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Comparing five simple vascular storage protocols

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ABSTRACT

Background: We aim to find a storage protocol for vessels that preserves their dimensional, histologic, and mechanical characteristics to facilitate reproducible anastomosis experiments and microsurgical training with constant quality.

Materials and methods: We compared stored rabbit aortas, harvested in a slaughterhouse, using five different protocols with fresh controls. Aortas were preserved for 125 d in (1) NaCl 0.9% at −18°C, (2) Roswell Park Memorial Institute 1640 90% with 10% dimethyl sulfoxide (RPMI/DMSO) at −18°C, (3) RPMI/DMSO at −70°C, (4) glycerol 85% at 4°C, and (5) glycerol in stepwise increased concentrations until 85% at 4°C. After preservation, we measured vessel diameter, wall thickness, and Young's Modulus indicating stiffness. Neurosurgeons compared stored vessels with fresh vessels, blinded for preservation subgroup. We performed histologic assessment blinded for preservation subgroup.

Results: Fresh rabbit aortas showed a mean diameter of 2.65 ± 0.14 mm, a mean wall thickness of 126 ± 22 μm, and a Young's Modulus of 11.4 ± 2.4 N/mm^2. NaCl 0.9%-preserved aortas showed a significantly increased vessel diameter and decreased stiffness. RPMI/DMSO-preserved aortas showed no significant differences from fresh aortas in dimensions and mechanical characteristics. Glycerol-preserved tissue showed a significant increase in wall thickness, a related significant decrease in diameter, and increase in stiffness. Neurosurgeons regarded RPMI/DMSO tissue as most comparable with fresh tissue. Histologic assessment revealed no differences between the different protocols and fresh control group.

Conclusions: Storage of rabbit aortas in RPMI/DMSO most adequately preserves their dimensional and mechanical properties.

1. Introduction

The *in vitro* development of microsurgical anastomosis techniques and preclinical training of microsurgical anastomosis techniques ideally are performed with vessels that have dimensions similar to patient tissue and have a minimal variation in mechanical and dimensional characteristics.

The development of a new microsurgical anastomosis technique requires a high number of *in vitro* anastomoses.

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For example, our development of the Excimer Laser Assisted Nonocclusive Anastomosis (ELANA) technique [1–3] required in some studies over 2000 anastomoses [4–6]. To make measurements trustworthy and repeatable, we prefer to use vessels with the same dimensional and mechanical characteristics as the artery on which the ELANA technique is used most in patients, the proximal middle cerebral artery (MCA) [1–3]. Hereby, the vessel wall was especially important because the technique uses a laser to penetrate the vessel wall [1–3].

Microsurgical training in general and specifically ELANA training starts *in vitro* on training models [5]. To maximize training quality, all vessels must have comparable dimensional and mechanical characteristics. Ideal vessel dimensions for ELANA training also equals MCA characteristics for obvious reasons.

However, fresh patient MCA's are not readily available for anastomosis development and training in high volumes. Alternative is the use of animal tissue. Hereby, the use of animals should be minimized and the tissue should be readily available in terms of cost and effort [7,8].

The (healthy) human proximal MCA has a mean diameter of 3 ± 0.5 mm and mean arterial wall thickness of 0.2 ± 0.1 mm [9,10]. A comparable artery to the MCA is the abdominal part of the rabbit aorta. This artery is readily available with relatively low costs and effort because of the presence of consumption slaughterhouses. We use rabbit aortas in our laboratories since more than a decade [5]. These are harvested a couple of times per year by specialized laboratory employees who are trained to treat the material very carefully. It is not possible to do this every day because of practical reasons. Therefore, we need to preserve these arteries. This preservation method should be nontoxic, inexpensive, and simple.

To determine the optimal preservation method for these aortas, we selected three suitable storage media; NaCl 0.9%, Roswell Park Memorial Institute 1640 90% with 10% dimethyl sulfoxide (RPMI/DMSO) and glycerol. We designed five different storage protocols using these media and compared thickness, stiffness, and histologic characteristics of the differently preserved rabbit aortas with fresh controls. With this study, we try to find the optimal storage protocol.

2. Methods

2.1. Tissue preparation

Trained laboratory employees harvested seventy-five abdominal aortas from rabbits in a regular consumption slaughterhouse. The aortas were treated delicately and never directly touched by instruments. They were kept in their adjacent retroperitoneal tissue and fat. Directly after harvesting, the aortas were cleaned from adjacent tissue with micro instruments under the microscope, keeping the adventitia adherent to the vessel meticulously intact. Thirty-nine of these vessels were sharply divided into a total of 492 ring segments, each 3 mm wide. Thirty-six of these vessels were kept intact.

2.2. Preservation protocols

The 492 segments and 36 full vessels were randomly divided over five different preservation protocols and one control group, resulting in 82 rings and six vessels per group. (Table 1). The first group of 82 rings was preserved in NaCl 0.9% at −18°C (NaCl-18). This solution has an osmolarity of 0.3 osm/L. The second group of rings was preserved in RPMI/DMSO at −18°C (RPMI-18). This solution has an osmolarity of 0.3 osm/L. The third group was preserved in RPMI/DMSO at −70°C (RPMI-70). The fourth group was preserved in glycerol 85% at 4°C (glycerol-85). This solution has an osmolarity of 9.2 osm/L. The fifth group was preserved in stepwise increased concentrations of glycerol until an ultimate concentration of 85% at 4°C (glycerol-stepwise). The glycerol-stepwise protocol was described previously [11]. In all five preservation groups, tissue was stored for 125 d. The sixth group of rings served as control. These rings were tested within 6 h after harvesting and were kept in NaCl 0.9% at 18°C before the experiments. All media were calcium free.

Before the experiments, the aortas from the NaCl-18, the RPMI-18, and RPMI-70 groups were thawed at 19°C. The aortas from the glycerol 85% group at 4°C (glycerol-85) and glycerol-stepwise group, also stored at 4°C, were rinsed for 10 min in 0.9% NaCL at 19°C. Each specimen was tested within 1 h after removal from its storage conditions and regularly moistened during testing with 0.9% NaCl.

Table 1 – Experimental groups.

Group	Ring segments (*N*)	Intact aortas (*N*)	Storage solution	Storage temperature (°C)	Total storage d (*N*)
1	82	6	0.9% NaCl	−18	125
2	82	6	RPMI 1640 (90%) + DMSO (10%)	−18	125
3	82	6	RPMI 1640 (90%) + DMSO (10%)	(1) −18 (24 h) (2) −70 (124 d)	125
4	82	6	Glycerol 85%*	4	125
5	82	6	(1) Glycerol 50%* (24 h) (2) Glycerol 70%* (30 d) (3) Glycerol 85%* (94 d)	4	125
6	82	6	0.9% NaCl	18	0–0.25

* All glycerol dilutions were performed with 0.9% NaCl.