Journal of Cranio-Maxillo-Facial Surgery xxx (2018) 1–6

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

A novel microsurgical anastomosis training model using gradually thawed cryopreserved microvessels of rat cadavers

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ARTICLE INFO

Article history:
Paper received 6 March 2018
Accepted 7 May 2018
Available online xxx

Keywords:
Microsurgery
Model
Rats
In vitro
Anastomosis
VCA

ABSTRACT

In consideration of the 3-R-rule (Refine-Replace-Reduce) as a guideline for promoting ethical use of animals for surgical training, we present a novel training model for microvessel anastomosis. In a rat cadaveric study, we evaluated the surgical anatomy of the common carotid artery (CCA), external jugular vein (EJV) and femoral vessels (FV) which were then used as templates for the present investigation. Anatomical dissection of 30 rat cadavers was performed. Two residents without prior microsurgical experience were included in the study and performed 5 CCA, 5 femoral artery, 5 EJV and 5 femoral vein anastomoses. Patency and leakage served as qualitative variables and operation time as a quantitative variable for efficiency control. The average time improved for arterial and venous anastomoses (45 min−22 and 60 to 32 min, respectively) for both surgeons. While both surgeons experienced patency failure or leakage within the first half of performed arterial and venous anastomoses, they could improve to a 100% patency rate without the occurrence of leakage for the last half of trials. The rat head & neck anatomy presents various characteristics related to the harvest of the vessels of interest. We provide anatomical knowledge about the topography related to the harvest of the CCA, EJV, and FV. Our model is an easily accessible, low-cost microsurgical simulation model, allowing a realistic and instructive performance of anastomoses. Since cadaveric vessels are used, an approval of the local ethics committee is not needed.

1. Introduction

Successful microsurgical procedures require sound training methods, which on the one hand address theoretical and on the other hand address the highly complex practical aspects (Egle et al., 2015). In this regard, in vitro and in vivo microsurgery models play a decisive role, as they allow pre-clinical training and improvement of surgical skills to ensure a good surgical outcome in the context of clinical patient management (Krishnan et al., 2004).

Anastomosis training models have vastly been described throughout literature and can be generally classified into three categories, namely a) non-biological/non-living, b) biological/non-living and c) in-vivo animal models (Krishnan et al., 2004). However, most of these models are characterized by either high costs, high technical complexity or poor similarity to human tissue (Kim et al., 1994). Nevertheless, promising surgical skill learning curves are frequently published, and their implementation into the basic surgical training of students or residents are suggested (Phoon et al., 2010; Cifuentes et al., 2016). However, in-vivo animal models should incorporate and consider the 3R (Replace-Refine-Reduce) principle in order to minimize the number of animals used (Galmiche et al., 2018; Dumestre et al., 2015).

Accordingly, we present our training model, which allows cost-efficient microsurgical training on organic mammal vessels and offers an objective performance control on the basis of patency and fluid leakage, simultaneously acting out the 3R-rule (Refine-Replace-Reduce). Additionally, we provide detailed knowledge

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https://doi.org/10.1016/j.jcms.2018.05.018
1010-5182/

about the harvesting procedure of the external jugular vein, common carotid artery and femoral vessels in the rat. These vessels allow supermicrosurgical training due to their small diameter and are relevant e.g. in the context of experimental facial VCA (vascularized composite allotransplantation) models (Kulahci and Siemionow, 2010). As rats are one of the most frequently used small animal models world-wide, propagation of knowledge about vascular head and neck anatomy is of great interest to the scientific community.

2. Material and methods

The study was conducted in the Institute of Experimental Neurosurgery of the University Hospital Cologne between January and March 2018. Two residents without microsurgical experience were introduced to microsurgical techniques and consecutively chosen for the present investigation. Anatomical terminology was used in accordance with Greene et al (Greene, 1935). In total 30 rat cadavers were used for the present investigation which consisted of an anatomical study of the vessel anatomy of the common carotid artery, the external jugular vein and the femoral vessels in rats as well as of the harvest of the vessels for establishment of the model and for consecutive evaluation of the feasibility and practicability. For the present investigation, we solely used rat cadavers that were generated in the context of other approved animal experiments e.g. as drop out animals or after reaching the determined endpoint. Therefore, none of the animals was specifically killed for the present investigation. In total, the head and neck vascular anatomy was evaluated at hand of 20 cadavers. The remaining 10 cadavers were decapitated and therefore only allowed anatomical dissection of the inguinal region. All animals that were subjected to the anatomical study underwent dissection at the same day of their death.

2.1. Biological, non-living microsurgery simulation model

For our model we used the common carotid artery (vessel diameter: 0.8–1 mm), the femoral artery (vessel diameter: 0.9–1.1 mm), the external jugular vein (vessel diameter: 1–1.5 mm) and the femoral vein (vessel diameter: 1.2–1.7 mm) of rats. The vessels were harvested from rat cadavers at a length of at least 2 cm. For the investigation, we used cryopreserved vessels or freshly harvested ones from rat cadavers.

The following materials were used for application of our model: 22G intravenous catheters (Braun company), basic microsurgical instruments (Scissors and forceps), straight rectangular stable ground, 0.9% sodium chloride, Phosphate-buffered saline, securing tapes, poly- and monofilament suture material (Vicryl 6/0 and Prolene 10-0) and a Wild Leitz surgical microscope allowing dissection at 8-, 16-, and 32 times magnification (Wild Leitz, Wetzlar, Germany).

The microsurgical biological/non-living simulation model was constructed as follows: A straight rectangular blue plane was covered in surgical drapes and the drapes were fixed in place with the help of securing tapes. Consecutively the 22G intravenous catheters were prepared in order to serve as the vessel's suspension. In order to guarantee that the vessels do not loosen after being mounted to the catheter, it is important to cut the tapered ending in order to attain a uniform longitudinal course of the catheter. The catheters can then be fixed to the small box with the help of securing tapes. Do not block the catheters adapter side in order to allow for manual application of PBS into the catheter for patency and leakage testing.

In the beginning only one catheter should be fixed to the ground. Consecutively the cryopreserved or fresh vessel was mounted onto the first (fixed) catheter with the help of the microscope which is then fixed with a suture (Vicryl 6-0). Then, the other side of the vessel is mounted onto the mobile 22G intravenous cannula and is stretched until a distance of 5 mm is acquired between the catheter tips. Hereafter, the mobile intravenous catheter is fixed in the same manner as on the other side (see Fig. 1). Now, the vessel is cut in the middle, perpendicular to its longitudinal course. Afterward, the anastomosis is performed in a standardized conventional technique with 8 sutures.

Firstly, a vessel approximator is applied and narrowed in order to acquire a distance of the vessel ends of less than 1 mm. The first suture is placed at the back wall and the second 180° distant from this site. The third and fourth suture is placed at 90° and 270°, respectively. After that, the following sutures are sewed between the existing ones. It is important to note, that the vessel should be kept moist during the whole procedure of anastomosis to prevent dehydration (Hino, 2003).

Patency and leakage were tested as qualitative variables (yes/no) 20 min after the anastomosis, by clamping one side of the vessel and applying 20 ml fluid into the vessel through an intravenous catheter (Bas et al., 2017; Kim et al., 1994). Time needed for anastomosis was measured in minutes as a quantitative variable.

2.2. Harvest of the vessels

The external jugular vein, common carotid artery and femoral vessels were visualized and harvested respecting the surgical approach as described by Kulahci et al. in the context of rat facial vascularized composite allotransplant grafting (Kulahci and Siemionow, 2010). Primarily the external jugular vein was dissected. After excision of the vein, dissection and consecutively the extraction of the common carotid artery was performed. Secondly, we harvested the femoral vessels. The evaluation of the exact vessel anatomy was part of the present investigation. Hence, the harvesting procedure will be described in the results section, simultaneously describing detailed anatomical knowledge necessary for the harvesting procedure. Dissection was carried out using the materials as described above. Vessels were dissected with the help of two micro-forceps under 16 times magnification, securing vessel integrity.

2.3. Cryopreservation of the vessels

After dissection of the vessels and removal of the adventitia under the microscope (16× and 32× magnification), the vessels were removed with a minimum of 2 cm in length and were rinsed with PBS (Phosphate-Buffered Saline). In order to conserve the vessels, they were cryopreserved at −20 °C in moistened medical gauze and were gradually thawed under room temperature,

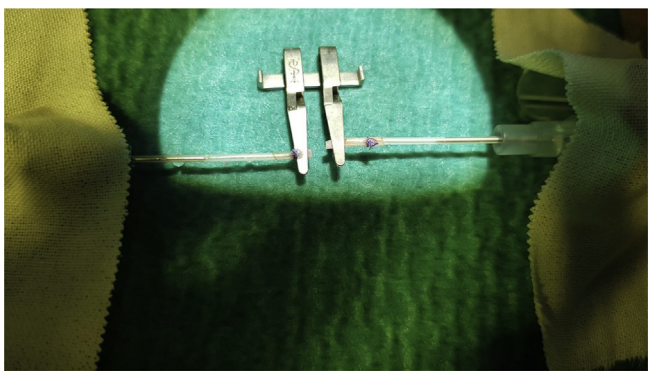

Fig. 1. Presentation of our biological non-living training model for microsurgical anastomosis.

Please cite this article in press as: Safi A-F, et al., A novel microsurgical anastomosis training model using gradually thawed cryopreserved microvessels of rat cadavers, Journal of Cranio-Maxillo-Facial Surgery (2018), https://doi.org/10.1016/j.jcms.2018.05.018