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ORIGINAL ARTICLE

An old-new problem: The impact of intraoperative tissue desiccation on the regeneration of vascular anastomoses

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Introduction

Multiple factors contribute to achieving a safe and successful anastomosis, extending beyond proper technique, suture material, and appropriate anticoagulants.[1,2] Careful attention is also required for atraumatic tissue preparation and general cautery practices. The fragility of these structures demands meticulous precision and a thorough understanding of tissue handling.[1-4] A classic surgical dogma is to moisturise the surgical site with body temperature physiological saline solution. However, besides empirical observations, objective data on this topic remain limited, relying primarily on empirical observations.

Moisture plays a critical role in ensuring the preservation of tissue viability, improving visualisation, and reducing tissue trauma, particularly in delicate microvascular surgeries. The hydration status of tissues is pivotal during perioperative microvascular anastomoses, as drying can result in various adverse effects.[5-7] Cell damage from tissue desiccation may impair viability and raise the possibility of necrosis. Additionally, dehydration can induce vascular spasms, complicating the anastomotic process and potentially hindering postoperative blood flow. Dehydrated tissues become brittle and lose their elasticity, heightening the risk of accidental damage during suturing or manipulation. Dry tissues also impair visibility by causing blood and surgical rem-

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nants to adhere to the surgical site, which obscures the field and complicates the procedure. Furthermore, dehydrated vessels are more susceptible to endothelial injury, raising the risk of thrombus formation, postoperative thrombosis, and graft failure. Finally, compromised healing caused by perioperative dryness impacts cell migration, proliferation, and differentiation, which are vital processes for wound healing.[8-10] Proper tissue moisturisation not only aids in maintaining tissue viability but also facilitates the manipulation and suturing of delicate structures during microsurgical procedures.[11] Neglecting tissue moisturisation during surgery can lead to increased complications, such as prolonged healing times, higher rates of postoperative complications, and compromised surgical outcomes.[12]

However, there the literature is limited in providing objective data on the histological, biomechanical, fluid dynamic, and hemorheological aspects in this field. Our hypothesis posited that improper tissue handling and insufficient moisturization would hinder anastomosis healing. This study aimed to generate objective evidence on the impact of tissue desiccation, caused by inadequate moisturization during surgery, on anastomosis remodelling.

Materials and methods

Animals, experimental groups, drugs

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

Twenty-four male Wistar (CRL:WI) rats (body weight: 305.44 ± 21.97 g, Toxi-Coop Zrt., Budapest, Hungary) were involved in this study. The animals were housed in the department's conventional animal facility. The animals were weighed weekly during the study, and general anaesthesia was administered using a mixture of ketamine and xylazine (100 mg/kg; 10 mg/kg).[13,14] Heparin was given as prophylactic treatment for thrombosis (40 IU/kg) through the lateral tail vein. Preoperatively, the animals were randomly divided into three groups (benchmark (BG), moist (MG), and desiccation (DG)), according to the operative protocol. On the 21st postoperative day, the MG and DG animals were over-anaesthetised with intravenous Ketamine-Xylazine (25 mg/kg; 2.5 mg/kg). The animals in the BG group were euthanized after the anastomosis was performed, without follow-up; only tissue samples were removed for different studies. The femoral arteries from these animals were dissected immediately after suturing and used as freshly anastomosed blood vessels for the study.

Operative and sampling protocol

After the induction of anesthesia, the animal's inguinal region was shaved with an electric razor, and then a depilatory cream (Veet Pure, Reckitt Benckiser, England) was applied. After washing off the cream, Betadine was used for skin disinfection. The lateral tail vein was cannulated (26 G) for blood collection (0.5 ml; K3-EDTA, Vacutainer®, Becton Dickinson GmbH, Franklin Lakes, New Jersey, USA). The cannula was then rinsed with an equal volume of physiological saline for replenishment. After making a skin incision above the inguinal ligament and isolating the operation field with gauze pads, then the atraumatic preparation of the femoral artery began. We preserved the superficial inferior epigastric artery, vein, and nerve, which were pulled aside without causing damage. After the isolation of the femoral artery (1 mm ± 0.1 mm), we administered physiological saline diluted (Isotonic Saline Solution 0.9%; B. Braun Avitum Hungary; Hungary) heparin (HEPARIBENE; Teva Hungary; Hungary; 40 IU/kg) through the tail vein cannula. The application of a safety clip proximally followed by an approximator. Subsequently,

the vessel was transected halfway through the isolated area and washed with heparin solution (2500 IU/mL). For the 8-stitch end-to-end femoral anastomoses, we used 10/0 polyamide-6 with a serosa needle (DACLON, SMI, Belgium). In every instance, the anastomoses' efficacy was evaluated through the application of the "milking test".[15] The quality of the anastomoses was also assessed by measuring the blood flow (ml/min) before, after, and at the end of the follow-up using a special flow probe (T206, Transonic Systems Inc., USA) designed for this purpose. The skin incision was then sutured using a continuous matrace suture with a 4/0 reverse cutting needle, polyglycolide-poly (e-caprolactone) copolymer (Simfra, KOLLSUT, Hauppauge, NY, USA) suture material, without leaving knots outside the incision to prevent autophagy during the healing process. The animals underwent routine examinations after surgery to aid in their healing.

All the animals underwent the same surgical procedures, as stated above, and an ALPHA ENT-4A (Elektro-Optika, Hungary) microscope was utilised. In our MG group, special care was taken to keep the surgical site moist during the operation (31.3 ± 5.1 min); this was done with body-temperature physiological saline solution (Isotonic Saline Solution 0.9%; B. Braun Avitum Hungary; Hungary), while in our other DG group, extra care was taken to not wet. The epigastric vessels in the surgical area were wetted according to the groups after the retraction.

Laboratory tests

Pre-operatively, on days 7, 14, and 21 postoperatively, blood samples were taken. The hematological measurements were performed by a Sysmex K-4500 automate (TOA Medicor Electronics Co., Ltd., Tokyo, Minato City, Japan). In this study, red blood cell count (RBC [10^{12}/L]), white blood cell count (WBC [10^{9}/L]), hematocrit (Hct [%]), haemoglobin concentration (Hgb [g/L], MCH [pg], MCHC [pg]), mean corpuscular volume (MCV [fL]), and platelet count (Plt [10^{9}/L]) were analysed.

A LoRRca MaxSis Osmoscan ektacytometer was used to assess erythrocyte deformability (RR Mechatronics International BV, Zwaag, The Netherlands).[16–18] Polyvinyl-pyrrolidone (PVP) and the phosphate-buffered saline (PBS) solution (viscosity: 40.6-43.0 mPas, osmolarity: 291-303 mOsm/kg, pH: 7.0-7.3) were diluted with 10 μL of blood. The deformability of the RBCs was examined by measuring the elongation index (EI) of the cells under increasing shear stress (SS [Pa]). For the parameterisation of EI-SS curves, the Lineweaver–Burk equation was used to express the maximal elongation index (EI_{max}) and the shear stress at half EI_{max} ($SS_{1/2}$ [Pa]).[18]

Red blood cell aggregation was tested with the method based on light transmission (Myrenne MA-1 aggregometer, Myrenne GmbH, Roetgen, Germany). Four aggregation parameters were determined using a modest sample size (20 μL/measurement): aggregation M index values under stasis (M 5 s and M 10 s; 5 and 10 secundum after stasis), and M1 values at 3 s^{-1} shear rate (M1 5 s, M1 10 s; 5 and 10 secundum after the application of the low shear rate condition, which roughly corresponds to physiological blood flow patterns).[16–18]

Tensile strength measurements

Before the termination, the anastomosed and the contralateral intact arteries were taken out for the tensile strength tests. We used a tensile strength measuring device developed in collaboration with our department, which consists of a load cell and a motor to provide tensile force.[16] In all cases, the distance between the jaws fixing the arteries was uniform (L_0=8 mm), and the anastomoses were always placed in the middle. During the measurements, the tensile force was increased at a steady, constant rate of 4.78 steps per second (1 step: 0.04 mm) until the moment of rupture. The measured data were exported to CSV, processed, and finally plotted in newtons as a function of the steps. The tensile strength curves were also analysed for the slope of the curves, which is related to the rigidity and maturation processes of the materials. The curves were examined between 0.001 Newton and the maximum point of the curve.[19,20]

Histomorphological investigations

Histopathological examination was also performed in our experiments, where we tested the morphology with different staining methods. We investigated the changes in the thickness of the layers of the arterial walls in hematoxylin-eosin-stained sections

The torn femoral arteries and the intact epigastric vessels were preserved in 4% paraformaldehyde until additional histological analyses.

The vessels underwent a physiological saline wash and were subsequently fixed in a 4:1 mixture of 40% formaldehyde and absolute ethanol before being embedded in paraffin. Serial sections were prepared (5 µm each), and morphological examination was done using hematoxylin-eosin staining (H&E, Sigma-Aldrich, St. Louis, MO, USA) and elastin visualization using orcein staining (Sigma-Aldrich, St. Louis, MO, USA). The staining procedures were followed in accordance with the manufacturer's guidelines. A DP74 camera (Olympus Corporation, Tokyo, Japan) was used to capture photomicrographs using an Olympus Bx53 microscope (Olympus Corporation, Tokyo, Japan). The collagen fiber orientation in the vasculature was examined using Picrosirius red staining (Sigma-Aldrich, MO, USA). Under polarized light, samples were examined using an Olympus Bx53 polarization microscope (Olympus Corporation, Tokyo, Japan) by turning the light plane with $\lambda/4$.[17]

ImageJ 1.40 g freeware was used to measure the thickness of the tunica intima, media, and adventitia on H&E-stained micrographs at 20 $\times$ magnification with perpendicular lines between the borders. For the tunica media, the area between the membrana elastica interna and externa was measured, while for the other two layers, the thickness beyond these membranes was measured. One section each of intact and anastomosed femoral arteries and intact epigastric vessels from an animal was prepared, and twenty independent measurements were made on these sections (17).

Statistical analysis

For estimating the necessary sample size (animals per group), Mead's resource equation method was used.[21] GraphPad Prism 9 software was used for statistical analysis. Normality was checked for all data distributions, and accordingly, Student t-test or non-parametric tests (Wilcoxon or Mann-Whitney rank sum tests), and two-way/repeated measures ANOVA tests were used. The significance level was set at $P < 0.05$.

Results

General observations

During surgical procedures, we observed increased difficulty in manipulating tissues that were not kept moist. In the DG group, there was an increased amount of denser connective tissue in the surgical site on day 21, as well as a thrombosis in one artery and an aneurysm in another (Fig. 1). The epigastric vessels were unaffected by the cutting and stitching injury, but the tissue portion containing them was the most exposed to DG. Animals showed a similar variation in weight during the follow-up period, however, the DG group had much higher standard deviations, and weight gain was also lower in comparison to the MG (14±6 % vs 16±3 %) group.

All anastomoses were successfully performed in both groups, as demonstrated by the arterial flow rates before and after surgery (Fig 2A). On day 21, no significant difference was found between the two groups or between the two sides, but a slightly increased blood flow was observed in the DG group (Fig. 2B).

The skin temperature measured on the feet and abdomen of the animals did not change significantly during the follow up period.

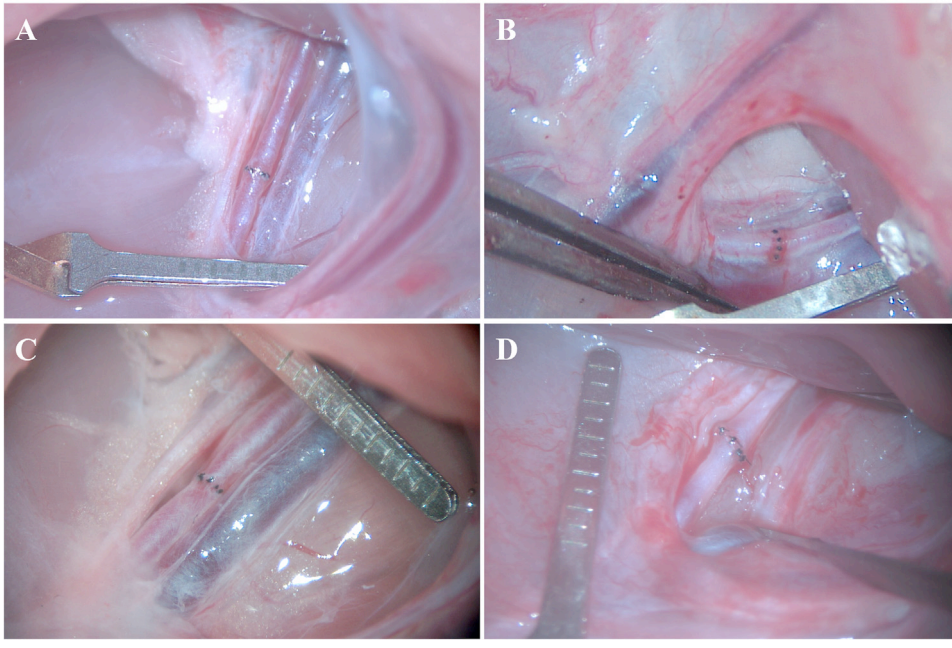

Fig. 1. Representative operative pictures in moistured (A, B) and in the desiccated groups (C, D) after anastomosis (A, C) and on the 21st day (B, D). (original magnification: 16 ×).

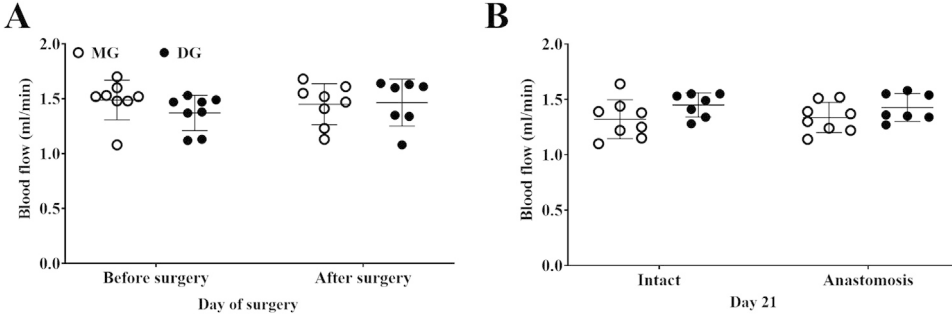

Fig. 2. Arterial blood flow in moistured and desiccated before and after surgery (A) and on the 21**st** day (B). The data are presented as the means ± SD.

Laboratory parameters

Hematological parameters showed nearly similar differences. There were no significant differences in the white blood cell count during the follow-up period. The red blood cell count in the second week in both groups showed a significant increase (MG $P = 0.0169$; DG $P = 0.0018$), and in the third week, only MG showed a difference ($P = 0.0418$). Hematocrit showed a significant increase ($P = 0.0030$) on week 2 in the DG group compared to the MG. In the hemoglobin concentration, the only significant difference happened in the first week in the MG group ($P = 0.0220$). The platelet count showed a slight increase in both groups, most notably in the MG, which caused a significant ($P = 0.0007$) difference in the first week in the DG group compared to the MG (Table 1).

Table 1
Alterations of selected hematological parameters.

Variable	Group	Base	7th p.o. day	14th p.o. day	21st p.o. day
WBC [10^9/L]	MG	10.6 ± 1.68	12.71±2.13	12.61±3.275	12.08±3.75
	DG	10.27 ± 2.31	10.51 ± 1.17	10.414 ± 1.820	11.08 ± 1.31
RBC [10^{12}/L]	MG	7.95 ± 0.34	7.66 ± 0.7	8.31 ± 0.37*	8.27 ± 0.34*
	DG	7.73 ± 0.3	7.89 ± 0.22	8.17 ± 0.25*	8.26 ± 0.6
Plt [10^9/L]	MG	796.83 ± 156.51	1012.9 ± 88.25	903.2 ± 169.66	857.25 ± 54.46
	DG	663.94 ± 186.33	756.33 ± 161.73#	818.08 ± 60.11	776.79 ± 159.54
Hct [%]	MG	44.87 ± 1.93	43.41 ± 4.82	46.21 ± 2.08	46.37 ± 1.76
	DG	44.57 ± 1.81	45.06 ± 1.63	46.34 ± 1.58*	45.65 ± 2.36
Hbg [g/L]	MG	15.35 ± 0.55	14.59 ± 0.94	15.22 ± 0.77	15.06 ± 0.57
	DG	15.03 ± 0.61	15.09 ± 0.31	15.38 ± 0.57	14.85 ± 0.49

Means ± S.D.; *$P < 0.05$ vs. base. #$P < 0.05$ vs. MG.
(white blood cell count - WBC; red blood cell count - RBC; hematocrit - Hct; haemoglobin concentration - Hgb; mean corpuscular volume - MCV; platelet count - Plt) in the MG and DG groups during the follow-up period.

Table 2
Changes of red blood cell deformability (EI_{max} and $SS_{1/2}$) and red blood cell aggregation parameters (aggregation indices M 5 s. M 10 s. M1 5 s and M1 10 s) in the MG and DG groups during the follow-up period.

Variable	Group	Base	7th p.o. day	14th p.o. day	21st p.o. day
EI at 3 Pa	MG	0.335 ± 0.025	0.336 ± 0.019	0.350 ± 0.027	0.364 ± 0.01*,#
	DG	0.349 ± 0.016	0.323 ± 0.044	0.327 ± 0.031	0.343 ± 0.03
EI_{max}	MG	0.549 ± 0.027	0.533 ± 0.023	0.570 ± 0.025	0.555 ± 0.023
	DG	0.573 ± 0.019#	0.554 ± 0.031	0.564 ± 0.023	0.567 ± 0.019
$SS_{1/2}$ [Pa]	MG	2.234 ± 0.208	2.158 ± 0.233	2.025 ± 0.326	2.158 ± 0.154
	DG	2.122 ± 0.268	2.477 ± 0.365*	2.335 ± 0.441	2.223 ± 0.298
$EI_{max}/SS_{1/2}$ [Pa^{-1}]	MG	0.254 ± 0.019	0.250 ± 0.029	0.273 ± 0.039	0.254 ± 0.019
	DG	0.273 ± 0.020	0.219 ± 0.026*,#	0.238 ± 0.039	0.265 ± 0.04
M 5s	MG	2.35 ± 1.07	2.11 ± 0.94	2.37 ± 0.99	2.54 ± 1.1
	DG	2.51 ± 0.92	2.86 ± 0.85#	3.18 ± 1.93	2.74 ± 1.32
M 10s	MG	7.49 ± 3.21	9.09 ± 2.91	8.13 ± 2.92	7.97 ± 3.84
	DG	8.36 ± 2.74	7.13 ± 2.34#	6.29 ± 3.92	7.73 ± 3.71
M1 5s	MG	2.79 ± 1.33	2.46 ± 0.85	2.52 ± 0.95	2.62 ± 0.91
	DG	2.86 ± 1.07	3.27 ± 0.76#	2.56 ± 1.45	2.78 ± 1.04
M1 10s	MG	6.62 ± 2.79	6.91 ± 3.19	7.58 ± 3.25	7.71 ± 2.93
	DG	7.22 ± 1.86	8.23 ± 3.01	5.58 ± 4.2	8.23 ± 3.66

Means ± S.D.; *$p < 0.05$ vs. base. #$p < 0.05$ vs. MG.

We also examined the deformability parameter of the red blood cells. The deformability of the red blood cells can be characterized by the elongation index, plotted as a function of the corresponding shear stress. The curves of the DG group showed the greatest decrease in the first week compared to the base, indicating a deterioration of their deformability. Interestingly, in the MG group, their curves showed an increase after the operation. The analyzed data are shown in Table 2. The EI 3 Pa showed a decrease in the groups after the operation but on the third week, the values increased, most importantly in the MG group ($P = 0.0061$ vs base). There were no significant differences in the EI_{max} and $SS_{1/2}$ values between the groups. The $EI_{max}/SS_{1/2}$ showed a significant decrease in the DG group on the first week compared to the base ($P = 0.0014$) and the MG ($P = 0.0471$) (Table 2).

The degree of red blood cell aggregation is expressed by the aggregation index. The M 5 s and M1 5 s index parameters increased in the DG group after the operation and were significant on the first week compared to the MG (M 5 s $P = 0.0190$; M1 5 s $P = 0.0026$) and then normalized until the third week. In the M 10 s parameter, the MG group after the operation showed an increase, while the DG group showed a decrease in the values. This caused a significant difference between the two groups on the first week ($P = 0.0423$) but by the third week, they normalized. The M1 10 s parameter only showed small, non-significant changes (Table 2).

Table 3
Numerical analysis of the H&E-stained femoral arteries.

Variable	Group	Intact	Anastomosis
Full layer thickness [μm]	BG	140.59 ± 18.03	132.31 ± 35.2
	MG	142.37 ± 17.39	154.28 ± 43.05
	DG	147.46 ± 5.78	151.1 ± 19.12
Tunica intima thickness [μm]	BG	3.5 ± 0.76	3.66 ± 0.63
	MG	3.21 ± 0.35	3.95 ± 0.68
	DG	2.74 ± 0.53	3.03 ± 0.86
Tunica media thickness [μm]	BG	65.64 ± 9.36	56.03 ± 9.59
	MG	66.31 ± 11.84	67.12 ± 9.75
	DG	61.1 ± 6.48	76.35 ± 11.5*
Tunica adventitia thickness [μm]	BG	75.42 ± 13.97	71.63 ± 7.4
	MG	74.59 ± 5.67	78.13 ± 17.06
	DG	80.56 ± 14.31	76.54 ± 12.45

Means ± S.D.; *$P < 0.05$ vs. BG;.

Tensile strength measurements

Stress-strain diagrams were obtained from the tensile strength measurements (Fig. 3A), which were also numerically analysed (Fig. 3B-E). The stress-strain curves of the arteries are similar to elastomers, which was also observed in our previous study.[16] The analysis determined the maximum tensile strength (Fig. 3B), for which the anastomoses of both groups were found to be significantly weaker compared to the intact contralateral artery (BG $P = 0.0146$; MG $P < 0.0001$; DG $P = 0.0006$). There were no significant differences between the groups, but the strongest were the freshly made BG arteries and the weakest were the MG arteries.

The slope of the curves was examined over the entire section (0.001N- maximum point; Fig. 3C) and separately over the 0-33% (Fig. 3D) and 33-100% (Fig. 3E) areas of the entire section. For the full curve, the BG group had the lowest value compared to its own contralateral artery ($P = 0.0024$), both in the initial part of the curve (0-33%) ($P = 0.0138$ vs own base) and in the terminal part of the curve (33-100%) ($P = 0.0138$ vs own base). For the DG and MG groups, the slopes were not significantly reduced in the full and final curve sections compared to the intact opposite vessel. However, an increase was observed at the initial stage compared to the opposite intact vessel for the DG group, which was found to be a significant ($P = 0.0175$) difference compared to the BG group (Fig. 5).

Histomorphology

There was no significant difference between anastomoses for full wall thickness and tunica adventitia thickness (Fig. 4A, B, Table 3). However, intimal thickness was not increased in the DG group alone. Tunica media thickness increased primarily in the DG group ($P = 0.0127$ vs BG) (Table 3).

Besides the layer thickness of the arterial wall, a general morphological analysis has been done. The endothelial lining was identifiable in both the control and desiccation groups without any pathological disorders. In the tunica media of the desiccation group, more swollen cells appeared and the overall number of cells increased compared to the control group. The tunica adventitia contains a slightly more condensed collagen matrix and a bit denser tissue, and it is likely that the fibroblast number also increased in the desiccation group. On the other hand, the staining intensity was higher in the tunica adventitia of the desiccation group. This stronger eosinophilic appearance may represent enhanced protein expression.

In the epigastric artery, only the tunica intima showed a significant increase in both MG ($P = 0.0073$) and DG group ($P = 0.073$) compared to the intact vessel. In contrast, for the epi-

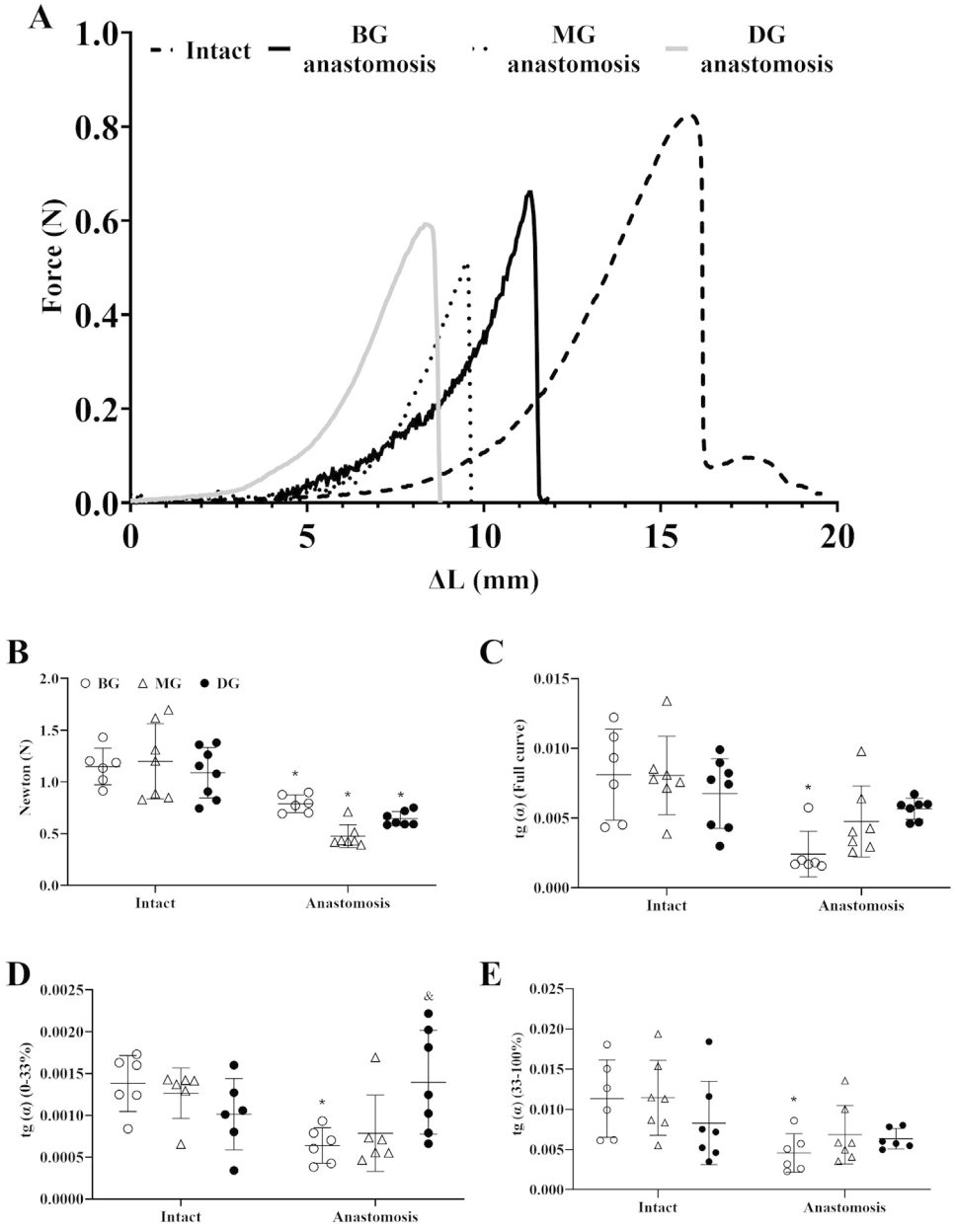

Fig. 3. Representative tensile strength measurement stress-strain curves of differently-treated arteries. (L_0 = 8 mm; motor speed: 1.95 mm/s) (A). Numerical analysis of the stress-strain curves of differently-treated arteries (B-E). B: maximum tensile strength; slope of the tensile strength in the region of the 0.001 N to the maximum point (C); in the region of the 33-100% of the curves (D); in the region of the 33-100% of the curves (E). Means ± *S*.D.; $^{*}P < 0.05$ vs. base; $^{\#}P < 0.05$ vs. Control; $^{\&}P < 0.05$ vs. Benchmark; (L_0 = 8 mm; motor speed: 1.95 mm/s).

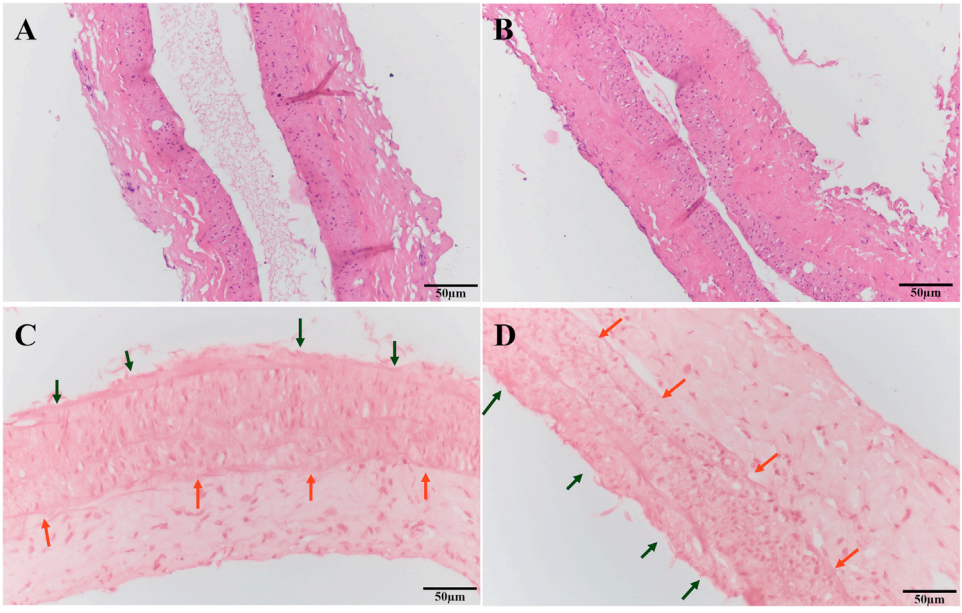

Fig. 4. Representative photos of H&E (A, B) and orcein (C, D) stained histological slides in moistured (A, C) and desiccated groups (B, D). Black arrows: membrana elastica interna, red: membrana elastica externa. (original magnification: 20 ×).

Table 4
Numerical analysis of the H&E-stained epigastric vessels.

Variable	Group	Intact	Anastomosis
Full layer thickness of the artery [μm]	MG	85.31 ± 22.15	97.53 ± 24.59
	DG	103.52 ± 22.38	94.95 ± 11.78
Tunica intima thickness of the artery [μm]	MG	2.42 ± 0.54	2.72 ± 0.54*
	DG	2.3 ± 0.42	3.11 ± 0.49*
Tunica media thickness of the artery [μm]	MG	41.12 ± 10.43	46.1 ± 13.72
	DG	47.6 ± 8.75	43.38 ± 8.67
Tunica adventitia thickness of the artery [μm]	MG	41.77 ± 15.16	48.72 ± 15.37
	DG	53.63 ± 14.25	49.34 ± 6.72
Full layer thickness of the vein [μm]	MG	74.78 ± 9.87	84.72 ± 15.19
	DG	84.83 ± 20.76	103.22 ± 31.2
Tunica intima thickness of the vein [μm]	MG	2.97 ± 0.51	3.11 ± 0.72
	DG	2.63 ± 0.28	2.88 ± 0.53
Tunica media thickness of the vein [μm]	MG	20.99 ± 3.57	22.67 ± 2.81
	DG	21.89 ± 3.95	27.12 ± 4.81
Tunica adventitia thickness of the vein [μm]	MG	50.01 ± 6.86	58.57 ± 12.98
	DG	55.49 ± 11.87	75.3 ± 26.35

Means ± S.D.; *$P < 0.05$ vs. base.

gastric vein, a non-significant thickening of the tunica media, adventitia, and total thickness was observed in the DG group (Table 4).

Picrosirius-stained sections in red show non-specific collagen staining. Rotating the plane of the polarized light by lambda/4 (birefringence structures represent collagen fibers, where shiny red structures are thicker while green structures reveal thinner collagen fibers). Both red (thick fibers) and green (thin fibers) light intensity showed an increase in the DG group compared to the intact arteries, and in the red light intensity, we observed a significant ($P = 0.0226$) increase compared to the BG anastomoses (Fig. 5, Table 5).

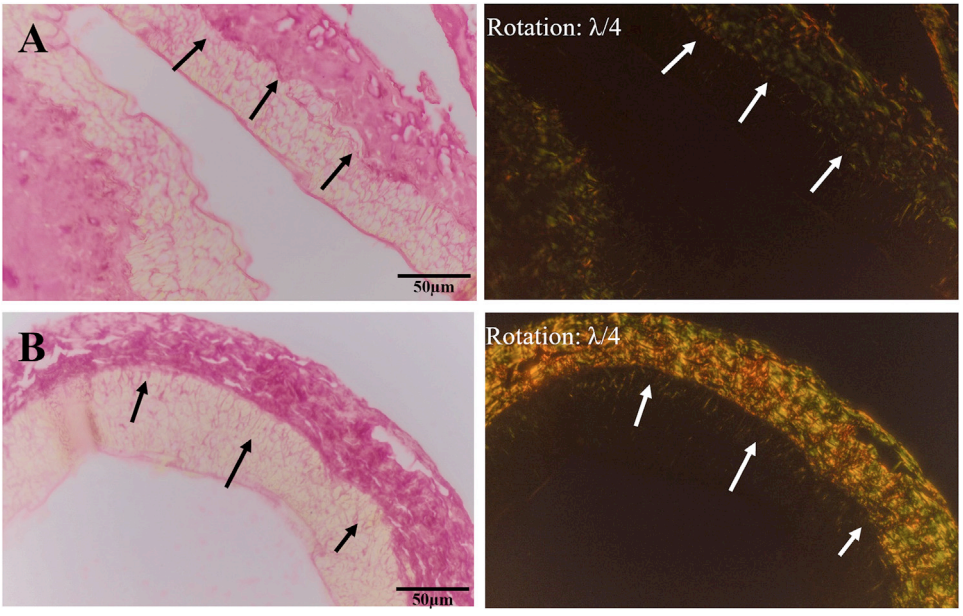

Fig. 5. Representative picrosirius-stained slides on the anastomosed moistured (A) and desiccated (B) arteries in the experimental groups. Black and white arrows are in the same position, pointing toward the tunica *m*edia. (original magnification: 20 ×).

Table 5
Numerical analysis of the picrosirius-stained femoral arteries.

Variable	Group	Intact	Anastomosis
Red light intensity [PN]	BG	578.54 ± 59.63	651.98 ± 246.68
	MG	725.6 ± 74.33	785.81 ± 304.96
	DG	623.34 ± 177.03	1361.48 ± 539.29*
Green light intensity [PN]	BG	292.81 ± 26.92	312.59 ± 129.49
	MG	319.92 ± 110.44	419.6 ± 140.2
	DG	404.78 ± 180.31	497.43 ± 67.35

Means ± *S.D.*; $^{*}P < 0.05$ vs. BG.

In sections stained with orcein, we examined the amount of elastic fibers and their arrangement into lamellae (Fig. 4C and D, Table 6). For femoral arteries, we did not see clear differences in the amount of elastic fibers and lamellae. In the epigastric artery, however, a non-significant increase was observed in the MG group, which was not observed in the DG group. Another small decrease was observed for the laminar quantity in the DG group (Table 6).

Discussion

In addition to clinical patient care, microsurgical research requires specific skills to ensure that research into vascular suture healing is free from technical bias. The learning of microsurgical anastomosis is a complex, gradually evolving process, for which it is essential to provide an appropriate practice model and a structured teaching environment. The amount of practice time and the reduction of technical errors are key factors for the steepness of the learning curve and the success of anastomosis. A biological, non-living training model consisting of cryopreserved cadaveric rat arterial models is cost-effective, ethically sustainable, and excellent for de-

Table 6
Numerical analysis of the orcein-stained femoral and epigastric arteries.

Variable	Group	Intact	Anastomosis
Integrated green light density of the femoral arteries [PN]	BG	148041.9 ± 14321.8	156312.43 ± 13557.55
	MG	164909.09 ± 24347.49	163000.28 ± 14676.05
	DG	171133.97 ± 19677.77	171628.83 ± 19031.97
Number of elastic membranes of the femoral arteries	BG	3.25 ± 0.96	3 ± 1
	MG	3.13 ± 1.36	2.75 ± 0.96
	DG	3 ± 1.22	2.83 ± 1.33
Integrated green light density of the epigastric arteries [PN]	MG	62073.42 ± 19461.56	107936.74 ± 11233.76
	DG	78402.12 ± 36852.39	71562.8 ± 44341.04
Number of elastic membranes of the epigastric arteries	MG	1.29 ± 0.76	1.2 ± 0.45
	DG	1.11 ± 1.05	0.75 ± 0.46

Means ± *S.D.*

veloping the manual skills of novice surgeons. Continuing the training on an in vivo animal model offers more lifelike tissue conditions and physiological responses, which aids preparation for subsequent clinical application, but comes with higher ethical, technical and financial requirements. The two models support the development of microsurgical competence in a complementary way: the biological, non-living model is ideal for safe technique acquisition in the early phase, while the living model allows for the practice of more subtle, dynamic situations in the later phase. an appropriate teaching structure and graded difficulty tasks, microsurgical skills can be developed to a significant extent, regardless of the initial expertise or specialization. With an appropriate teaching structure and graded tasks, microsurgical skills can be developed to a significant extent, regardless of the initial expertise or specialization, which should precede any research and clinical intervention.[22-24]

Most research on wound healing disorders highlights the importance of local as well as systemic influences such as inflammation, associated underlying diseases, diabetes, hypertension, cancer, oxygenation, age, etc..[25] In general, local tissue wetting as a variable factor was not found among the parameters studied in relation to wound healing disorders. Where it has, the research has been mostly in the field of dermatology and pharmacology, where various hydrogels and other local wound healing modalities have been investigated.[26] These studies also highlight and support the importance of tissue hydration in relation to wound healing.

Dehydrated or poorly moisturized tissues can become less pliable, fragile, and more prone to injury, leading to increased tissue trauma, reduced vessel patency, and compromised surgical outcomes. The precise nature of microsurgery necessitates an optimal surgical field to ensure the highest levels of accuracy and success, with adequate moisturization of the surgical site playing a crucial role in achieving this clarity and success.[6,27,28] In microvascular anastomosis, maintaining moisture at the vessel ends can provide easier manipulation and decrease the likelihood of vessel spasm, thrombosis, aneurysm, and eventual failure of the anastomosis.

Our research is based on the basic surgical dogma that tissue drying can be associated with several unwanted complications, such as wound healing disorders, connective tissue adhesions, and suture failure. In such cases, wounds may reopen, become infected, and may be associated with increased pain and secondary wound healing of connective tissue.[29] In abdominal surgery, fistulas, perforation, adhesions, and mechanical ileus, among others, may develop, and therefore it is of paramount importance to keep the surgical site moist during surgery.[30]

We aimed to support and clarify this empirical observation with objective data. We did this by making microsurgical vascular anastomoses since the healing of blood vessels and animal studies meant good reproducibility, speed, and standardization.[31] Wound healing is a complex process involving inflammation, revascularization, and connective tissue remodeling in the body.[34,35] Therefore, we selected parameters that can adequately represent and give us well-tracked feedback on the healing process as well as on the lesions that appear in the dried tis-

sues. These were therefore hematological, hemorheological, histological, tensile strength, and, of course, macroscopic observations.[17,25,36]

Macroscopically, we observed that anastomoses and instrumental manipulation were significantly more difficult to perform during surgery in the DG group. The tissues became paler by the end of the surgery and lost their slight shiny surface that is revealed by microscope light. When we examined the surgical area after the animals were terminated on the third week, we found that the healed area was more adhesive, which made it difficult to clean the blood vessels again. Most importantly, during reoperation, aneurysms and thrombosis were found only in the DG group. These results are consistent with the literature, where mainly the intestines, peritoneum, and cornea were affected.[5,32,33,37]

In our study, the stitched arteries always ruptured at the anastomosis as the weakest point, without rupture or disbanding of the sutures. Of the anastomoses, the freshly stitched vessels were found to be the strongest, but had the lowest slope of the curves. Since maturation was not yet possible, the only connection was formed by the sutures, which caused the cut in and tearing of the arterial wall, resulting in an elongated curve. In the DG group, the tensile strength was slightly higher compared to the MG, which may be related to the thickened tunica media and the higher amount of collagen fibers. For the slope of the curves, a steeper slope was observed in the first third of the curve section than in the DG group, which would be mainly related to the elastic fibers. Although there was no clear difference in the amount of elastin produced, the increased collagen could have had an effect. These findings align with existing literature, which reports an improvement in mechanical properties after the inflammatory phase (first days) of anastomoses.[17,38]

Examining hematological and hemorheological parameters allowed us to quantitatively element the systemic effects of a possible inflammation.[39] Here, our experience showed that white blood cell count and red blood cell count were almost similar in the postoperative period in both groups. The lack of increase in white blood cell count is not proportional to the extent of trauma and limited inferences can be made about the extent of trauma in the DG group.[40]

Acute phase reactions following surgical trauma and the fact that white blood cells accumulate locally during sterile inflammation may also have influenced the change in parameters.[41,42] As well as in the MG group, the capillary suction effect of the gauze in the surgical site, which was kept permanently moist, may have influenced the degree of white blood cell change.

When red blood cells were examined, we observed a worsening in the deformability and aggregation in the DG group. From these parameters, we can conclude that postoperative inflammation and possibly changes in flow parameters were more significant in the DG group.[43]

Scars are made up of the same extracellular matrix (ECM) molecules as the tissue they replace, but the proportions in the scar tissue are significantly different from normal tissue. The levels of collagen I and III, fibronectin, and laminin in scars are all increased. Collagen synthesis in keloids is 20 times greater than in normal intact skin and three times greater than in hypertrophic scars. Not only is collagen production high in hypertrophic scars and keloids, but the proportion of type I and type III collagen is also high. This overproduction of collagen and the change in ratios can be attributed to a stronger proliferative activity of keloid fibroblasts and higher type I and type III collagen production by fibroblasts.[44] This was supported by the histological results of our study.

Histopathology was used to examine the anastomosed vessels, where we observed that the intimal thickening present as part of normal wound healing was absent in the dried group. We further found that the amount of type I collagen was significantly increased in the dried group, while the number of elastic membranes decreased. The elastic property of blood vessels is very important, as one of the pillars of arterial flow is the pulsatile dilation and contraction of blood vessels, the lack of which may also contribute to an increased impairment of normal vessel function.[45] Without adequate blood, and therefore oxygen and nutrient supply, wound healing is generally said to be impeded, which is also true for the healing of the blood vessels' tissues.

In our study, we also examined epigastric vessels exposed to desiccation without trauma caused by the cut and manipulation. We saw a significant increase in the epigastric arteries intimal thickness in both groups, with a greater increase in the DG group. Since the epigastric

vessels originate lateral to the femoral anastomosis, ischaemia-reperfusion effects may have influenced the data in addition to desiccation.[46] The epigastric vein, which was only affected by desiccation, showed a non-significant thickening in the tunica media and adventitia, moreover, the amount of elastic membranes also showed a decreasing tendency. These changes may also have influenced the hemorheological parameters and called for careful wetting of the entire surgical site.

Conclusion

Tissue desiccation during surgery significantly affected the regeneration of vascular anastomoses. Inflammatory reactions, altered tensile strength curves, and histomorphological changes indicated disturbed maturation. While the instruments, surgical techniques, and surgeon's skills are integral to the success of microsurgery, maintaining adequate hydration of the surgical site is equally important. Especially in microvascular surgeries, where the margin for error is minuscule, ensuring the surgical field remains optimally moisturized can be the difference between a successful outcome and post-operative complications. The objective data that was provided served to support the classical doctrine. Further research is needed to optimize the rate of moisturization and the length of time between each moisturization in order to define the reversible/irreversible limit of tissue desiccation.

Limitations to the study

This study has several limitations. The sample size per group was relatively small ($n = 8$), potentially underpowering subgroup analyses, despite the prior use of Mead's resource equation. The moistening protocol lacked standardization in terms of frequency and volume, making reproducibility difficult. Additionally, the benchmark group was not subjected to longitudinal follow-up, restricting comparative analysis of the laboratory parameters. The study could have included a group where femoral vessels were desiccated intact (without anastomosis) and then followed up.

Author contributions

Conceptualization and methodology, L.Á.F., T.J. and N.N.; microsurgical operations, L.Á.F., D.H. and T.L.D.L; investigation and data analysis, L.Á.F., D.H., T.L.D.L., A.V., A.A.M., C.F., M.W.A. and N.N.; histological investigations, L.Á.F., C.F., A.T. and T.J.; writing—original draft, L.Á.F., T.J., and N.N.; writing—review and editing, L.Á.F, M.W.A., M.Q.A. and N.N.; supervision and funding acquisition, N.N. All authors have read and agreed to the published version of the manuscript.

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