0.1 mm vs. 2.00±0.29 mm, p=0.0041). Also, the femoral artery and vein was

also significantly enlarged compared to the other side. In case of the loop there were no differences between the left and right side.

It was clearly visible that the blood wasn't evenly colored in the caudal vena cava, we observed a distinctive thin brighter red line in the vein. When we traced back that line we found it was originating from the AVF where the SIEV joins the femoral vein. This means that the blood flow becomes almost instantly laminar at the SIEV femoral vein junction, and it is hardly mixing. We took blood samples from this blood, the result of that will be presented in that blood-gas measurements chapter.

In the fistula group the all of the outer vessel diameter significantly enlarged. The SIVE from the arterio-venous anastomosis until the SIEV-femoral vein junction showed a gradual but highly notable dilatation (proximal section vs. distal section: 1.40±0.22 mm vs. 2.67±0.55 mm, $p<0.0001$). The shape of the fistula is also significantly changed, because the horizontal axel elongated, however the vertical didn't show notable change. In addition, the length of the SIEV elongated after five weeks of maturation.

Contrary to the fistula group, the loop showed no significant dilatation in the course of the vessel at the measured point. The only notable change was measured distal to the distal anastomosis site (before surgery vs. PO5 week: 1.09±0.09 mm vs. 1.19±0.09 mm, $p=0.0216$). I would like to explain the cause of this at the *histological findings* chapter. However, the shape of the loop like the fistula also have changed, both the vertical and the horizontal axes become shorter. The angle where the loop crosses itself also decreased. In accordance with the previous findings the length of the graft has also significantly decreased (before surgery vs. PO5 weeh:19.03±1.40 mm vs. 16.13±2.03 mm, $p= 0.0015$).

Histological finding

During the explorative laparotomy we haven't found any macroscopic morphological changes on the rats' heart. However, the histological examination showed that in the fistula group the venous pressure must've been high because we found that the right ventricle's wall notably thickened (control vs. fistula: 0.81±0.13 mm vs. 0.95±0.17 mm, $p=0.0046$). In addition. the fistula caused elevated load on the heart because the histology also showed left ventricle enlargement (control vs. fistula: 2.2±0.22 mm vs. 2.47±0.30 mm, $p<0.0001$). In the loop group we have not found any such alterations.

In the Sham-operated group we found no changes, only around the vessel we saw increased amount of connective tissue, which we suspected was the scarification after the surgery. All of the arterio-venous fistulae matured during the follow-up period. In the Loop group the grafts arterialized. The tunica media enlarged significantly in both operated groups compared to the Sham vessels, however the tunica intima enlarged significantly only in the loop .

Within the groups we compared different locations. The fistula distal inner dimeter enlarged compared to the proximal end (proximal vs. distal: 0.448±0.124 mm vs. 1.062±0.191 mm, p=0.0079). We divided the anastomosis site in to an upper and lower wall. The venous part of the anastomosis at the upper wall, we observed significantly enlarged tunica media, while in the lower fall the tunica media was much thinner it was still significantly enlarged compared to an intact vessel (fistula vs. intact vessel: 178.0±47.58 μm vs. 61.82±16.27 μm, $p<0.0001$).

In case of the AVF group we examined the femoral artery proximally and distally to the anastomosis and at the anastomosis site, where the vein was also visible. We found a small patch of intimal hyperplasia opposite to the anastomosis orifice, and also another one distally to the anastomosis in the artery. Intimal hyperplasia formation was found in the fistula only at the junction of the superficial inferior epigastric vein and the femoral vein. It was located in the femoral vein where the blood rushes against the vein wall from the SIEV.

In the Loop group the diameter became roughly the same throughout the graft after the arterialization. The tunica media significantly thickened, almost in the entire area of the looped venous graft. Gradual enlargement towards the distal anastomosis was seen in the tunica intima layer, and the difference of the thickness was significant at the two ends. This difference was significant between the proximal and the distal ends.

The outer and inner side also showed asymmetrical intimal hyperplasia. The intima was much thinner in the area of the outer curvature at the distal section. At the proximal anastomosis we did not found intimal hyperplasia formation, however at the distal anastomosis the tunica intima thickening was even in both sides like the wall shear stress pattern. This intima thickening was also observable distal to the distal anastomosis at the area of the femoral artery, however the lumen diameter remained the same. This can explain our finding at the morphological measurements.

Scanning electron microscopy

Using scanning electron microscopy, we examined the surface of the plastic castings. The surfaces of the plastic castings of the loop, before and after the arterialization were notably different. After five week of maturation the surface become much smoother. Interestingly even though that the anastomosis showed no narrowing before and after the arterialization viewed from the outside, there was a circular indentation at the anastomosis sites. At the anastomos we also observed the smoothing effect of the maturation.

3D Flow simulation findings

The simulations revealed how different is the flow in the operated vessels then in an untouched vessel. However, there were no alterations in the sham-operated vessels flow simulations.

In case of the AVF, we observed two "hot-spots" of WSS, one was found in the upper wall of the vein at the anastomosis site. Here the WSS showed much higher values at the upper venous wall then the lower venous wall (Upper vs. Lower; 131.25 Pa vs. 34.97 Pa). The other was at the junction of the SIEV and the femoral vein. In this location the high WSS was seen on the wall of the femoral vein opposite to the side branch opening.

The pressure drop was much higher in the fistula then in the loop (Fistula vs. Loop; 22.39 mmHg vs. 5.74 mmHg). In the first millimeters (mm) of the fistula the pressure was at the arterial level and the vein showed no dilatation after maturation. In the matured fistula after approximately 2 mm away from the anastomosis the vein suddenly dilated, distal to the dilation point the pressure and WSS notably decreased.

In the fresh fistula the high WSS was observed in a much longer section then in the matured one, but the pressure decreased gradually before the maturation. In the loop there is a clear difference in WSS in the outer and the inner curve (Outer vs. Inner; 11.92 Pa vs. 3.08 Pa), the shear-stress becomes even again only at the distal anastomosis location. The diameter of the original vein graft slightly enlarges which causes the decrease of WSS at the distal part of the loop. Compared to the matured vessel the geometry drastically changes. Because the diameter becomes even throughout the vein graft and the size of the loop decreases. This result in a homogenous distribution of WSS at the peak of the pulse wave. The pressure drop is similar, at

the two and of the vessel, to the untouched vessel in the same length (Before vs. After maturation; 5.74 mmHg vs. 4.76 mmHg).

Comparison of the 3D flow simulation and the histological findings

The simulations showed homogenous WSS and gradual decrease of pressure in the sham-operated vessels, accordingly we found no changes in the histology either.

In the fistula group the asymmetrical WSS pattern at the AVF anastomosis site and in the distal arterial and venous branch with the histological finding. At the venous part of the anastomosis site and at the distal arterial branch where the simulation showed asymmetrical WSS, asymmetrical tunica media enlargement was found in the vein and asymmetrical tunica intima in the arterial branch. Where the pressure suddenly dropped we found major dilatation of the vein. The distal "hot-spot" mentioned in the simulation coincided with the IH found at the SIEV-femora vein junction.

In case of the loop we also saw the high correlation of the WSS and the IH. The outer and inner curvature intima pattern matched the asymmetrical WSS. Where the simulation showed circular WSS at the distal anastomosis site, we saw even tunica intima enlargement in the histological slides.

Hematological measurements

The hematological values showed the biggest changes at the PO1 week. The Hct, RBC count, and the Hgb values all decreased significantly within each group. The loop group values were significantly lower at the PO1 week compared to the other groups. The values normalized by the PO5 week.

The MCV values also increased significantly at the PO1 week, this increase was notable compared to the other groups too, however a slow but steady decrease was seen after the PO1 week.

Interestingly the Hct, RBC count, and Hgb showed a distinctive elevation at the PO5 week within the fistula group and also compared to the other two groups. The Plt count significantly elevated in the loop group at the PO1. The WBC values increased after the surgery, and significantly decreased in the sham and loop groups. In the fistula groups the WBC count remained in higher level. The MCH and MCHC values showed no discernable differences.

Blood-gas and electrolyte measurements

Several parameters were measured but most of them didn't show any notable changes during the observation period. Only the oxygen and carbon dioxide partial pressures changed significantly in the fistula group. The pCO2 elevated after the surgery (After OP vs. PO5 week; 35.67±5.81 vs. 47.54±11.22; p=0.0076), and accordingly the pO2 decreased (Before OP vs. PO5 week; 67.08±4.93 vs. 57.08±3.19; p=0.461). At the PO5 week the fistula group had the highest pO2 values, but the change wasn't significant. We also took blood samples in the fistula group from the inferior caudal vein where the venous blood mixed with the arterial blood from the fistula. This was done at the PO5 week when we terminated the animals. Most notably the pO2 (venous vs. mixed; 57.083±3.20 vs. 63.12±11.14; p=0.0099) and glucose concentration (venous vs. mixed; 18.95±3.61 vs. 24.93±2.31; p=0.0109) were increased. Other acid-base or metabolic parameters and electrolytes did not change significantly.

Hemorheological measurements

In conjunction with the hematological result, the most prominent changes were observed at the PO1 week in the loop group. The red blood cell deformability significantly impaired by the PO1 week in the loop group, and compared to the other groups the changes were significant, too. The largest differences were seen between 1.69 Pa and 5.33 Pa shear stress. However, in all groups the deformability values were almost the same by the end of the postoperative period. The fistula showed significant elevation at the PO5 week only above 16.87 Pa compared to the values before the operation.

The EI_{max} and EI_{max} /$SS_{1/2}$ values also normalized at the end, except in the fistula group where the values significantly increased (EI_{max}: Before OP vs. PO5 week; 0.55±0.023 vs. 0.57±0.015; p=0.0015). The other calculated values didn't show notable changes.

In case of the red blood cell aggregation the results showed a significant decrease after the surgery in the loop and fistula groups. The sham-operated group values behaved irregularly after the surgery, and returned back to the original range in sham-operated and loop groups, except in the fistula group, where the values were significantly higher at the PO5 week. This was consistent with the hematological findings, because at the PO5 week in the fistula group the Hgb, RBC, Htc values also significantly increased.

Hemodynamic and microcirculatory parameters

When the SIEV was clamped down the mid arterial pressure suddenly elevated, then the removal of the clamp normalized the blood pressure. This showed the significant cardiovascular effect of the fistula.

These results showed that in the sham-operated group, as the rats grew, the microcirculatory values increased in both foot over the follow-up period, this increase was significant. In the loop group this kind of increase was missing from the non-operated foot, the fistula notably disturbed the microcirculation, because both foot microcirculation remained in lower levels. Both hind limbs in the fistula group had significantly lower values than of the other two groups.

These results were supported by the foot surface temperature results, as well. In the loop group the non-operated foot surface temperature was significantly lower than the other foot and it was also lower than the sham non-operated foot surface temperature (operated vs. non-operated: 32.22±0.7 °C vs. 30.65±1.35 °C, p=0.0009; sham vs. loop; 32.28±1.15 °C vs. 30.65±1.35 °C, p=0.0003). Because of the steal mechanism caused by the fistula we suspected that the surface temperature of the operated foot would be notably lower. The temperature measurement confirmed that the values were lower than on the other foot and the sham operated foot, as well (operated vs. non-operated: 30.57± 1.4 °C vs. 31.51± 1.03 °C, p= 0.0283; sham vs. fistula: 32.33±1.11 °C vs. 30.57± 1.4 °C, p<0.0001).

MAIN FINDINGS AND CONCLUSIONS

1. The experiments carried out on the biopreparates end *ex vivo* vessels showed by comparing the biomechanical properties of the simple interrupted, continuous and the modified ”Lauritzen”-type sleeve techniques, that the fastest is the sleeve method, however it shows the biggest pressure drop. The simple interrupted and the continuous suture showed no notable differences in the tensile-strength parameters. The simple interrupted suture is the most to able to restore the vessel original condition, with the least amount of morphological alterations. The needle and the thread handling with the correct knotting technique is what influences the tensile*strength the most in case of the suture number.
2. We successfully designed a rat model which is suitable to examine microvascular anastomoses’ regeneration, and the complex functional and morphological alterations in an arterio-venous fistula and in a loop shaped venous graft interposit.
3. The created arteriovenous fistula and the loop shaped venous graft interposit caused significant morphological, histological alterations in the operated vessels in the femoral region, however distal circulatory changes only occurred in the fistula group. The fistula and the loop created distinct areas with different wall shear-stress and pressure ratios, which were easily measurable with the 3D flow simulation and easily comparable with the histological findings. Intima hyperplasia occurred where the wall shear-stress and the pressure were present in an inverse relationship. Although tunica median enlarged only when both parameters were present and showed highly elevated values. The uneven flow pattern shown by the 3D flow simulation highly correlated with the tissue alteration found on the histological slides. These shows the technique precision and predictive capabilities
4. In case of the hemorheological and hematological parameters, the most notable changes were found in the loop group. These changes normalized by the end of the 5^{th} postoperative week, except in the fistula group where we experienced significant late alterations. The fistula, directly by the steal-mechanism and indirectly by the alterations of the hemorheological values, decreased the microcirculation.

DEBRECENI EGYETEM
EGYETEMI ÉS NEMZETI KÖNYVTÁR
H-4002 Debrecen, Egyetem tér 1, Pf.: 400
Tel.: 52/410-443, e-mail: publikaciok@lib.unideb.hu

Registry number: DEENK/371/2022.PL
Subject: PhD Publication List

Candidate: Balázs Szabó
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. **Szabó, B.**, Gasz, B., Fazekas, L., Varga, Á., Kiss-Pápai, L., Matolay, O., Rezsabek, Z., Al-Smadi, M. W., Németh, N.: Heterogeneous Maturation of Arterio-Venous Fistulas and Loop-Shaped Venous Interposition Grafts: a Histological and 3D Flow Simulation Comparison.
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DOI: http://dx.doi.org/10.3390/biomedicines10071508
IF: 4.757 (2021)

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DOI: http://dx.doi.org/10.1016/j.injury.2020.02.104
IF: 2.586

3. **Szabó, B.**, Tánczos, B., Varga, Á., Baráth, B., Ghanem, S., Rezsabek, Z., Al-Smadi, M. W., Németh, N.: Micro-rheological changes of red blood cells in the presence of an arterio-venous fistula or a loop-shaped venous graft in the rat.
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DOI: http://dx.doi.org/10.3389/fphys.2020.616528
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DEBRECENI EGYETEM
EGYETEMI ÉS NEMZETI KÖNYVTÁR
H-4002 Debrecen, Egyetem tér 1, Pf.: 400
Tel.: 52/410-443, e-mail: publikaciok@lib.unideb.hu

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Clin. Hemorheol. Microcirc. 71 (2), 225-236, 2019.
DOI: http://dx.doi.org/10.3233/CH-189414
IF: 1.741

Total IF of journals (all publications): 26,696
Total IF of journals (publications related to the dissertation): 11,909

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.

19 July, 2022

AGKNOWLEDGEMENT

During my studies, in the Department of Operative Techniques and Surgical Research, Faulty of Medicine, University of Debrecen, as a student, as a student research fellow, and as PhD fellow I received nothing but support to and encouragement. I had the opportunitiy to work independently and accomplish my ideas.

Special thanks to ***Prof. Dr. Norbert Németh***, who helped me since the beginning as the head of the department and more importantly as my supervisor. He supported me, guided me along the road and if I wondered away helped me with patience to find my way back to the right track. He greatly contributed to the growth of my surgical, clinical experience and helped me to achieve several scientific honors.

It is an honor that I had the opportunity to study from ***Prof Dr. István Furka (1935-2021)*** and ***Prof. Dr. Irén Mikó,*** who gave me countless advice.

I owe my thanks to ***Dr. Enikő Tóth***, who noticed me and my affinity to microsurgery, and set me off on this great journey.

I would like to thank the assistance of ***Dr. Ádám Deák,*** as the veterinarian of the university, gave me a huge amount of help and knowledge about animal research.

My greatest gratitude to ***Dr. Balázs Gasz and Dr. Levente Kiss-Pápai*** (University of Pécs), without their knowledge of 3D flow simulation, this scientific work would not have been possible.

The imaging techniques during my experiment are were made possible by ***Dr. György Trencsényi*** and ***Dr. Lajos Daróczi***, for that I am truly grateful.

I thank ***Dr. Orsolya Matolay*** from the Institute of Pathology, University of Debrecen. Her contributions allowed that this thesis can have such a great quality of histological images.

To my many colleagues, ***Bence Tánczos, Ádám Varga, Ádám Mátrai, Barbara Baráth,*** countless thank to their tireless laboratory work.

My student researcher, who helped me during my PhD studies deser special thanks: ***Dr. Zsófia Rezsabek, Dr. László Fazekas, Dr. Mohammad Walid Al-Smadi***.

I have great gratitude to ***Györgyné Gödény,*** who through all of those hard research days tenaciously stood beside me, and tirelessly helped a supported me with her colorful personality.

The quality images which are present in this thesis and in my published works are the work of ***Róbert Füzesi***, I would like to thank him for his kind help.

An absolute gratitude to my ***Wife***, who during the carrying of our unborn child showered me with countless support, encouragement, and helped me overcome the hardships to be able to finish this scientific work.

Finally, I send my sincere appreciation to all of the colleagues of this Department and everybody else who helped mi in any way to make this thesis.