Microvascular Research 118 (2018) 128–136

Contents lists available at ScienceDirect

Microvascular Research

journal homepage: www.elsevier.com/locate/ymvre

Vascular architecture in free flaps: Analysis of vessel morphology and morphometry in murine free flaps

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ARTICLE INFO

Keywords:
Microvascular surgery
Free flap
Vascularization
Vascular architecture
Sprouts
Pillars
Intussusceptive angiogenesis

ABSTRACT

The aim of this study was to analyze the development of vascular architecture as well as vascular morphometry and morphology of anastomosed microvascular free flaps.

Free pectoral skin flaps were raised in 25 rats and anastomosed to the femoral vessels in the groin region. CD31 immunohistology was performed after 3, 7 and 12 d (each 5 animals each) to analyze microvessel density (MVD), microvessel area (MVA) and microvessel size (MVS). Microvascular corrosion casting was performed after 7 and 12 d (5 animals each) to analyze vessel diameter (VD), intervascular distance (IVD), interbranching distance (IBD), and branching angle (BA). Further on, sprout and pillar density as hallmarks of sprouting and intussusceptive angiogenesis were analyzed. Pectoral skin isles from the contralateral side served as controls.

A significantly increased MVD was found after 7 and 12 d (p each < 0.001). MVA was significantly increased after 3, 7 and 12 d (p each < 0.001) and a significantly increased MVS was analyzed after 3 and 7 d (p each < 0.001). VD and IVD were significantly increased after 7 and 12 d (p each < 0.001). For IBD, a significantly increase was measured after 7 d ($p < 0.001$). For IBA, sprout and pillar density, no significant differences were found (p each ≥ 0.05).

Significant changes in the vascular architecture of free flaps after successful microvascular anastomosis were seen. Since there was no evidence for sprout and pillar formation within the free flaps, the increased MVD and flap revascularization might be induced by the receiving site.

1. Introduction

Microvascular free flaps, such as the radial forearm flap, latissimus-, ALT-flap (anterolateral thigh flap), or fibula free flap are considered to be the gold standard for complex reconstructions of soft- and hard tissues in the head and neck region. These flaps achieve functionally convincing and aesthetically pleasing outcomes with a high flap success rate of nearly 95–96% (Al-Dam et al., 2014; Preidl et al., 2015; Mücke et al., 2016). Next to the head and neck region, free flap surgery is also indicated in a variety of scenarios, such as breast cancer reconstruction, lower extremity trauma reconstruction and many more (Las et al., 2016).

A selection of recent innovations in microvascular surgery and free flaps are extracorporeal perfusion systems in patients with vessel depleted necks, the intraoperative fluorescence angiography for flap perfusion monitoring, the coverage of donor site defects by porcine collagen membranes, or mini perforator flaps (Preidl et al., 2015; Byun et al., 2016; Fichter et al., 2016; Hitier et al., 2016; Wolff et al., 2016; Wolff, 2017). Beside single flaps, double-flap techniques – two or more flaps to cover one extended defect – became more and more popular and widely used to cover and restore extensive defects in the head and neck region (Weitz et al., 2015).

Unfortunately, microvascular free flaps require advanced surgical skills and training for flap raising and for the microvascular anastomoses of the vessels to ensure the blood perfusion of the flap as well. In general, possible risk factors for flap failure include a prolonged ischemia time, insufficient arterial and venous anastomosis, thrombus formation and vessel obliteration (Yang et al., 2017). Also, previous radiotherapy in the head and neck region has been reported to be a relevant risk factor for flap failure (Zhou et al., 2017). Further on prior and multiple attempts of microvascular transplants and the length of hospitalization can be considered as possible risk factors for flap failure

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https://doi.org/10.1016/j.mvr.2018.03.006
Received 19 August 2017; Received in revised form 16 March 2018; Accepted 16 March 2018
Available online 22 March 2018
0026-2862/

(Mücke et al., 2016).

Regarding the survival- and success rate of free flaps, a continuous and sufficient blood perfusion is the most relevant factor, which is primarily ensured by the flap pedicle during the first 10–12 days after surgery. Simultaneously the revascularization of the flap starts from the surrounding tissues of the recipient site. In this context, Wolff et al. reported, that free flaps in vessel depleted necks with an extracorporeal perfusion system can be separated from the extracorporeal blood supply after 10–13 days (Wolff et al., 2016). These observations may give evidence that free flaps could be sufficiently revascularized from the recipient site and independent from the pedicle perfusion after nearly two weeks. But it is still unclear whether this represents the norm. In contrast, reliance on the pedicle years after transfer is also possible. Further, the time required to become independent from the pedicle may also be dependent on various factors, such as the flap size, the flap thickness or the composition of the flap (e.g., bone versus soft-tissue). Next to neovascularization, defined as a *de-novo* creation of blood vessels by endothelial stem cells, two different and important ways of angiogenesis in adult (non-malignant) tissues can be observed: (1) sprouting angiogenesis by the creation of outbranching new vessels from preexisting vessels and (2) intussuscepted angiogenesis via creation of triangular pillars in preexisting vessels and the division of one preexisting vessel into two new vessels alongside (Ackermann et al., 2014a, 2014b).

Recent studies used intra- and postoperative fluorescence angiography and laser Doppler spectrophotometry to control the blood perfusion of the flap (Hitier et al., 2016; Ludolph et al., 2016; Mücke et al., 2017). Further laser speckle contrast imaging (LSCI), fluorescence-mediated photoplethysmography (FM-PPG) and iontophoresis were used to investigate the microvascular function (Cordovil et al., 2012, Ansari et al., 2017, Flower and Kling, 2017, Loader et al., 2017). As a limitation, these methods are not suitable to visualize the vascular architecture, morphometry and morphology of free flaps in more detail. In addition, it is still unclear, if the flap revascularization – independent from the perfusion of the flap pedicle – is based on revascularization from the recipient site or on remodeling processes of the vascular system within the flap.

To the best of our knowledge, there is no literature on the exact mechanisms regarding the revascularization of free flaps. Also, there is no information available about the development of two- and three-dimensional (2D, 3D) vascular morphometry and morphology after anastomosis in microvascular free flaps. Therefore, the aim of this study was to analyze the vascular architecture in microvascular free flaps in a murine free flap model.

2. Material and methods

2.1. Animal care

All animal experiments were approved by the local animal care protection authority (Landesuntersuchungsamt Rheinland-Pfalz, Koblenz, Germany; No. 23 177-07/G 12-1-091). Animal care was in accordance with institutional, national and international guidelines, and with the ARRIVE guidelines. Twenty-five Sprague-Dawley rats (Envigo, Rossdorf, Germany), male, average age 14 (± 2) weeks, average weight 480 (± 30) grams, were used. Animals were housed in an animal house in cages (two animals each) with an automatically 12-hours dark/light rhythm and unlimited water and food. Wood chips were used as bedding material. Control and weighing of the animals was performed daily by the same person to reduce strain and stress. Prior to experiments, animals acclimatized for 4 weeks and were randomly divided in 5 study groups, 5 animals each.

2.2. Surgery

Surgical procedures were performed in the operating room of our animal laboratory under sterile conditions. Animals were anesthetized with isoflurane (Isofluoran 99.9%; Provet AG, Lyssach b. Burgdorf, Switzerland) combined with an intraperitoneal (i.p.) injection of ketamine (Ketavet® 100 mg; Pfizer, Berlin, Germany) and xylazine (Rompun® 2%; Provet AG). The right pectoral- and groin region was shaved and disinfected. Pain therapy was performed with a subcutaneous (s.c.) injection of buprenorphine (Buprenex® 0.3 mg/ml; Bedford, USA; 0.01 mg/kg body weight b.w.). Besides anticoagulation with heparin s.c. was administrated (Clexane®; Sanofi-Aventis, Frankfurt, Germany; 1 mg/kg b.w.).

A modified free pectoral skin flap was raised from the right pectoral region as described before (Miyamoto et al., 2008; Pabst et al., 2015).

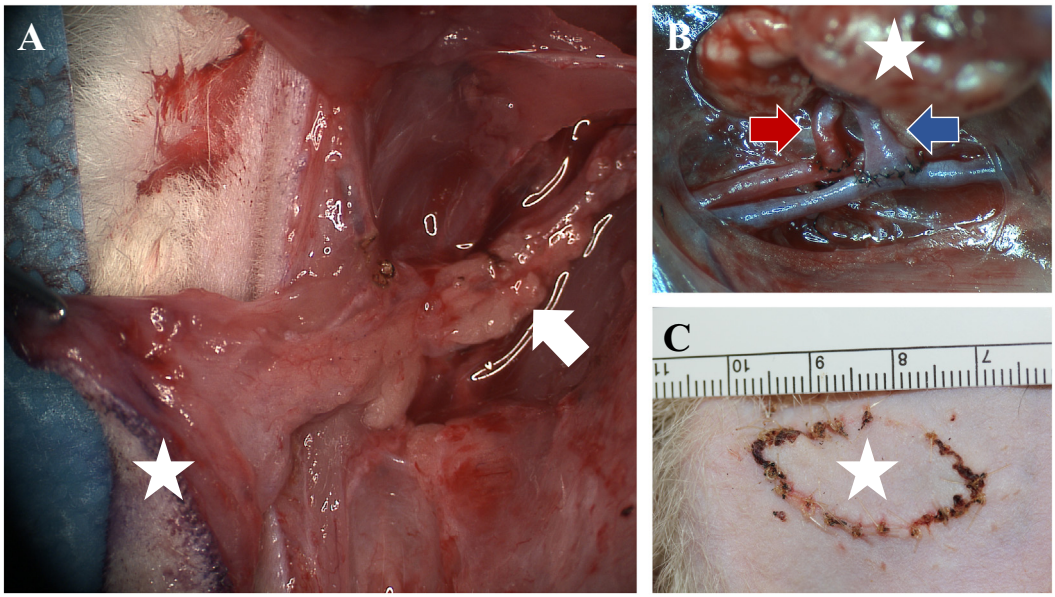

Fig. 1. Free pectoral skin flap. (A) Modified free pectoral skin flap raised in the right pectoral region of a rat consisting of the skin isle (white asterisk) and the flap pedicle (white arrow) including the common and long thoracic artery and vein. (B) Common thoracic artery (red arrow) and vein (blue arrow) of the raised flap (white asterisk) after end-to-side anastomosis to the femoral artery and vein in the right groin region. (C) Flap in the right groin region on day 7 postoperatively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)