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Morphology and Function of Preserved Microvascular Arterial Grafts: An Experimental Study in Rats

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The aim of this study is to examine the morphology and function of small-caliber, arterial grafts after preservation in the University of Wisconsin solution (UW). Rat carotid arteries were stored in UW (n = 10) or in phosphate-buffered saline (PBS) (n = 10) for 1, 3, 7, and 14 days and were examined with light microscopy (LM) and scanning electron microscopy (SEM). Rat aortic preparations were stored in UW or PBS for 1 hour, 24 hours, 72 hours, 7 days, and 14 days and assessed for functional responses (stimulated contraction and endothelium-dependent relaxation). Segments (5 mm) of rat carotid arteries were stored in UW or PBS for 3 days, 7 days, and 14 days and orthotopically implanted as autografts and allografts. No immunosuppressive or anticoagulant agents were used. After 28 days of implantation, the grafts were assessed for patency and excised for LM and SEM. In UW, the endothelial layer remained intact up to 9 days of storage. In PBS, the endothelial layer showed deterioration after 1 day and was completely lost after 3 days. Functional responses were demonstrated to exist for as long as 7 days storage in UW. In PBS, no responses could be evoked after 24 hours storage. Autografts preserved in UW for 3 days (n = 6), 7 days (n = 6), and 14 days (n = 6) showed patency rates of 83.3%, 66.6%, and 66.6%, respectively, whereas patency rates of allografts were 66.6%, 33.3%, and 33.3%, respectively. Autografts stored in PBS for 3 days (n = 6), 7 days (n = 6), and 14 days (n = 6) showed patency rates of 33.3%, 33.3%, and 50%, respectively, whereas patency rates of allografts were 16.7%, 0%, and 33.3%, respectively. The UW preserved autografts showed normal morphology. All other groups showed vessel wall degeneration which in the allograft groups, were accompanied by lymphocellular infiltration. In conclusion, the endothelial layer and vessel wall of arteries are adequately preserved in UW. Functional responses are retained up to 14 days storage in UW, but, are lost after 24 hours storage in PBS. Autograft implantation studies accordingly show good performance of arterial segments preserved in UW, whereas allografts are subject to degradation as a result of rejection. (Ann Vasc Surg 1997;11:284–291.)

INTRODUCTION

Digital and limb reimplantation has become a common procedure in plastic reconstructive surgery. To avoid tension at the anastomotic sites, grafts are needed to bridge both the arterial and venous defects. A large number of prosthetic, microvascular graft materials have been evaluated for this purpose including PTFE,[1] glutaraldehyde tanned vessels,[2] bioabsorbable polyurethane grafts,[3] and freeze-dried vessels.[4] None of these materials have consistently produced an acceptable patency rate. Thus, the ideal vascular prosthesis for small caliber peripheral or microvascular reconstructions has not yet been established.

A vascular transplant possibly offers an attractive

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alternative. In the past, investigators have looked at possibilities to use venous[5-7] as well as arterial[8-14] allografts. Arteries are the preferential substitute for arteries, because they have the advantage of similar longitudinal elasticity and closely matched mechanical properties. However, transplantation of allogeneic arteries has been hampered by severe rejection and aneurysmatic degeneration.[10,11] An additional problem in case of allogeneic transplantation is the need to preserve arteries in order that these vascular conduits can be ''banked.''[9,15,16]

Considering the advances in preservation techniques and immunosuppression, the concept of arterial transplantation may be readdressed. Potent immunosuppressive drugs, such as cyclosporine A, have greatly increased long term survival of organ transplants. This notion has changed the prospects of allogeneic artery transplantation.

A major advance in organ preservation has been the introduction of the ''University of Wisconsin'' (UW) solution.[17] This solution has expanded the limits of storing organs and is currently considered the preservation solution of choice. This technique opens up possibilities for banking of arterial grafts providing a readily available vascular prosthesis.

The aim of this study is to assess the efficacy of the University of Wisconsin preservation solution for prolonged preservation of rat arteries. Stimulated functional responses of the endothelial layer and the media of the vessel wall were examined in rat aortic preparations to assess their viability. The effects of storage on morphology of UW-preserved segments of carotid arteries were investigated. For comparison, arteries were stored in phosphate buffered saline (PBS), a solution often used for intraoperative storage of free vascular grafts. Patency studies were performed on UW-preserved segments of carotid arteries after orthotopic implantation in allogeneic and syngeneic rat strains. Histological studies were performed in these grafts to study perigraft and tissue reaction with regard to the allograft implantations.

MATERIALS AND METHODS

Preservation of Arterial Grafts

For morphological examination and transplantation common carotid arteries were harvested from female Wistar rats (200 g) and stored in the University of Wisconsin solution (ViaSpan™, Du Pont Pharmaceuticals, Wilmington, DE, USA)(UW)[17] (n = 10) at 4°C (Table I), or for comparison, in an indifferent solution, i.e., phosphate buffered saline (0.9%), pH 7,4, 4°C, with the addition of Eusaprim (co-trimoxazol) (Wellcome™, Zeist, The Netherlands) 0.5mg/ml (PBS) (n = 10).

Assessment of Stimulated Isometric Contractile Responses and Endothelium-Dependent Relaxation

For assessment of functional responses after preservation infrarenal aortas of male Wistar rats (350-400g) were carefully excised, divided into two segments, and stored immediately at 4°C in UW (n = 6) or in PBS (n = 6). For isometric recording of tension, a ring of aorta (3 mm segment) was cut, following 1 hour, 24 hours, 72 hours, 7 days, and 14 days of storage. The rings were mounted vertically between stainless steel hooks in an organ bath (5 ml). The initial resting tension was set at 10 mN and was readjusted throughout the experiment. The upper hook was connected to an isometric force transducer (Kyowa™, Tokyo, Japan). The signal was amplified (Kyowa™, Tokyo, Japan) and recorded by means of a thermal pen recorder (WKK™, Kaltbrunn, Germany).[18]

The medium was maintained at 37°C and pH was kept at 7.4 by bubbling the solution with carbogen O_2/CO_2 (95:5). The medium used was a Tyrode's solution of the following composition (mM): NaCl 124, KCl 4.0, $CaCl_2$ 0.9, $MgCl_2$ 1.1, NaH_2PO_4 0.42, $NaHCO_3$ 24.9, glucose 5.5.

In the aortic preparations, endothelial cell function was measured by means of methacholine-(100 µM) stimulated release of endothelium-derived relaxing factor (EDRF/NO). Vascular wall function, as reflected by stimulated contraction, was assessed by measuring the response to depolarization induced by potassium chloride (40 mM), and the receptor-dependent contraction evoked by the α_1-adrenoceptor agonist, L-phenylephrine (100 µM).

Experimental protocol: after 1 hour of equilibration, the rings of the isolated aortas were exposed for 10 min to a high potassium concentration (40 mM) to induce membrane depolarization and contraction. When a maximal contraction developed, the solution was replaced by normal Tyrode's solution. This procedure was repeated twice with 15-min. intervals. The rings were, subsequently, contracted with L-phenylephrine (100 µM). As soon as a steady state had been reached, methacholine (100 µM), a stable derivative of the endogenous agonist acetylcholine, was added to induce endothelium-dependent relaxation. To confirm the ability of the vascular smooth muscle to relax in response to EDRF/NO, the aortas were exposed to the endothelium independent NO-donor Na^+-nitroprusside

Table I. Composition of the University of Wisconsin solution

Potassium lactobionate	100 mmol/L[a]
KH_2PO_4	25 mmol/L
$MgSO_4$	5 mmol/L
Raffinose	30 mmol/L
Adenosine	5 mmol/L
Hydroxyethyl starch	50 g/L
Glutathione	3 mmol/L
Allopurinol	1 mmol/L
Osmolarity	320 mOsm/L
pH	7.4

[a]The final sodium and potassium concentration of the UW solution is Na 25 mmol/L and K 120 mmol/L.

(100 µM), after constriction by KCl (40 mM) and L-phenylephrine (100 µM).

The contractions induced during exposure to 40mM KCl and 100µM L-phenylephrine were expressed as absolute force of contraction in mN. Baseline values for the responses to KCl and L-phenylephrine were established by calculating mean values of the responses of a control group of freshly obtained aortas (n = 29). Results at 1 hour, 24 hours, 72 hours, 7 days, and 14 days are presented as means ± standard error of the mean (sem). The endothelium-dependent relaxation is expressed as the percentage decrease in tension from the preconstricted level, presented as mean values ± sem.

Preparation of Grafts for Light Microscopy (LM) and Scanning Electron Microscopy (SEM)

After between 1 and 14 days of storage, segments of preserved artery were cut. For LM, specimens were placed in 10% buffered formaldehyde and subsequently embedded in paraffine. Five-micron sections were cut and stained with Hematoxilin-Eosin (HE) and Giemsa's elastin stain. For SEM, slices of preserved artery were cut and placed in Karnovsky's fixative for 24 hours. After fixation, the specimens were dehydrated in graded ethanols. After this procedure, the specimens were critical point dried (CPD). Subsequently, they were mounted on stubs with conductive glue, sputter coated with 20nm gold-palladium and viewed with an ISI SS40 scanning electron microscope (ISI™, Tokyo, Japan) at 10 kV accelerating voltage.

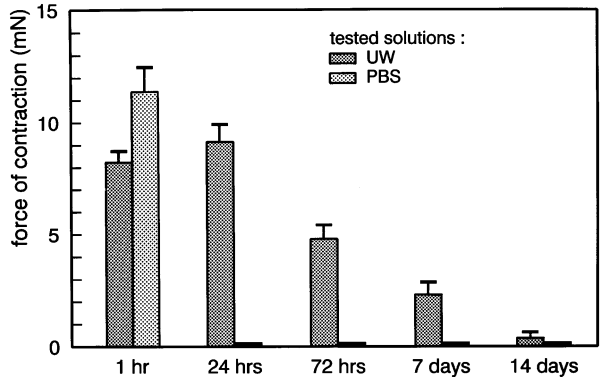

Fig. 1. Graph showing the mean values (± SEM) of stimulated contractile responses to 40 mM KCl in aortic preparations, examined at 1 hour, 24 hours, 72 hours, 7 days, and 14 days of storage at 4°C. UW: University of Wisconsin organ preservation solution; PBS: phosphate buffered saline (pH 7.4, NaCl 0.9%).

Transplantation Experiments

Five millimeter segments of carotid arteries stored in UW or PBS were, orthotopically, implanted in female rats (n = 72, 200 g). The animals were premedicated with pentobarbital 1 ml/100 g body weight, administered intraperitoneally. Subsequently, the animals were given inhalation anesthesia with a mixture of NO_2/O_2 (1:1) and halothane (Fluothane™, Zeneca, Ridderkerk, The Netherlands) 1%. The operations were performed using an operating microscope (Zeiss™, Oberkochen, Germany) at low power. Using a median incision in the neck, the left carotid artery was exposed by splitting the sternocleidomastoid as well as the long neck muscles. The artery was dissected free taking care not to damage the adjacent vagal nerve. Subsequently, the artery was crossclamped with a Biemer clip (Aesculap™, Tuttlingen, Germany) and a 5-mm segment was excised. The arterial graft was removed from the preservation solution and immediately interpositioned into the defect of the host carotid artery. A sample of the preservation solution was obtained for culture. All implants were anastomosed end-to-end with 11-0 Dermalon™ (Cyanamid, Gosport, Hampshire, UK) interrupted sutures (BV-4 needle) (Cobbet technique). After hemostasis was secured by gentle pressure, patency was determined by direct inspection. The wound was then irrigated with saline and closed with 3-0 Dexon™ sutures. The rats were given 1.5 ml Hartmann's solution, subcutaneously, together with 0.005 mg buprenorphine (Temgesic™, Reckitt and

Colman, Kingston-upon-Hull, UK) as pain medication. The rats had free access to standard food and water.

Autografts and Allografts

Arteries were used from an inbred strain of syngeneic Wistar rats (200 g), for autograft implantation studies. Grafts were stored in UW or PBS at 4°C during three- ($2 \times n = 6$), seven- ($2 \times n = 6$), or fourteen days- ($2 \times n = 6$). For allograft implantation studies, arteries from Brown Norway rats weighing 200 g, were grafted into Wistar rats. These grafts were also stored in UW or PBS at 4°C for three- ($2 \times n = 6$), seven- ($2 \times n = 6$), or fourteen days ($2 \times n = 6$). Control studies were performed using fresh arterial autografts ($n = 6$). After 28 days of observation, the grafts were assessed for patency by inspection, excised, and prepared for LM and SEM.

STATISTICAL ANALYSIS

The data of viability studies were analyzed with the unpaired, double-sided Student's t-test. Statistical significance was considered at $p < 0.05$.

RESULTS

Functional Responses after 1 Hour, 24 Hours, 72 Hours, 7 Days, and 14 Days of Storage

In freshly dissected aortic rings ($n = 29$), baseline values for the contractile responses to 40 mM KCl and 100 µM L-phenylephrine were 9.5 ± 0.4 mN and 10.3 ± 0.5 mN, respectively.

Smooth Muscle Contractile Response to 40 mM KCl

After 1 hour of storage in PBS, aortas showed a stronger contractile response than UW-stored aortas ($p < 0.05$). Mean values were 8.2 ± 0.5 mN for UW and 11.4 ± 1.0 mN for PBS. After 24 hours in UW, the contractile response was 9.2 ± 0.6 mN, whereas no contractile response could be evoked in the PBS-preserved aortic rings. Values established beyond 24 hours of storage in UW were 4.8 ± 0.6 at 72 hours, 2.3 ± 0.6 mN at 7 days, and 0.5 ± 0.2 mN at 14 days (Fig. 1).

Smooth Muscle Contractile Response to 100 µM L-phenylephrine

Mean values of α_1-receptor mediated contractions in rat aortic rings stored in UW and PBS for 1 hour amounted to 8.5 ± 0.5 mN and 12.8 ± 1.1 mN, respectively. A significantly stronger contractile response was observed in the PBS preparations than in the UW group ($p < 0.05$). In the UW-stored aortas, a decrease in contractility was measured compared to baseline values ($p < 0.05$). After 24 hours, the contractile response had further decreased to 6.4 ± 0.6 mN in the UW group whereas aortas stored in the PBS group showed no response to phenylephrine at all. Beyond 24 hours of storage in UW, values were 3.9 ± 0.6 mN at 72 hours, 2.7 ± 0.5 at 7 days, and 0.5 ± 0.2 at 14 days (Fig. 2).

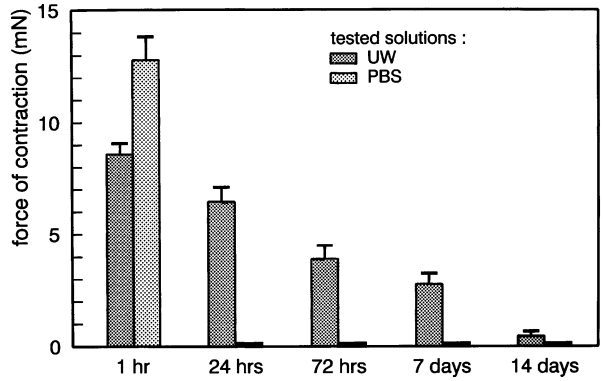

Fig. 2. Graph showing the mean values (± SEM) of stimulated contractile responses to 100 µM L-phenylephrine in aortic preparations, examined at 1 hour, 24 hours, 72 hours, 7 days, and 14 days of storage at 4°C. UW: University of Wisconsin organ preservation solution; PBS: phosphate buffered saline (pH 7.4, NaCl 0.9%).

Endothelium-Dependent Relaxation to 100 µM Methacholine

Rat aortas stored in UW and PBS for 1hour showed endothelium-dependent relaxation of 94.1 ± 2.8% and 74.6 ± 4.2% of the preconstricted level, respectively. These results indicate, that already one hour of storage in PBS, induced a significant loss of endothelial function ($p < 0.05$), as reflected by the blunted response to methacholine. The relaxant effect of methacholine was better preserved in UW. After 24 hours of storage, values were 96.1 ± 2.1% in UW. In the PBS group, at this stage, experiments were abandoned, since no initial contraction could be evoked. Beyond 24 hours of storage in UW, values were 84.6 ± 5.1 after 72 hours, 59.1 ± 13.1% after 7 days, and 55.3 ± 4.0 after 14 days (Fig. 3).

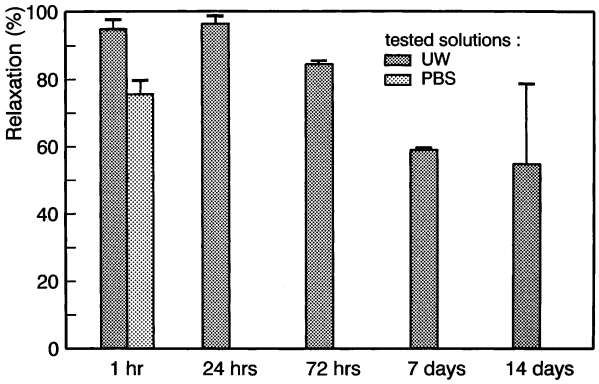

Fig. 3. Graph showing the mean values (± SEM) of 100 µM methacholine stimulated endothelium dependent relaxation in aortic preparations, examined at 1 hour, 24 hours, 72 hours, 7 days, and 14 days of storage at 4°C. UW: University of Wisconsin organ preservation solution; PBS: phosphate buffered saline (pH 7.4, NaCl 0.9%).

Morphology after 1-14 Days Storage

Light microscopy. The vessel wall of UW-preserved carotid arteries (n = 10) was largely unaffected after 7 days of storage. The media showed smooth muscle cells (SMCs) with normal nuclei. The lamina elastica interna (LEI) was constricted as evidence of normal contractility of the vascular wall in response to the fixative. Elastin content and structure of the LEI as well as the elastin fibers in the media appeared unaffected. PBS-stored grafts (n = 10) showed severe destruction of the vascular wall as of the first day of storage, consisting of disintegration of SMC nuclei in the media, disappearance of cytoplasm, and edematous degeneration of the vessel wall. The LEI and the elastin fibers in the media also showed disintegration.

Scanning electron microscopy. The endothelial layer, examined by SEM, was largely intact after 9 days of storage in the UW-preserved arteries. In the PBS-stored arteries, however, loss of endothelium was apparent at day one. After 3 days of storage in PBS, no remaining endothelium could be identified. Thus, the University of Wisconsin solution preserved function and morphology of carotid arterial grafts up to 7 days of storage. After this time, a gradual decline in quality was observed. The grafts stored in PBS, showed an immediate decline of function and morphologic quality, as of the first hour of storage, this process being completed at 24 hours.

Transplantation Experiments

One-month patency (28 days). After opening the wound the carotid artery was carefully exposed and pulsations as well as the direction of blood flow were established in the grafted segments. One-month patencies are summarized in Table II. Best results were found in the UW-preserved autografts. Of 18 implanted UW-preserved autografts 13 grafts were patent at one month implantation. In the PBS autografts, seven of 18 implanted grafts were patent after one month. In the UW-preserved allografts, eight of 18 implanted grafts were patent and in the PBS group only three of 18 implanted allografts were patent at one-month implantation. In the control group (n = 6) one graft was occluded.

Morphological evaluation of grafts. Occluded grafts were usually thrombosed or reduced to a white-yellowish string of tissue that could not be retrieved for morphologic evaluation. Morphological evaluation, therefore, was based on patent grafts (see Table II).

Light microscopy. Control fresh autografts showed normal morphology. The vessel wall of the UW-preserved autografts appeared normal. SMCs looked normal and elastin structure was intact. In contrast, cellular elements were lacking in the PBS-stored autografts, being reduced to an empty matrix. The elastin structure was damaged. Allografts preserved in UW showed lymphocellular infiltrates in the intima, subintimally, and in the media after 28 days of implantation. In the adventitia, perivascular infiltration also contributed to the rejection response of the blood vessel. The SMCs were in poor condition showing signs of media necrosis. The LEI and elastin structure of the media were severely damaged. Destruction of the vascular wall was still more evident in the PBS-stored allografts, although lymphocellular infiltration was of milder intensity.

Scanning electron microscopy. All patent grafts, either syngeneic or allogeneic, showed a smooth, continuous layer of endothelial cells covering the anastomoses and the luminal surface of the graft. In the UW-preserved autografts, morphology of the endothelial cells was normal with endothelial cell nuclei protruding into the lumen and aligning to the direction of blood flow. In the UW-preserved allografts the endothelial layer, although confluent, showed involution of microvilli as well as nuclei. In the patent, PBS-stored autografts and allografts, endothelial cells, obviously of host origin, covered the entire length of the graft.

In summary, the UW-preserved autografts per-

Table II. Patency rates of experimental group

Storage[a]	UW-auto[b]	PBS-auto[b]	UW-allo[b]	PBS-allo[b]
3 days	5/6 (83.3)	2/6 (33.3)	4/6 (66.6)	1/6 (16.7)
7 days	4/6 (66.6)	2/6 (33.3)	2/6 (33.3)	0/6 (0)
14 days	4/6 (66.6)	3/6 (50)	2/6 (33.3)	2/6 (33.3)
Controls[c]	5/6 (83.3)			

All groups: $n = 6$, morphological evaluation was performed on patent grafts.
[a]Time of storage.
[b]Patency (%) (28 days).
[c]Fresh arterial autografts.

formed best in terms of patency and morphology, however, rejection was histologically more pronounced in the UW-preserved allografts than in the PBS-stored allografts.

DISCUSSION

Carrel[8] was the first to transplant arteries and organs. Initial enthusiasm, however, was tempered when Szilagyi and coworkers demonstrated that the ultimate fate of transplanted arteries was disintegration due to rejection phenomena.[10,11] When in 1948, Kunlin[19] performed the first peripheral reconstructions with autologous vein, the concept of arterial transplantation for *peripheral arterial reconstructions* became obsolete.

Another major obstacle, apart from rejection, was the need to preserve arteries for transplantation.[9,15,16] In this area two concepts can be discerned: preservation of a blood vessel with maintained viability and hence, maintained antigenicity, or preservation of a blood vessel in which viability is diminished and only the connective tissue matrix is preserved. In this study the artery as a whole is preserved as is the goal of solid organ preservation. The UW solution was used for the preservation of arteries since it is the preservation solution of choice in organ transplantation.[20]

It was demonstrated that the UW solution preserves rat arteries morphologically as well as functionally, at least up to 7 days. Grafts that were stored in PBS showed functional and morphological signs of deterioration within 1 hour, resulting in complete disappearance of all functional responses after 24 hours. In accordance with these observations, performance of UW-preserved autografts after implantation, was superior to PBS autografts. UW-preserved allografts clearly showed morphological signs of rejection. The PBS-preserved allografts showed milder lymphocellular infiltration accompanied with a further disintegration of the vascular wall.

In coronary and peripheral arterial bypass surgery, the condition of the endothelial layer of autologous grafts is recognized to be important for early and late graft function.[21-23] In this study, assessment of the retained endothelium-dependent response to methacholine, together with the assessment of the stimulated smooth muscle contractile responses to KCl and phenylephrine, proved a valuable tool in determining viability of arteries after storage. UW-preserved aortic preparations were functional at 3 days of storage. PBS-stored grafts lost 30% of stimulated contractile and relaxation responses in the first hour. These observations corroborate the notion of other investigators, that saline is not an appropriate intraoperative solution for the storage of free saphenous vein grafts.

Scanning electron microscopy is the most appropriate method to study the morphological quality of the endothelial layer of the vessel whereas light microscopy is useful to assess the quality of the vessel wall. Evaluation with light and scanning electron microscopy showed that morphological integrity was preserved after 9 days of storage in UW. In grafts stored in PBS, structural integrity was seriously compromised within the first day of storage.

The advent of potent immunosuppressive agents has greatly attributed to improved graft survival in organ transplantation. Hence, arterial transplantation should be feasible too, since the survival of an organ, in part, depends on the integrity of *the main artery in the vascular pedicle of the organ.* In the present study, syngeneic grafts and allogenic grafts were used to distinguish between factors that were a result of preservation and rejection. As could be expected on the basis of results obtained after evaluation of preservation efficacy, the UW-preserved autografts showed better patency and morphological results than PBS-stored autografts (see Table II). The UW allografts showed severe

lymphocellular infiltration in all layers of the grafts, indicative of rejection. The PBS-stored allografts showed a similar, but milder pattern of rejection. The results in the allograft groups can be explained by the fact that UW-preserved allografts were implanted as viable grafts eliciting a stronger rejection response due to the retained cellular elements. Since cellular components had been lost during storage in PBS, a milder cellular rejection was seen at 28 days of implantation of PBS-stored allografts. In the implantation studies performed in this study, quite a few failures occurred in UW autografts despite the fact that these grafts were implanted in a high-flow artery, i.e. the carotid artery, and no immunologic responses were in effect. Thrombus formation at the microanastomotic region may have been the cause.[24]

In all patent grafts, confluent endothelial layers were seen. As is known of rat endothelial healing,[25] repopulation with endothelial cells readily occurs. In the UW-preserved allograft groups, it seemed that donor endothelium was persisting, since after host reendothelialization, a new, better quality endothelial layer would have been expected. In the PBS-stored autografts and allografts, it was observed before implantation that the endothelial layer was lacking. In these groups, the neoendothelial layer was of good quality, obviously, of host origin. Host lateral ingrowth of smooth muscle cells and fibroblasts has been described.[14] At 28 days implantation it was unclear whether the observed smooth muscle cells in the UW autografts belonged to an implanted population of cells or were replaced by ''new'' cells. The same question stands for the fresh autologous control grafts.

In conclusion, the endothelial layer and vessel wall are preserved, functionally and morphologically, in the UW solution for up to 7 days of storage. After storage in PBS, 30% of function of the endothelial layer was lost in the first hour of storage and after 24 hours no function could be detected. The best patency results were accordingly obtained with UW-preserved autografts. UW-preserved allografts were subject to degradation as a consequence of rejection. Future research should focus on some degree of immunosuppressive protection since the use of preserved arterial allografts may be feasible when immunologic rejection is controlled.

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