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Morphology and Function of Dog Arterial Grafts Preserved in UW-Solution*

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Objectives: *To assess the function of arterial grafts after prolonged preservation in the University of Wisconsin solution (UW),* in vitro *and* in vivo.
Methods: *Carotid arteries were harvested from dogs and stored for 1–21 days at 4°C in UW (n = 10) or in PBS (0.9% NaCl, pH 7.4), (PBS) (n = 10). Slices were examined by lightmicroscopy (LM) and scanning electron microscopy (SEM). For viability testing, specimens were connected to an isometric force transducer (2 × n = 9). Contractile and relaxation responses were examined by adding phenylephrine (200μM) and metacholine (200μM), respectively. For* in vivo *studies (n = 41), 2.5cm carotid artery segments were implanted orthotopically, as autografts and allografts, after 14 days of storage in UW or in PBS. Autologous veins were used as controls. After 28 days or 56 days, arteriography was performed and the grafts were excised for LM and SEM.*
Results: *The arterial endothelial layer remained intact after up to 14 days of storage in UW. In PBS, the endothelium was lost after 3 days. The functional response after 14 days storage in UW was approximately 50% vs. 0% after 14 days in PBS. In the autografts, total patencies (28 days + 56 days) were 100% (8/8) and 63% (5/8) for UW and PBS stored grafts, respectively. In the allografts, the UW and PBS preserved grafts showed total patencies of 86% (12/14) and 83% (5/6), respectively. Microscopically, the allografts showed fibrotic degeneration.*
Conclusions: *Arteries are well preserved in UW up to 14 days of storage. Arterial autografts preserved in UW showed good patency and better integrity of the vessel wall after implantation, than grafts stored in PBS or allografts (without immunosuppressive therapy).*

Key Words: Viability; Tissue preservation; Arterial; Transplantation; Autograft; Allograft.

Introduction

In patients with critical limb ischaemia, the autologous vein is the conduit of choice in distal bypass surgery.[1] Absence of autologous vein following previous bypass surgery is a reason to search for lesser saphenous or arm veins, since these grafts have a higher patency rate compared to prosthetic grafts.[1–3] Thus, the ideal alternative to the autologous vein graft is still lacking. A vascular transplant is an attractive alternative and this concept has evoked a large body of research. Procured venous allografts are being applied extensively, although all with modest results,[4–6] partly due to intimal hyperplasia[7] and rejection.[8,9] Less attention, however, has focused on the use of arteries for grafting.[10] Arterial grafts when retaining viability, have the potential advantage of displaying normal longitudinal elasticity and more favourable flow related variables and anastomotic behaviour.

Transplantation of viable arteries, however, is hampered by graft rejection.[11,12] Other problems are the retrieval of arterial allografts and also the preservation of these arteries in order to bridge the time between harvesting and implantation. Recent advances in immunosuppressive therapy[13,14] and techniques in organ preservation[15,16] have shed new light on these problems and have renewed interest in what seems, a valid concept. The advent of the University of Wisconsin (UW) preservation solution, currently the organ-preservation solution of choice,[17] has opened-up the possibility of the cold storage of whole organs for prolonged periods of time. Applied to arterial grafts,

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this technique, but also other preservation methods such as cryopreservation,[10,18] could lead to a blood vessel bank to meet the needs and demands in vascular reconstructive surgery. The storage of blood vessels for prolonged time periods with preservation of vessel wall viability and endothelial function, is crucial to this concept. With this in mind we initiated two series of studies in dog carotid arteries. The first series involved *in vitro* evaluation of the influence of the UW preservation solution on arteries. The efficacy of this solution was compared to an indifferent solution, i.e. phosphate buffered saline. The latter solution is often used for storage of free autologous vein grafts during reconstructive, vascular procedures. The second series included implantation studies in which UW- as well as PBS stored arterial grafts were implanted during one- and two months as autografts and as allografts. Autologous veins served as controls.

Material and Methods

Preservation of arterial grafts

Carotid arteries were harvested from female mongrel dogs (24kg + /– 3kg) and preserved in the University of Wisconsin solution (Viaspan TM, Du Pont Pharmaceuticals, Wilmington, DE, U.S.A.) (UW) at 4°C, 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) (Welcome™) 0.5mg/ml (PBS). The composition of the UW solution has been described elsewhere.[19] Viability of the preserved arteries during storage was tested by assessing the contractile and relaxation response of the blood vessel. Morphological changes in the vessel wall were assessed by light microscopy (LM) and the endothelium was investigated by scanning electron microscopy (SEM).

In vitro studies

Viability assessment

Contraction as a measure of vascular smooth muscle function was examined as a parameter of viability. Relaxation through the stimulated secretion of endothelium derived relaxing factor (EDRF) was assessed as a measure of endothelial function. Arterial segments were randomised and stored either in UW ($n = 9$) or PBS ($n = 9$) and kept at 4°C for a maximum period of 63 days. At 1, 7, 21, 28 and 63 days, respectively, a slice was cut off for testing. In this fashion each artery served as its own control. Slices were mounted as ring preparations between a rigid rod and an isometric force transducer.[20] The slices were then placed in thermostatically controlled (37°C), 5 ml organ baths containing 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, $NaHCO_3$ 24.0, glucose 5.5. The medium was maintained at 37°C and pH was kept at 7.4 by gassing the solution with carbogen (95% O_2 : 5% CO_2). The initial resting tension was set at 20 mN and was continued throughout the experiment. Isometric contractions of the preparations were measured by means of force transducers (Kyowa™, Tokyo, Japan) connected to amplifiers (Kyowa™, Tokyo, Japan) and recorded on a thermal pen recorder (WKK™, Kaltbrunn, Germany). The experimental protocol was as follows: After 60 min of equilibration the preparations were exposed for 10min to a high potassium concentration (120 mM), to induce contraction. Subsequently, this solution was replaced by normal Tyrode's solution. With 60 min intervals, this procedure was repeated twice with the high potassium solution and once with phenylephrine (100μM), an α_1-adrenoreceptor agonist. In order to test endothelium-dependent relaxation, metacholine (100 μM), a stable derivative of the muscarinic agonist acetylcholine, was added upon this contraction. The contraction induced during the third exposure to high potassium and the phenylephrine-induced contraction were expressed as absolute force of contraction in mN. The endothelium-dependent relaxation induced by methacholine was quantified as percentage of the phenylephrine contraction. The data are presented as the mean values (± S.E.M.) of nine experiments.

Preparation of grafts for LM and SEM

Segments of preserved artery were cut and for LM, placed in 10% buffered formaldehyde and subsequently embedded in paraffine. 5μm sections were cut and stained with Hematoxilin-Eosin (HE). For SEM, slices of preserved artery were cut and placed in Karnovsky's fixative for 24 h. 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™, Japan) at 10 kV accelerating voltage.

Transplantation experiments

Segments of UW and PBS stored carotid arteries were

orthotopically implanted into female, mongrel dogs (24kg + /− 3kg)(Table 1). The animals were premedicated with ketamine (Ketalar™) 5mg/kg, xylazine (Rompun™) 2mg/kg and atropine 0.5mg. Subsequently, they were intubated and were given inhalation anaesthesia with a mixture of NO_2/O_2 (1:1) and halothane (Fluothane™) 1–1.5%. Sufentanyl (Sufenta™) 0.6μg/kg/h was administered intravenously. Using a median incision in the neck, the right and/or left carotid artery was exposed by splitting the sternocleidomastoid and long neck muscles. The artery was dissected free taking care not to damage the adjacent vagal nerve, crossclamped and a 2.5 cm segment excised. The arterial graft was removed from the preservation solution and immediately interpositioned into the defect of the host carotid artery. All implants were anastomosed end-to-end with a running 7-0 Prolene™ suture. At the level of the proximal and distal anastomosis, surgical clips were fixed to the surrounding tissue as markers during angiography. After completion of the interposition graft, suction drains were positioned near the graft. Postoperatively, long acting Clamoxyl™ (amoxicillin) 150mg/10kg was administered three times for 1 week. Postoperative pain was managed by buprenorphine (Temgesic™) 0.01–0.02mg/kg for 2 days. No immunosuppressive or anticoagulant agents were given postoperatively. The dogs were observed for 28 and 56 days (Table 1). The grafts were assessed for patency on a daily basis by placing a Doppler probe (Parks Electronics Lab., Beaverton, Oregon, U.S.A.) in the neck at the site of the implanted blood vessel. Before sacrifice, angiography of the carotid arteries was performed (Seldinger technique, 30ml of contrast medium (1:1 H_2O) (Radiopaque™)).

Autograft and allograft studies

For autotransplantation, segments of 2.5cm length were excised from both carotid arteries. The right segment was stored in UW at 4°C, the left segment in PBS at 4°C (Auto UW, Auto PBS) (Table 1). The vessel stumps were approximated and were temporarily anastomosed, end-to-end with a running 7-0 Prolene™ suture. After 14 days of cold preservation, the grafts were re-implanted after excision of the site of the temporary anastomosis. Each preservation medium was cultured for bacterial growth. For allograft studies, donor arteries were obtained from Mongrel dogs in the laboratory at sacrifice, or during an allograft implantation procedure. The allogeneic arteries were implanted after 14 days of storage in UW in PBS both at 4°C (Allo UW, Allo PBS)(Table 1). Control experiments, were performed by excising autologous, external jugular vein segments ($n = 5$) and using them as interposition grafts in the carotid artery (Auto vein)(Table 1).

Table 1. Summary of experimental groups and patency rates

Group	*n*	28 days	56 days	Total patency rate
Auto UW	8	100% (5/5)	100% (3/3)	100% (8/8)
Auto PBS	8	40% (2/5)	100% (3/3)	63% (5/8)
Allo UW	14	71% (5/7)	100% (7/7)	86% (12/14)
Allo PBS	6	67% (2/3)	100% (3/3)	83% (5/6)
Control	5	100% (5/5)		

For *in vivo* studies, UW and PBS stored grafts were implanted as autografts and as allografts. Autologous veins served as control.

Preparation for microangiography

At sacrifice, a barium contrast solution (Micropaque™) was flushed through the aorta of the animal replacing all its blood. The grafts were then excised and fixed in 10% formaldehyde. 3mm rings were cut from the midgraft area and placed on Kodak™ high resolution glass plates and exposed with 25 kV and 25 mA for 20min. The plates were viewed with a low-power microscope.

At sacrifice, the implanted grafts were grossly inspected. An assessment for patency was performed palpating the graft *in situ*. Signs of rejection, inflammation and scar tissue formation were taken into account. After excision, the implants were prepared for SEM and LM evaluation as described above.

Morphometric analysis

Morphometric analysis was performed in order to compare the various groups of vascular preservation and implanted grafts (Table 1). The intima was examined for endothelium covering the graft and quality of the endothelial cells, i.e. flattening of the nuclei and subintimal hyperplasia. The media of the vessel wall was assessed taking into account the quality of the smooth muscle cells, tissue integrity, presence of oedema and cellular infiltration. The adventitia was examined for cellular infiltration and the presence of fibrotic tissue. The proximal, middle and distal third were examined in up to five locations. The specimens were scored according to a 0–3 scale: 0 = bad quality; 1 = poor quality; 2 = moderate quality; 3 = good quality.

Statistical analysis

The data were analysed with the unpaired, double-sided Student's *t*-test. The null hypothesis was rejected at the 5% level.

Results

In vitro experiments

Viability assessment

After 24 h of storage in PBS-solution, no functional response could be evoked from the vessel ring preparations. Therefore, no observations beyond 24 h were made with regard to the grafts stored in PBS. In the UW group of grafts, significant preservation of contractile response to KCl and phenylephrine and relaxation response to methacholine (Fig. 1), was observed ($p < 0.0001$). The functional response to KCl, phenylephrine and metacholine after 21 days storage in UW, expressed as percentage of the response on day one, was 35 ± 13%, 70 ± 17% and 51 ± 16% (means ± S.E.M., $n = 9$), respectively.

Microscopy

The vessel wall of UW-preserved arteries ($n = 10$) was unaffected after up to 14 days of storage. PBS stored grafts ($n = 10$) showed severe destruction of the vascular wall after the first day of storage, with disintegration of nuclei, disappearance of cytoplasm and edematous degeneration.

The endothelial layer, examined by SEM, was largely intact after up to 14 days of storage in the UW preserved arteries. In some parts, damage to the endothelial cytoplasm was observed, occasionally disclosing the underlying basal lamina (Fig. 2). In the PBS stored arteries, however, loss of endothelium was apparent at day one and after 3 days of storage no endothelium could be identified. (Fig. 3).

Implantation experiments

At the time of implantation, after 14 days of preservation, the UW-preserved arteries were in good condition. The cultured preservation media were all free from contamination. Patent grafts generated Doppler signals in the arterial frequency range. When occlusion occurred, it usually presented within the first 7 days of implantation.

Angiography

Table 1 shows the patency rates of auto- and allograft implantation. After 28 days, all UW preserved autografts were patent (5/5), whereas in the PBS group only 40% was patent (2/5). Patency of the UW stored and PBS stored allografts was 71% (5/7) and 67% (2/3), respectively. When patent, the angiography showed smooth and widely patent anastomoses (Fig. 4). At long term observation (56 days), both the UW and PBS stored autografts also showed patency rates of 100% (7/7) and 100% (3/3), respectively. To summarise, in the autograft groups, total patencies (28 days group plus 56 days group) were 100% (8/8) (UW Auto) and 63% (5/8) (PBS Auto), respectively. In the allograft groups, total patencies were: 86% (12/14) (UW allo) and 83% (5/6) (PBS Allo).

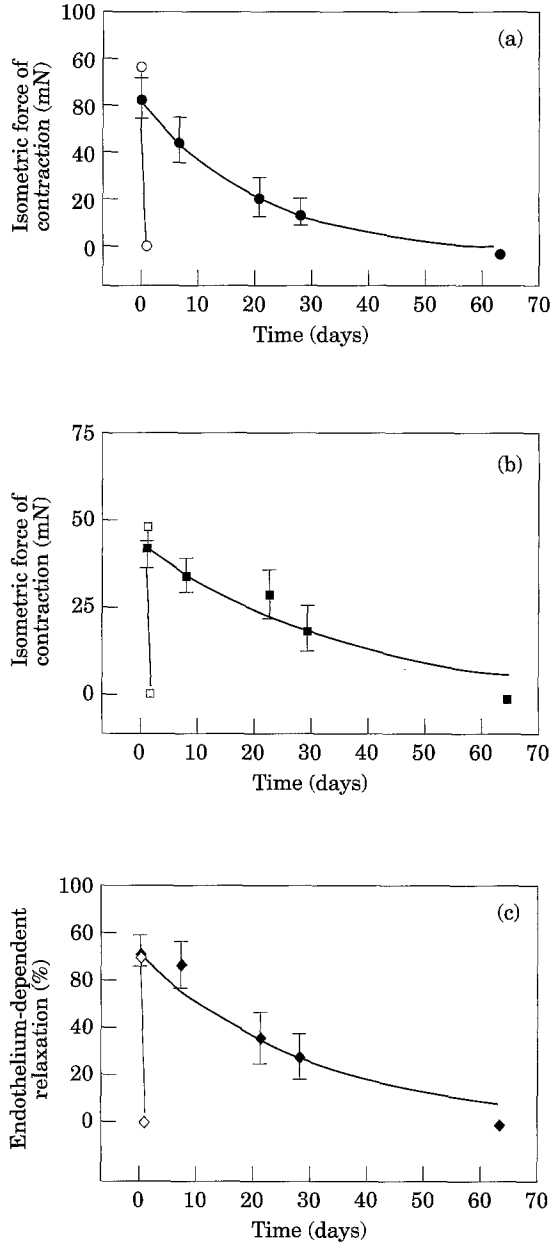

Fig. 1. Responses to KCl 120 mM (a)(●) l-phenylephrine 100 μM (b)(■) and methacholine 100 μM(□) (c)(◆) of isolated arterial segments stored in UW solution and stored in PBS (◇). The functional response to KCl, phenylephrine and metacholine after 21 days storage in UW, expressed as percentage of the response on day one, was 35 ± 13%, 70 ± 17% and 51 ± 16% (means ± S.E.M., $n = 9$), respectively.

Macroscopical examination

At gross examination, a clear difference was visible between the UW stored autografts and the PBS stored autografts. The UW stored autografts, after excision, showed a normal resilient vessel wall with a glistening luminal surface. The PBS stored autografts were dilated and on cutting the grafts open, showed a weak, distended vessel wall that had lost its elasticity. The luminal surface, however, had the same glistening aspect, suggesting a smooth neo-endothelial lining. Both the UW and PBS stored allografts showed various stages of fibrous tissue reaction and also the vessel wall was thinned and dilated as compared to the native vessel. At long term observation similar results were obtained except that the fibrous reaction surrounding the grafts was less extensive as compared to the short term series.

Microscopical examination

On SEM, all patent grafts showed a smooth, continuous layer of endothelial cells covering the luminal surface of the graft. The quality, however, differed in the following respects: The UW autografts, after short term as well as long term implantation, displayed a continuous endothelial layer with good quality endothelial cells protruding into the lumen and aligning to the direction of the blood flow. The UW allografts also displayed intact layers except that the quality of cells was poor, characterised by flattened nuclei and an overall disappearance of contour. In the patent, PBS stored grafts (PBS auto- and allografts) the neo-endothelial cells presumably of host origin, were elongated and seemed to slide over the previously denuded surface (Fig. 5). In the autologous vein grafts (Augo vein) a normal venous endothelial layer was visible.

On light microscopy, the vessel wall of the UW stored autografts after 28 days of implantation had a normal appearance with viable cells amidst the connective tissue fibres. The adventitia and perigraft

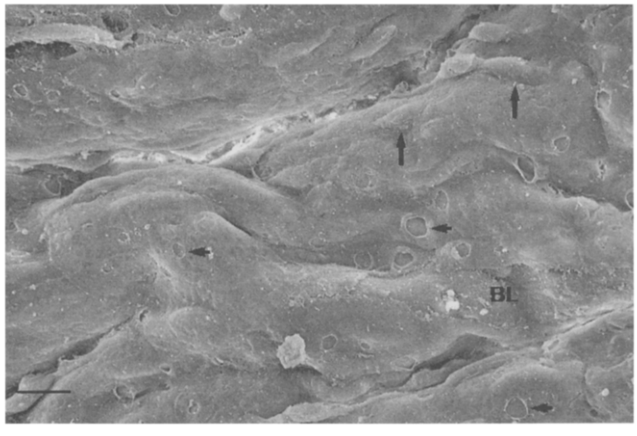

Fig. 2. Scanning electron micrograph of the endothelial surface of an arterial segment preserved in UW for 14 days (scale: 10μm). The endothelial layer was largely intact. In some parts, damage to the endothelial cytoplasm was observed (arrow heads), occasionally disclosing the underlying basal lamina (BL). Nuclei: (arrows)

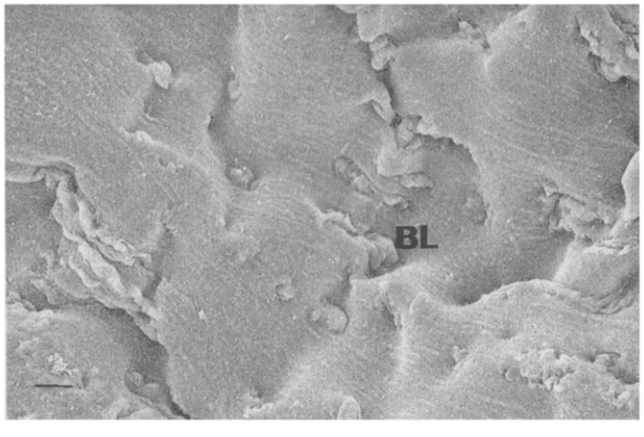

Fig. 3. Scanning electron micrograph of the endothelial surface of an arterial segment stored in PBS for 14 days (scale: 10μm). The complete loss of endothelium is evident. Examination of the denuded luminal surface shows the basal lamina only.

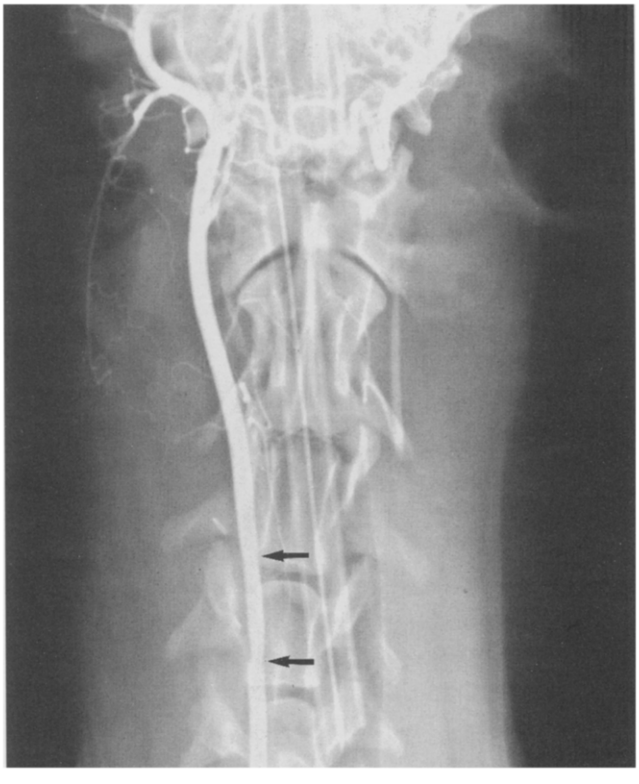

Fig. 4. Angiography of a patent graft preserved in UW for 14 days, after 28 days of implantation. Site of anastomoses shown by arrows. Visualisation of the right carotid artery only.

region showed minimal fibrotic reaction. After 56 days of implantation, these UW preserved autografts still looked normal. In UW preserved allografts, the quality of nuclei of the smooth muscle cells was poor, combined with loss of integrity and slight oedema. The grafts in this group showed a mononuclear cellular infiltration in the vessel wall and perigraft region. Fibrosis was severe at 1 month, although the intensity of the reaction varied between dogs. After 2 months infiltration was less, indicating disappearance of antigenic elements.

In the PBS stored autografts the media showed further deterioration as compared to directly post-preservation. An empty matrix was seen without any cellular elements and poor integrity of the connective tissue matrix. At long term observation, an extensive fibrotic degeneration of the vessel wall had developed. The adventitia in this group showed slight fibrosis. The PBS allograft group also lacked cellular elements and showed loss of vessel wall integrity. A mild lymphocellular reaction could be observed. At long term observation, further vessel wall degeneration was evident. In the adventitia, fibrosis was similar to the autografts at 1 month. A mild lymphocellular infiltrate was visible at this time, that had disappeared after 2 months.

Control autologous veins (Auto vein) displayed an intact luminal surface with endothelial cells of typical venous phenotype and slight subintimal hyperplasia. These control grafts showed normal integrity and cellular composition of the vascular wall. A moderate fibrotic reaction was invariably observed in the perigraft region.

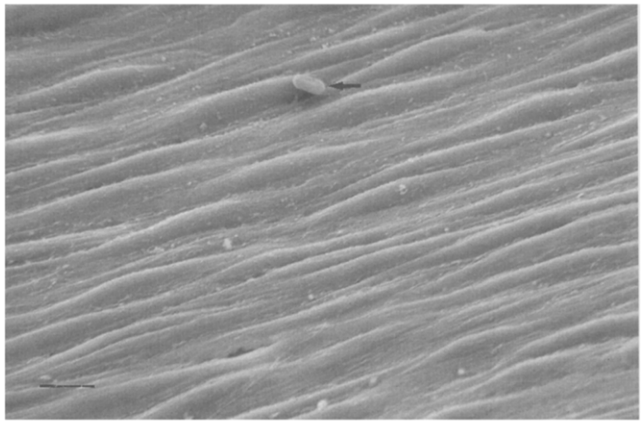

Fig. 5. Scanning electron micrograph of an autograft which had been stored in PBS during 14 days. The midgraft region is shown after 28 days of implantation, displaying a confluent, active endothelial layer with microvilli (scale: 10μm). The endothelial cells are obviously of host origin, since after 14 days of storage, all native endothelial cells were demonstrated to have been lost. Arrow at erythrocyte.

Morphometric analysis

The morphological results are summarised in Table 2. In all groups an endothelial layer was observed. The UW autografts (Auto UW) showed the best quality endothelial layer. An endothelial layer was seen also in the UW allografts (Allo UW), although this was of inferior quality. Obviously, a neo-endothelial layer had developed in the PBS groups (Auto- and Allo PBS), since prior to implantation, this layer was found to be absent. The smooth muscle cells in the media of the vessel wall were largely intact in the UW preserved autografts in contrast to the other groups. No fibrosis was seen in the adventitial layer of the UW preserved autografts. Data are shown as average values (± S.E.M.).

In the UW autograft group, the microvasculature in the media and adventitia appeared normal as compared with controls (Fig. 6). The microvasculature showed less density in the PBS autografts. In the UW allografts, the microvasculature was present predominantly in the adventitial layer. In the PBS allografts, the microvasculature was also limited to the adventitial layer, but, was less densely arranged.

Discussion

The idea of substituting a diseased artery with an arterial transplant, is not a new one.[21,22] An artery would seem to be the ideal graft for replacing an artery since it allows for unaltered mechanical and flow related characteristics of the vessel. In addition, a graft of arterial origin might adapt to growth changes when used as arterial conduits in children. Fresh, allogeneic vessels have never found widespread application, however, since graft rejection invariably leads to graft failure.[11,12]

The advent of potent immunosuppressive agents, have greatly contributed to improved graft survival in organ transplantation and this puts the issue of arterial grafting in a new light. Since the vascular connections of the transplanted organ in the recipient is via middle-large vessels such as the renal or hepatic artery, long term patency of the artery is a prerequisite for extended graft function. That these vessels can remain patent for many years is exemplified by reports of kidney and liver grafts showing continuous function for as long as 27 years.[23]

Harvesting of segments of artery or even whole arterial trunks during organ procurement for use as vascular grafts, is a feasible prospect, just as it is common to procure heart valves, bone, skin and cornea. For cadaveric arteries to become useful, there

Table 2. Summary of morphometric analysis

Group	Auto UW (n=8)	Auto PBS (n=5)	Allo UW (n=12)	Allo PBS (n=6)	Auto vein (n=5)
Intima					
Intact layer (%)‡	100	100	81	100	100
End. Cells§	2.7±0.2*	2.2±0.3††	1.1±0.2	3±0.0††	3±0.0
Media					
S.M. cells‖	2.8±0.2†	0.4±0.2	0.8±0.2	0.5±0.2	2.8±0.2
Wall integrity¶	2.6±0.2†	0.6±0.2	1.0±0.2	0.3±0.2	2.8±0.2
Adventitia					
Fibrosis**	0±0.0†	2.0±0.5	2.6±0.2	2.2±0.4	1.8±0.2

* $p<0.0001$ compared to Allo UW.
† $p<0.0001$ (compared to the other experimental groups).
‡ Intact layer (%): percentage of interior graft surface covered with endothelium.
§ End. cell quality: 0=no endothelial cells, 1+=poor quality, 2+=moderate quality, 3+=good quality.
‖ Smooth muscle cell quality: 0=no S.M. cells, 1+=poor quality, 2+=moderate quality, 3+=good quality.
¶ Wall integrity: 0=no integrity, 1+=poor integrity, 2+=moderate integrity, 3+=good integrity.
†† Neo-endothelium.
** Fibrosis: 0=no fibrotic tissue, 1+=slight fibrotic tissue, 2+=marked fibrotic tissue, 3+=severe fibrotic tissue.
Morphometric findings are expressed as mean values (±S.E.M.) of total scores using a 0–3+ scale. Experimental groups and their patencies are summarised in Table 1. The presented data are cumulative after 28 days and 56 days implantation. Control autologous veins were implanted for 28 days only.

are two practical issues that need to be resolved. First is the question of preserving the artery, thus to allow storage of the vessel for longer time periods. Secondly, the question of immunosuppression arises which needs investigation into the dose of immunosuppression required in view of the reduced antigenic load of a single artery in comparison to a solid organ. One may speculate that a lesser dose of immunosuppression is needed for a single arterial graft resulting in a decreased risk of potential side effects of this therapy. Another point that may be of relevance, is the development of graft tolerance, ultimately obviating the need of immunosuppression. This phenomenon has been observed in hepatic allografts after prolonged periods of continued graft function.[24] The present study addresses the first question, i.e. the possibilities for extended preservation of arterial grafts without loss of viability.

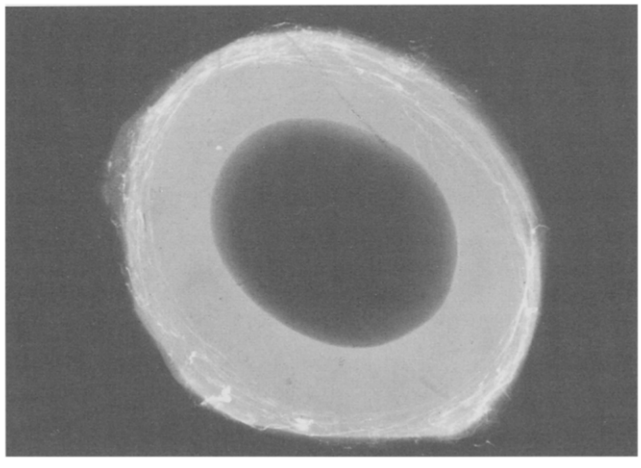

Fig. 6. Microangiography of a 14 days UW preserved autograft after 56 days of implantation. The vasa vasorum are well preserved and show the same configuration as in controls.

Following the concepts outlined above, an artery can be looked upon as organ tissue and consequently, should be treated according to the same principles that are applied in organ preservation. The "gold standard" in organ preservation to date is hypothermic storage of the organ in the preservation solution developed by the University of Wisconsin (UW).[16,25] In this solution, liver grafts can be preserved for 24h, kidneys for 48h and thyroid organ tissue for 7 days with retained function.[26,27] In the present study, arteries could be preserved at hypothermia in UW solution for 14 days whilst remaining morphologically and functionally intact. The efficacy of the UW solution for the preservation of blood vessels has been demonstrated previously but only for short periods of time (< 72 h).[28,29]

Measurement of muscle contractility of the vessel wall and relaxation as a parameter of endothelial cell function, proved a valuable tool in assessing the retained function of the artery during preservation. Scanning electron microscopy is the most appropriate method to study the quality of the endothelial layer of the vessel and light microscopy is useful to assess the vessel wall. The condition of the endothelial layer is important for early graft function and the prevention of thrombus formation, whereas the quality of the vessel wall determines late aneurysmatic degeneration.[11,12] PBS which is an indifferent solution with no preservation properties at all. The endothelial layer largely remained intact up to 14 days of storage in UW

solution and the functional response to contraction inducing and relaxing agents was at that time, more than 60% of its control value. The results of this test of function were consistent with the obtained morphological data.

Autogenous arterial grafts stored in UW solution were all patent after short term (28 days) and long term (56 days) implantation. In the group of dogs with UW stored allografts, receiving no immunosuppression, the patency of the grafts after short term and long term implantation was 71% (5/7) and 100% (7/7), respectively, showing no obvious differences. Since patency is dependent on the length of observation time and implantation periods in this study did not exceed 56 days, patency is not the only parameter to describe what occurred in the transplanted arteries.[13] Morphologically, a severe perigraft reaction with cellular infiltration and vessel wall degeneration was evident compatible with graft rejection. A similar fate of allografted vessels has been described in the literature.[11,12] Interestingly in this study, however, these phenomena had decreased after long term implantation, presumably because the allogeneic cells in the graft by that time had been cleared as the result of the elicited immune response. A remarkable finding in the PBS stored allografts was the lack of cellular infiltrate in the vessel wall, due to the fact that the native cells had been considerably diminished during the 14-day storage period, reducing the antigenic potential of the graft.

A fair number of grafts remained patent in spite of bad preservation (PBS groups) and/or rejection phenomena (allograft groups). Equally remarkable is the observation that the previously denuded luminal surface of the PBS stored grafts had been repopulated by host endothelial cells after implantation as was observed in the patent grafts. Daily Doppler assessment of the grafts showed that when occlusion occurred, it usually took place during the first week of implantation, presumably as a result of thrombosis of the graft. Therefore, when the graft remained patent during this first crucial period, a new endothelial lining could develop that permitted maintained patency of the graft. The observations described above, may at least in part be explained by the short length (i.e. 2.5cm) of the grafts and the relatively limited observation time (i.e. 56 days). Studies using 6cm UW preserved grafts and 4-6 months implantation time are currently underway, in conjunction with allograft studies applying three dose regimens of an immunosuppressive agent (Cyclosporin). The *in vitro* methods of graft assessment and the animal model described in this study, furthermore, provide the basis for coming studies focusing on modified preservation solutions, prolongation of preservation time and on other modalities of preservation such as cryopreservation.

In conclusion, the endothelial layer and vessel wall of arteries are well preserved in UW up to 14 days of storage, showing 60% of smooth muscle contractability as well as endothelial secretory capacity. The results of long-term, autograft implantation, accordingly show high performance of grafts preserved in UW. UW preserved allografts are subject to degradation as a result of rejection, in the absence of immunosuppressive therapy.

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