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Surface enlargement of a new arterialised venous flap by the surgical delay method

Povećanje površine novog arterijalizovanog venskog režnja metodom hirurškog odlaganja

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Abstract

Background/Aim. The delay method is a surgical, pharmacological and combined method that includes two or more time separated phases, which gives bigger flap surface. In our research we explored the possibility of flap surface enlargement in a new arterialised venous flap (AVF) on an experimental rabbit ear model by the delay surgical method. The aim of this research was to establish vitality surface of our AVF and to maintain the difference in flap vital surface between AVF flaps, with or without performing the delay surgery method. **Methods.** We used both ears of "Big Chinchilla" rabbits in 10 experimental male animals, divided into two groups, average weight 3–3.5 kg, and average age 8–10 months. In the first (experimental) group, a venous flap was arterialised by our method. In the second (control) group, the venous flap was arterialised 14 days after the delay surgical method. AVF surface was measured on the 1 and 14 days by the method of trapezoid rule. **Results.** Vital surface on our AVF experimental model was bigger than 87% of elevated flap surface after the delay surgical method. Vital surface on AVF without delay on our experimental model was bigger than 30% of elevated flap surface ($p < 0.001$). **Conclusion.** Analysis of previous experimental models on the rabbit ear, non-delayed and delayed (to enlarge flap surface) led us to conclusion that previously created experimental models of non-delayed AVF are hemodynamically negative. Our experimental non-delay AVF model is hemodynamically more positive than previously created models of non-delay AVF and provides better conditions for AVF survival and enlargement of vital flap surface of elevated flap. On the other hand, surgical delay method significantly enlarges vital surface of AFF.

Key words:
surgical flaps; reconstructive surgical procedures; methods; rabbits.

Apstrakt

Uvod/Cilj. Metoda odlaganja je hirurška, farmakološka i kombinovana metoda koja obuhvata dve ili više vremenski razdvojenih faza, što daje veću površinu režnju. U istraživanju je ispitivano povećanje površine novog arterijalizovanog venskog režnja (AVR) na eksperimentalnom modelu uha kunića metodom hirurškog odlaganja. Cilj rada bio je da se utvrdi vitalna površina našeg AVR i utvrdi da li postoji razlika u veličini vitalne površine AVR sa ili bez primene metode hirurškog odlaganja. **Metode.** U eksperimentalnom istraživanju upotrebljena su oba uha kunića rase *Big Chinchila* u 10 eksperimentalnih životinja muškog pola, težine od 3–3,5 kg, starosti 8–12 meseci podeljenih u dve grupe. U prvoj (eksperimentalnoj) grupi, arterijalizovan je venski režanj po našoj metodi, a u drugoj (kontrolnoj) grupi arterijalizovan je venski režanj nakon 14 dana od metode hirurškog odlaganja. Površina AVR određivana je 1. i 14. dana metodom trapezoidnog pravila. **Rezultati.** Vitalna površina AVR na našem eksperimentalnom modelu nakon metode hirurškog odlaganja bila je veća od 87% površine odignutog režnja. Vitalna površina AVR koja nije odložena na našem eksperimentalnom modelu iznosila je više od 30% površine odignutog režnja. ($p <$ 0,001). **Zaključak.** Analizom dosadašnjih eksperimentalnih modela AVR na uhu kunića, neodloženih i onih kod kojih je radi povećanja vitalne površine primenjena metoda odlaganja režnja, utvrdili smo da su dosadašnji kreirani modeli neodloženih AVR hemodinamski nepovoljniji. Naš eksperimentalni model neodloženog AVR je hemodinamski povoljniji od prethodno kreiranih modela neodloženog AVR i omogućava povoljnije uslove za preživljavanje AVR i povećanje vitalne površine odignutog režnja. S druge strane, metodahirurškog odlaganja značajno povećava vitalnu površinu AVR

Ključne reči:
režnjevi, hirurški; hirurgija, rekonstruktivna, procedure; metodi; zečevi.

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Introduction

Delay flap methods have been used for many centuries in various models, but remained not completely pathophisiologically explained until today. The term "delay phenomenon" was introduced by Blair [1] in 1912 for decryption of preliminary stages of flap elevation, and is used until today. In the 16th century Gaspare Tagliacozzi delayed brachial flap, making parallel incisions in skin and subcutaneous tissue for nose reconstruction [2].

The delay method is a surgical, pharmacological and combined method that includes two or more time separated phases, which gives bigger flap surface.

In the first phase, the skin and subcutaneous tissue are cut *via* marked lines of a planed flap. The aim of delay in this phase is to interrupt peripheral circulation, for making independent orientation of blood vessels in the part of connected tissue which was a flap basis.

In the second phase, after 10 to 14 days, the flap is completely divided (except flap basis) from undersurface and moved into defect. This is an optimal period in the surgical delay flap method, confirmed by Cheng et al. [3], Kelly et al. [4], Kushima et al. [5] and used in this research, too.

Arterialized vein flap (AVF) delay was demonstrated for the first time by Nakayama et al. [6] in 1981, in epigastric skin flap in rats. The results were confirmed in a similar study by Voukidis [7].

Another researches were directed to arterialisations of venous flap without previous delay (so-called acute arterialization of venous vessel space) [8]. Nichter and Haines [9] developed experimental AVF model on rabbit ear, making arteriovenous communication side-to-side, between the artery and vein, preserving both marginal vein for drainage.

Usage of central vein path and preservation of symmetrical marginal drainage veins increases survival rate of an AVF [10–12].

To clear up some of dilemmas, we studied that situation, discussing the delay surgical method in AVF. The aim of this study was to explore the possibility of surface enlargement in an AVF using the delay surgical method of flap.

In this work, we introduced an original experimental AVF model hemodynamically better than the previously demonstrated delay surgical method of flap [11].

In the first (experimental group) of study animals, AVF was applied by the surgical method.

In the second (control group) of experimental animals, AVF was applied by the delay surgical method.

We measured and compared AVF in both groups of animals, and, according to the results, we established the difference in size between surfaces of AVF performing one or another method.

Methods

In this experimental research we used both ears of "Big Chinchilla" male rabbits, weight 3.2–4.5 kg, age 12–18 months, from the experimental animals farm of Military Medical Academy in Belgrade (Figure 1).

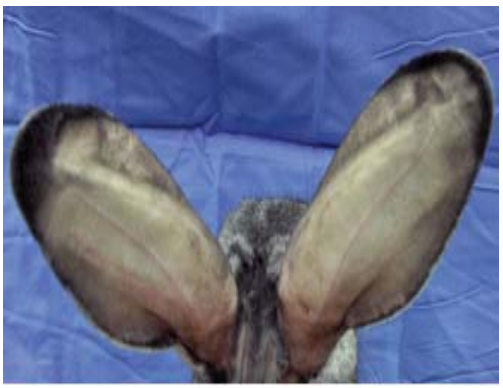

Fig. 1 – Experimental model.

Before the experiment, animals were kept in the vivarium of the Institute for Medical Research of Military Medical Academy, Belgrade, for the period of adaptation. Animals were nourished and watered "*ad libidum*".

For intravenous anesthesia, we used ketamine chloride in the dosage of 35 mg/kg of body mass, acepromazine maleate 1 mg/kg of body mass in 500 mL 0.9% NaCl and 100 IU heparine *per* kg of body mass. An intravenous cannula with 0.5 mm diameter was placed in the anterior marginal vein, and with infusion, drop by drop, anesthesia was maintained for approximately 4 h (Figure 2).

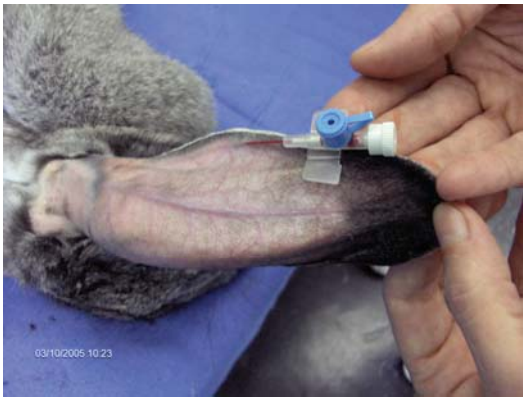

Fig. 2 – Intravenous cannula at site.

First, incision on the skin and subcutaneous tissue was done under the communicant vein, and access made to the vascular pedicle, thus preparing blood vessels. Emptiness test of prepared vessels was used to determine anatomic position of the central artery and vein. Central nerve and blood vessels were cut. A distal part of central vein and proximal part of central artery were tied. After establishing end-to-end anastomosis, we used Acland test to estimate transience, and then the ear was cut at all levels, with preservation of anterior marginal vein in which was intravenous cannula placed. Wound was sutured on anatomical layers. In this way, arterial blood supplied through venous vessel net a block of tissues, consisting of skin, subcutaneous tissue and cartilage. Preserved anterior marginal vein represented outflow vessel of AVGF. Flap vitality was estimated on the days 1 and 14, recording all parameters in experimental protocol for each animal (Figure 3). All animals were treated with heparine in

the dosage of 100 IU *per* kg of body mass during 14 postoperative days. To prevent infection, all animals were treated with 100 mg oxitetracycline in a single daily dosage.

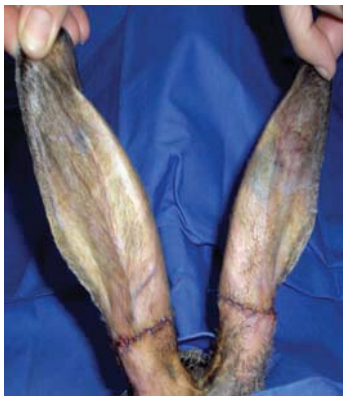

Fig. 3 – Arterialised vein flap formation.

Five experimental animals were used and an AVF was applied to both rabbit ears (10 samples).

The ear was cut at all levels, preserving the anterior marginal vein (Figure 4). The microsurgical technique was

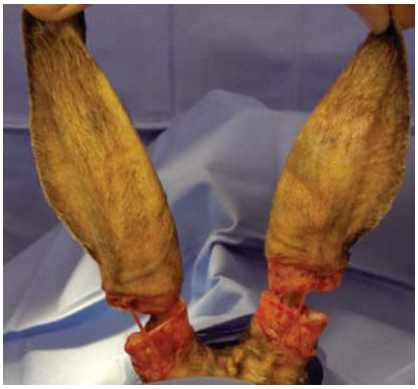

Fig. 4 – Arterialised vein flap preparation.

performed under 20 times of magnification, and the central artery and the central vein were anastomosed by single stitches (Figure 5). For the microsurgical end-to-end anasto-

Fig. 5 – End-to-end arterial-vein anastomosis.

mosis technique we used ethilon 10.0 monofilament polyamide black suture material, with a nontraumatic rounded curved needle 3.75 mm long, and 75 μ in diameter, manufactured by Ethicon Ltd. Blood vessels were tied with surgical thread monofilament polypropylene prolene blue 6–0, and wound was sutured with prolene blue 3–0 by single stitches, manufactured by Ethicon Ltd.

The same number of experimental animals (5) was used and before vein flap arterialisation the surgical delay method was performed on both rabbit ears also, so we had the same number of samples (10 samples).

Ear skin was cut only (Figure 6). Subcutaneous blood vessels and the central nerve were identified and all cut (Figure 7) except the anterior marginal vein, central artery and central vein, which still nourished rabbit ear. After 14 days from performing the surgical delay method (Figure 8), arterialisation of a venous flap was done by microsurgical end-to-end anastomosis technique, using the previously explained procedure.

Fig. 6 – Ear skin incision.

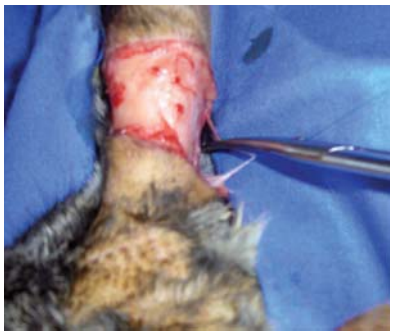

Fig. 7 – Cut of the nerve.

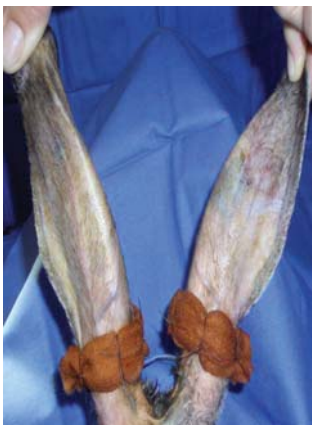

Fig. 8 – Delayed arterialised vein flap.

For surgery we used a binocular operational microscope OPTION I, with limited magnification of 5 to 25 times, with 200 mm focus optical distance. Standard microsurgical instruments and the above mentioned suture material were used.

The experimental animals were numbered previously and marked by experimental groups in experimental protocol.

After arterialisation of the venous flap, clinical estimation of microanastomosis transience was done. Early transience was evaluated by inspection and Acland test, immediately after microanastomosis was completed, exactly after 20 minutes. Microanastomosis transience was evaluated after 24 h by palpation and enlightening technique of the rabbit ear using neon light.

Palpable pulsations in regular time interval and fast fullfillness of blood vessels after vessel emptiness, were treated as a previous sign of clinical anastomosis. Previous anastomoses were included in further investigation.

AVF surface size in the experimental groups, on the days 1 and 14 were defined as: flap length in cm, measured by the longest axis; flap wideness in cm, measured by the widest axis; flap surface in cm^2.

Flap surface was measured after previously marked flap borders on a transparent foil with regions of flap necrosis (Figure 9). The marked surfaces were measured by computer program with integrals and trapezoid rules, as well as statistical program set. After the described process, transparent foils were put in the experimental animal evidence documentation.

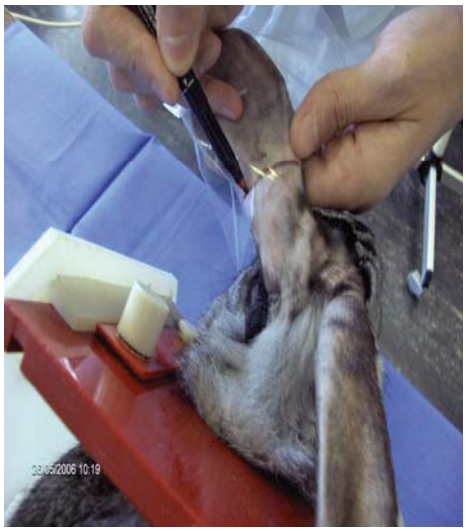

Fig. 9 – Marking flap surface borders on transparent foil.

Necrosis surface was defined as a region of full thickness of the rabbit ear without bleeding (sterile needle test) (Figure 10), as well as surfaces which did not epithelise and were covered by crusts. Measurements were performed on the days 1 and 14 after venous flap arterialisation in both groups.

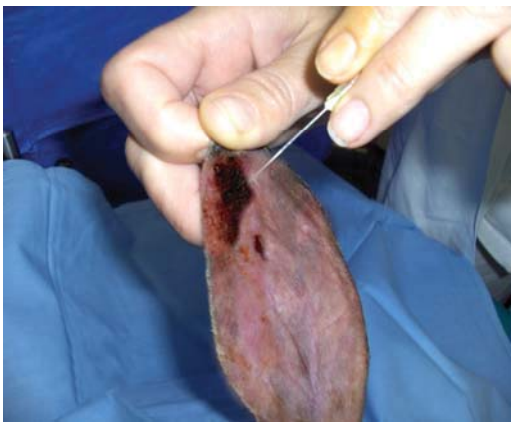

Fig. 10 – Sterile needle test.

Statistical methods

For statistical evaluation of the differences between some characteristics of experimental groups, the examined parameters were presented as mediana, minimal and maximal values, standard deviation (SD) and coefficient of variation (CV).

A statistical significance between some characteristics of experimental groups was defined by the Student *t*-test for independent samples.

The 3 levels of statistical significance were formed: $p < 0.05$, $p < 0.01$ and $p < 0.001$.

The commercial statistical software for PC (Stat for Windows, R.4.5, Stat Soft, Inc., SAD, 1993) was used.

All procedures were done according to the ethic principals of scientific research work on experimental animals in Military Medical Academy, Int. No. 282-12 from December 20, 2002.

Results

The final results of both groups of experimental animals in which venous flap arterialisation was done without the surgical delay method (group I) or with the surgical delay method (group II), are presented in Table 1.

Table 2 shows the surface of both sides of AVF in cm^2, surface of necrosis in cm^2, and a total percentage of necrosis and survival tissue AVF. Measurements were done on the days 1 and 14 after vein system arterialisation.

Discussion

According to the experimental research, the delay method in axial flap can be performed by one of the mentioned ways: artery, vein and nerve cutting; nerve cutting with arterial and vein adventitia cut; artery and vein cutting with adventitia preservation; cutting only artery with the biggest preservation of adventitia; cutting only vein (adventitia preserved).

According to the traditional Krough method, oxygen transportation is done through terminal arterioles. During blood circulation through the lungs, carbon dioxide diffuses into alveoli. That decreases carbon dioxide partial pressure in

Table 1

Characteristics of arterialsed venous flaps in the group without (I) and with (II) the surgical delay method

Studied group	Parameters	Days after surgery					
		1			14		
		$\bar{x}$ ± SD	min–max	CV (%)	$\bar{x}$ ± SD	min–max	CV (%)
I	Left ear						
	viable surface (cm^2)	111.42 ± 11.32	99.29 –125.35	10.16	65.59 ± 12.44	52.13–80.27	18.96
	necrotising surface (cm^2)				45.83 ± 6.06	38.92–52.45	13.08
	necrosis (%)				41.13 ± 6.76	31.54– 52.38	16.16
	Right ear						
	viable surface (cm^2)	102.43 ± 9.14	90.28–112.57	8.92	63.41 ± 8.46	51.07–73.12	13.34
	necrotising surface (cm^2)				39.01 ± 1.58	36.29–40.18	4.05
	necrosis (%)				38.17 ± 6.35	26.77–48.99	16.63
	Total						
	viable surface (cm^2)	106.92 ± 10.80	90.28 ± 125.35	10.10	64.50 ± 10.10	51.07–80.27	15.65
	necrotising surface (cm^2)				42.66 ± 5.68	36.26–52.45	13.31
	necrosis (%)						
II	Left ear						
	viable surface (cm^2)	106.13 ± 11.52	95.54–120.11	10.85	96.85 ± 10.72	86.01–111.73	11.06
	necrotising surface (cm^2)				9.28 ±2.09	6.52–12.24	22.52
	necrosis (%)				8.58 ± 3.31	4.49–13.38	38.57
	Right ear						
	viable surface (cm^2)	99.98 ± 7.98	91.18–109.43	7.98	91.23 ±7.95	79.53–99.01	8.71
	necrotising surface (cm^2)				8.75 ± 2.63	6.07–11.64	30.05
	necrosis (%)				8.76 ± 3.80	4.08–15.45	43.37
	Total						
	viable surface (cm^2)	103.06 ± 9.89	91.18–120.11	9.59	94.04 ± 9.37	79.53–111.73	9.96
	necrotising surface (cm^2)				9.01 ± 2.25	6.07–12.24	24.97
	necrosis (%)				8.67 ± 3.47	4.08–15.45	40.02

$\bar{x}$ – average flap surface; SD – standard deviation; min – minimal; max – maximal; CV – coefficient of variation.

Table 2

Tissue survival on the days 1 and 14 after arterialisation of venous flaps on both rabbit ears surfaces

Parameters	Flap surface			
	1st day		14th day	
	anterior	posterior	anterior	posterior
Surface (cm^2), $\bar{x}$ ± SD	55.50 ± 5.38	51.42 ± 5.77	22.02 ± 3.66	20.64 ± 4.11
Necrotising surface (cm^2),			33.48 ± 4.85	29.78 ± 4.62
$\bar{x}$ ± SD [%]			[39.89 ± 6.98]	[40.11 ± 6.70]
Survival tissue (%), $\bar{x}$ ± SD			60.11 ± 6.97	59.89 ± 6.97

$\bar{x}$ – average flap surface; SD-standard deviation.

blood, as well as hydrogen ions with decreases of carbon acid concentration. Both factors move oxyhemoglobin dissociation curve to the left. That increases oxygen amount, which bonds to hemoglobin on that partial pressure level, and increases oxygen amount for tissues. When that blood reaches the tissue capillary net, a completely different phenomenon appears. Carbon dioxide leaving tissue moves dissociation curve to the right, facilitating oxygen release from oxyhemoglobin, which improves tissue supply with more oxygen compare with situation in oxygen release during that partial pressure in blood. This phenomenon is known as Bohr`s effect and is the key for understanding non-conventional circulation [12]. Noreldin et al. [13] experimented on rats inferior epigastic flap and discussed about small number of capillaries in areolar tissue that surround vein during flap vein elevation. They think that this periareolar tissue can give enough arterial blood for survival. They concluded that monopedicle vein flap survive primary on small arterial inflow from arterial plexus that surrounds vein pedicle. There is an opinion that any peri-vein vessel that is included in nutritional vein pedicle (arteriole, capillary loop, small vein) can bring enough oxygen to flap, if oxygen partial pressure in blood is higher than oxygen partial pressure in flap. According to the ischemic state of flap at the beginning, it is expected that periareolar blood vessels provide gas diffusion in flap until arterial neovascularisation. On the AVF example, this survival mechanism is not adequate. Vein mono- and bipedicle flap is completely separated from surroundings, and with vein separation completely transferred as free, and with microanastomosis between the recipient artery and flap vein, circulation in a flap is “reconstructed”. So, periareolar blood vessels are initially cut.

At the beginning of ischemic state, the flap is nourished by plasmatic imbibition's mechanism, using diffusion of oxygen from a recipient bed, with higher oxygen partial pressure, as in the case of free tissue transfer. These mechanisms provide gas diffusion and oxygen usage on precapillary level, until conventional neovasularisation is established [14].

The most of today's theories about survival mechanism of nonconventional flaps, consider that gas transportation on the capillary bed is essential for flap vitality until neovascularisation happen. In explaining blood flow through capillary system, the "rolling" mechanism of blood is considered, in regard to turns of blood flow forming. In total vein and AVF, blood enters capillary bed with the help of the already existing arteriovenous anastomosis.

Harris et al. [15] suggested that because of the parallel arrangement of bigger arterioles and venules in skeletal muscles, where arteriovenous anastomoses are less developed, mutual oxygen exchange appeared. They supported that diffusion between arterioles and venules can decrease oxygen partial pressure in tissue and increase in venulas. This mutual exchange is considered harmful for oxygen delivery. According to that theory, a part of oxygen in arterial blood diffuses in vein blood, making diffusion gradient in shunt. In the presence of Borh`s effect, mutual exchange increases oxygen partial pressure in tissues, according to mathematical models. This effect is especially emphasized in hypoxia, which is intensified by lactic acidosis. During hyperoxaemia, Borh`s effect does not have a big influence on oxygen partial pressure, because oxihemoglobin is completely saturated. Because of that, mutual oxygen exchange behaved as oxygen diffusion shunt, decreasing oxygen partial pressure and preventing toxic influence of hyperoxaemia. Mutual gas exchange, according to the previously explained diffusion gas transport, can be the another mechanism which helps in surviving of nonconventional miocutaneous flaps in the initial ischemic state without relying on the exchange between oxygen and carbon oxide on the capillary level [16].

Clinical appearance of AVF at the beginning is characterized with edema and congestion which withdraw slowly, between the postoperative days 5 and 10. Congestion is understandably the result of neovascularisation and the vascular net adaptation to the flap. Blood vessels neovascularisation is inducted by hypoxia, and adaptation by arteriovenous shunts opening and pressure increase in vein vascular net. Oscillated veins play a big role in these processes [17].

On our experimental model this phenomenon was also seen. The vein system is not anatomically adapted to high blood pressure. In newly developed conditions, filtration pressure on the ending part of venous net is increased. Exudation of albumins and other proteins through capillary fenesters in interstitial happens. Protein fractions moved electrolytes and water, which makes edemas. Lymphatic system in normal conditions takes albumins, electrolytes and water, and brings them again into the vein drainage system. In those conditions the lymphatic net is not capable to take over transported role, because a muscle pump is missing. Edema brings cascade reaction of hypoxia and tissue adaptation in new conditions. On our experimental model, in both groups during arterialisation of the vein system, the nerve was cut. Denervation in this case widens the venous vascular net and decreases vascular wall tonus. It is considered that opening of arteriovenous shunts and precapillary sphincter are more expressed when denervation action is done [18].

The experimental rabbit model with demonstration AVF by Byun et al. [19] carries out "shorted" circle of perfusion in the rabbit ear with anastomosis of afferent central artery and anterior branch of central vein without the delay method. Arterial blood perfusion in venous vascular space has no possibilities for perfusion in distal ear parts. Total flap necrosis occurs in all AVF**,** despite the previous T-T micro anastomosis. A high percentage of AVF necrosis is similar to that reported in 1997 by Cho et al. [20], in 2003 by Morhammer et al. [21] and in 2005 by Baser et al. [22], on pedicle venous flap on rats.

According to this, it was established that denervation and ischemia improve surface enlargement of a flap with the delay method performed [23–25]. There are other explanations, based on the fact that surface enlargement of flap by the delay method is directly connected to the level of denervation. Since denervation in the elevated flap is bigger, the surface and level of necrosis is smaller, and the opposite way round. Delay of AVF was for the first time demonstrated by Nakayama et al. [6] in 1981, on the epigastic skin flap model in rats. The results were confirmed in the similar study of Voukidis [7]. Despite these promising results, the performed experimental model was not appropriate for two separate microsurgical procedures; venous flap arterialisation and delay method, with significant artery manipulation on the donor site with possible morbidity.

Clinical usage of big AVFs, was not achieved due to complex tissue. The first successful AVF in 1981 had the surface of only a few cm^2. Enlargement of the surface of this flap by the delay method had a limited success. In 1995 Byun et al. [19] examined the delay method of AVF on the rabbit ear. Both ears in 25 New Zealand white rabbits (n = 50) were randomized in three experimental groups. In the first one, AVFs were performed. In the second and the third one surgical delay method was performed, marked as limited and extensive. Two weeks after arterialisation of venous flap was done as in the first group, by end-to-end anastomosis of central blood vessels. The central vein above communicant branch with anterior marginal vein was excluded from blood flow by ligation. After two weeks flap surface was compared in all groups after arterialisation of venous system. In the non delay group, first one, all flaps necrotized completely (100%). Tissue vitality was 0%. In the second group the surface of AVF was 67.9% and in the third 94%, so the surface size of AVF was significantly increased by the delay method. During analysis of previous experimental models of AVF on the rabbit ear, in which the delayed method was performed to enlarge flap surface, we concluded that designed AVF models were hemodynamically inviolable. The mentioned author excluded some parts of the venous system of the rabbit ear from circulation (in our

opinion) making better conditions for AVF survival (delayed method performed).

After a preliminary research we designed AVF simply for experimental work using the surgical delay method. In this way it was more realistic to view surface enlargement of AVF after surgical delay method. AVF survival is optimized by using smaller-caliber veins for inflow and reserving larger-caliber veins for outflow. This regulates inflow and eliminates high blood pressure, and AVF behaves as physiologic flaps do, by not relying on neovascularisation for survival [26].

Conclusion

In the first experimental animal group a venous flap was arterialised with no surgical delay method. The average flap surface was 64.50 cm^2, and the average necrosis surface 42.66 cm^2. In the second experimental animal group a flap was arterialised with the surgical delay method. The average flap surface was 94.04 cm^2, and the average necrosis surface 9.01 cm^2. This clearly points out that surgical delay method usage significantly improves AVF circulation and vitality, as well as AVF surface.

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