Interactive CardioVascular and Thoracic Surgery 23 (2016) 300–309
doi:10.1093/icvts/ivw056 Advance Access publication 10 April 2016

Cite this article as: Winkler B, Reineke D, Heinisch PP, Schönhoff F, Huber C, Kadner A *et al*. Graft preservation solutions in cardiovascular surgery. Interact CardioVasc Thorac Surg 2016;23:300–9.

Graft preservation solutions in cardiovascular surgery

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Received 30 September 2015; received in revised form 23 January 2016; accepted 10 February 2016

Abstract

Vein grafts are still the most commonly used graft material in cardiovascular surgery and much effort has been spent in recent years on investigating the optimal harvesting technique. One other related topic of similar importance remained more or less an incidental one. The storage solutions of vein grafts following procurement and prior to implantation are, despite their assumed impact, a relatively neglected theme. There is no doubt that the endothelium plays a key role in long-term patency of vein grafts, but the effects of the different storage solutions on the endothelium remain unclear. In a review of the literature, we could find 20 specific papers that addressed the question, of which the currently available preservation solutions are superior, harmless, damaging or ineffective. The focus lies on saline and autologous whole blood. Besides these two storage media, novel or alternative solutions have been investigated with surprising findings. In addition, a few words will be spent on potential alternatives and novel solutions on the market. As there is currently no randomized clinical trial regarding saline versus autologous whole blood available, this review compares all previous studies and methods of analysis to provide a certain level of evidence on this topic. In summary, saline has negative effects on the endothelial layers and therefore may compromise graft patency. Related factors, such as distension pressure, may outbalance the initial benefit of autologous whole blood or storage solutions and intensify the harmful effects of warm saline. In addition, there is no uniform consent on the superiority of autologous whole blood for vein graft storage. This may open the door to alternatives such as the University of Wisconsin solution or one of the specific designed storage solutions like TiProtec™ or Somaluthion™. Whether these preservation solutions are superior or advantageous remains the subject of further studies.

Keywords: Saphenous vein graft • Storage solutions • Coronary artery bypass surgery

INTRODUCTION

Coronary artery bypass grafting (CABG) surgery is widely performed, with an estimated 800 000 procedures worldwide each year. As is general known, several types of bypass grafts can be used. This procedure is one of the most studied medical treatments in the history of surgery with a tremendous number of trials, studies and registries addressing almost every detail of the operation. One of these is the type of graft. Many questions have been formulated: how to harvest a vein graft, the radial artery and one or both internal thoracic arteries, and which graft to choose for which coronary vessel to be bypassed?

For every CABG procedure, it is necessary to harvest at least one graft, but more frequently several grafts. These conduits are prepared with clips or ligations, flushed and stored at least for a short period of time between procurement and construction of the anastomosis. In contrast, the internal mammarian artery is often left *in situ* or wrapped in a cloth immersed in papaverin [1, 2].

When compared with other fields of bypass grafting, only a few studies have addressed the optimal conditions for storage of vascular grafts during surgery. The results of such studies remain contradictory with a focus on two substances, namely heparinized autologous blood and physiological saline (PS) as storage media (Tables 1–4).

Despite continuous progress in the field of percutaneous coronary interventions, CABG continues to be a mainstay of cardiac revascularization. The saphenous vein as a graft material will possibly retain its area of application in the near future, but its use and patency rate are under constant debate. Full arterial revascularization has been gaining more influence and is practised widely [23, 24]. There are multiple reasons for vein graft failure in the long term following CABG: in particular, intimal hyperplasia, graft atherosclerosis or smooth muscle cell-triggered stenosis. The role of initial trauma during harvest has not been fully elucidated so far [25, 26]. The status of the vein graft prior to implantation is therefore of great importance.

Storage conditions with focus on the various types of media, their outcome and the potential for novel agents will be discussed in the present review article.

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Table 1: Grouped summary of available literature analysing distension pressure and surgical preparation

Study	Publications analysing distension pressure and surgical preparation			Conclusion
	Specimens/method	Storage solution	Key results	
Gundry *et al.* [3]	*Sample*: human SVGs (30 patients) *Storage*: effects of I—NS and II—autologous whole blood (AWB) at cold (4°C) and warm (28°C) temperatures, and two distension pressures (100 and 300 mmHg)	I—NS II—AWB	*Warm saline*: massive endothelial cell loss *Warm blood*: moderate damage *Cold blood*: fully preserved *Cold saline*: mural oedema *Distension to 300 mmHg*: severe endothelial damage and oedema	The authors concluded that optimal harvesting techniques comprise immersing veins in cold blood and avoiding distension above 100 mmHg
Kurusz *et al.* [4]	*Sample*: human SVG (n = 5) *Storage*: five storage groups: I—NS, II—AWB, III—heparinized cardioplegic solution, IV—distended with heparinized saline solution but no pressure limit, and V—not distended. All solutions were kept at 10°C and the pressure limit was set to 200 mmHg	I—NS II—AWB III— Cardioplegic solution	No morphological differences in the endothelium of veins distended to 200 mmHg with saline solution, blood or cardioplegic solution. Veins distended without pressure control showed massive endothelial disruption	The choice of solution used to distend the saphenous veins is not as important as limiting the distending pressure
Dumanski *et al.* [5]	*Sample*: expression of surface adhesion factors (VCAM1/ICAM1) analysed from SVGs of 48 patients undergoing coronary artery surgery *Storage*: preparation fluid used either I—AWB or II—saline	I—AWB II—NS	*VCAM1* (40.23 and 42.71% for blood and saline, respectively, versus 11.51% control) *ICAM1* (48.42 and 50.63% for blood and saline, respectively, versus 12.60% control)	Damage occurs to the veins regardless of the preparation used, simply as a result of the pressure required to flush the vein grafts
Unal *et al.* [6]	*Sample*: SVGs from 11 patients were divided into three segments. Segments separated into three groups as the control group and the storage solution groups: I—NS and II—lidocaine group. Nitric oxide synthase (NOS), nitric oxide (NO) pool, superoxide dismutase (SOD) and thiobarbituric acid reactive substance (TBARS) levels were measured	I—NS II—Lidocaine	Histological examination of the lidocaine groups and the control were similar, but histological scoring of NS was statistically higher than the control (P = 0.008). NOS activity and NO pool were higher in the storage solution group than in the control (P = 0.010). SOD activity was higher in the lidocaine group than in the NS (P = 0.008) group. SOD activity was lower in the Group NS than in the control group (P = 0.047)	Primary damage might occur during surgery due to traumatic handling of the graft. Following injuries could occur due to ischaemia–reperfusion injury during the waiting period. Adding lidocaine to the preservation solution will avoid later injury

AWB: autologous whole blood; ICAM1: intercellular adhesion molecule 1; NO: nitric oxide; NOS: nitric oxide synthase; NS: normal saline = sodium chloride; SOD: superoxide dismutase; SVG: saphenous vein graft; TBARS: thiobarbituric acid reactive substance; VCAM1: vascular cell adhesion molecule 1.

INFLUENCE ON THE ENDOTHELIUM AFTER IMPLANTATION

Within 24 h after implantation, endothelial cells are sandwiched between adherent luminal and infiltrating subendothelial polymorphonucleocytes with platelet deposition on the endothelial surface. In addition, there is extensive subendothelial oedema, which reflects a combination of ischaemia/reperfusion damage, increased transmural influx and stretch damage due to distension of the vein graft by arterial blood pressure. In the experimental setting, vein graft smooth muscle cell proliferation occurs within the first 72 h and continues for at least 7 days after insertion [11].

COMPARISON OF PREVIOUS STUDIES

A Medline search from 1940 to September week 1 in 2015 using the OVIDSP interface (exp Saphenous Vein/ or vein graft OR Saphenous vein) AND (preservation or Tissue Preservation/ or storage AND / OR CABG AND/OR bypass graft AND / OR grafting AND/ OR coronary artery bypass grafting AND /OR vascular graft) was conducted. The existing studies on this topic are discussed in the following section and grouped into sections according to their main findings. No study was found showing a clearly superior effect of saline over autologous whole blood (AWB) and is therefore not represented by a separate section below.

INFLUENCE OF DISTENSION PRESSURE AND PREPARATION

This section discusses studies that investigated different storage solutions but in addition harvesting technique, handling or the distension pressure during preparation. Brisk surgical handling or overdistension of the vein segment can mask damaging effects of the storage solution (Table 1).

The first directly related study in 1980 by Gundry *et al.* compared human saphenous vein grafts (SVGs) of 30 patients in terms of storage in AWB and normal saline solution at 4 and 28°C as well as distension pressures of 100 and 300 mmHg. The findings were interesting in terms of storage solution and the influence of the distension pressure. The use of warm normal saline and distension above 300 mmHg independently were responsible for massive endothelial damage. However, most of the endothelium was

Table 2: Grouped summary of the available literature analysing autologous whole blood

Study	Autologous blood superior			Conclusion
	Specimens/method	Storage solution	Key results	
Lawrie *et al.* [7]	*Sample*: human SVG segments (n = 139) *Storage*: Room temperature I—NS or room temperature II—AWB. Effects of pressure and temperature changes were also measured (endothelium-dependent relaxation factor [EDFR])	I—NS II—AWB III— Plasmalyte	*Room temperature saline and pressurization to 400 mmHg*: EDRF relaxation 10.6 versus 32.4% for control segments (P < 0.05) *Room temperature saline alone*: 17.4 versus control of 29.6% (P < 0.05) *Room temperature heparinized blood*: EDRF relaxation 31.4 vs 34.1% (P < 0.05) *Plasmalyte solution*: 28.4 vs 30.1% (P > 0.05) *Stored at 2–4*°C: 18.2 vs 34.0% (P < 0.05) *Pressurization to 400 mmHg*: 20 vs 34% (P < 0.05)	It is evident that saline is inferior to blood and plasmalyte. High pressures and low temperatures also appear to be detrimental to graft function
Zerkowski *et al.* [8]	*Sample*: human SVG (n = 30) *Storage*: isolated SVG rings were incubated for 60 min in I—AWB, II—Bretschneider's cardioplegic solution (HTK), III—human albumin solution (HAS) or IV—Ringer's solution (RS) compared with the results obtained immediately after the removal of untreated control samples (C) taken from the same patients	I—AWB II—HTK III—HAS IV—RS	Samples stored in AWB (13.4 ± 0.4 mN) showed similar maximal contractions with NE to those in the control group (14.4 ± 0.5 mN). Relaxation due to ACh was found in 72.4% of the samples after HWB, in 44% of the HTK samples, but in none of the HAS and in only 1 RS sample. In 76.9% of the HAS and 83.3% of the RS samples, paradoxical contractions in response to ACh were observed	AWB is significantly better for maintaining functionally intact endothelium than HTK, HAS and RS, use of which leads to severe impairment of endothelial function
Wilbring *et al.* [9]	*Sample*: human SVGs (n = 36) *Storage*: I—NS or II—AWB for ∼30 min at room temperature. Preconstriction with norepinephrine, concentration–relaxation curves was assessed for bradykinin and sodium nitroprusside. Endothelium- and smooth muscle-cell-dependent vasorelaxation. Availability of ATP was determined based on liquid chromatographic measurements of nucleotide tissue levels	I—NS II—AWB	Receptor-dependent and receptor-independent maximum of developed vessel wall tension was significantly reduced in the NS group (P = 0.05 and 0.045, respectively). ATP levels were significantly (P = 0.046) better preserved after AWB (74 ± 1%) in comparison with NS (68 ± 2%). Endothelium-induced vasodilatation in response to bradykinin reached only 12.3 ± 2.5% in NS, but 19.3 ± 5.2% in AWB (P = 0.033). EC_{50} concentration of bradykinin was significantly lower in AWB than in NS (log EC_{50} −7.08 ± 0.3 and −5.91 ± 0.4, respectively; P = 0.046)	AWB better preserves vascular contractile and endothelial functions of the saphenous vein graft. Normal saline should no longer be recommended for intraoperative storage of harvested grafts

ACh: acetylcholine; AWB: autologous whole blood; EDRF: endothelium-dependent relaxation factor; HAS: human albumin solution; HTK: histidine–tryptophan–ketoglutarate; NS: normal saline = sodium chloride; RS: Ringer's solution; SVG: saphenous vein graft; NE: noradrenalin; HWB: human whole blood.

preserved when the storage was performed under warm or cold AWB and distension pressures were below 100 mmHg. The study presented results with a beneficial effect for AWB and low distension pressures. Furthermore, when cold normal saline was used, it created an intramural oedema independently of the applied pressure, but when combined with higher pressure again massive endothelial damage was observed. This shows a negative effect of saline independently of the applied pressure, but when combined with higher pressure there was a direct damaging effect on the endothelial layer. The authors concluded therefore that storage in cold AWB and distension of the graft at a maximum pressure of 100 mmHg are acceptable conditions [3].

Kurusz *et al.* reported a quite sophisticated study on storage solutions and distension pressure in human vein samples only 1 year later addressing similar questions. The group used whole blood, heparinized saline (HNS) and a custom-made cardioplegic solution (25 mEq of potassium per litre, pH 7.8, 300 mOsm) as a storage medium. All solutions were kept at 10°C and the distension pressure was limited to a maximum of 200 mmHg in all three groups. Distension pressure again seemed to be the most important factor for adverse outcome. Veins distended with cold saline, the cardioplegic solution or blood at pressures below 200 mmHg showed a similar morphology with areas of endothelial damage. As expected, the veins distended without pressure control showed even severer extensive disruption of the intima with destruction of cells over large areas regardless of the medium used [4].

The study by Dumanski *et al.* analysed the expression of adhesion molecules and their correlation with the type of media used during preparation of the segments. Additionally, the effect of distension pressure was investigated. After flushing the conduits with heparinized blood or saline, similar damages on endothelial surfaces, expression of CD31 and expression of related markers of cellular adhesion molecules, in particular vascular cell adhesion molecule 1, intercellular adhesion molecule 1 and P-selectin, were studied. Expression of adhesion molecules was higher in the vein segments exposed to pressure. Damage of the endothelial surface represented by CD31-positive stained cell coverage of the lumen was equal in segments flushed with blood or saline. Therefore, the group concluded that damage may be

Table 3: Grouped summary of the available literature revealing no difference

Study	Publications showing no difference			Conclusion
	Specimens/method	Storage solution	Key results	
Bush *et al.* [10]	*Sample*: external jugular veins of mongrel dogs (n = 75) bilaterally were dissected *Storage*: three different groups I—tissue culture medium 199 at 37°C (Group I), II—AWB at 37°C (Group II) or III—NS at 4°C and 200 mmHg (Group III). Vein grafts were reconstructed in the carotid circulation of each dog and then studied for biochemical and morphological features before or after arterialization	I—Medium 199 II—AWB III—NS	After arterialization, Group I developed an abnormal surface by 24 h. Group II showed significant damage in 24 h greater than that of Group I. The healing process was for about 4 weeks in Group I and 6 weeks in Group II. Despite the abnormal surface before arterialization in Group III, the morphological changes and repair process were similar to those of Group II	This study highlights that although there are some clear advantages to AWB in the early stages of vein harvesting, this difference does not persist post-arterialization
Chester *et al.* [11]	*Sample*: 210 ring segments of SVG (24 patients) *Storage*: I—AWB, II—NS, III—199-TC solution, IV—St Thomas' cardioplegic solution (STCS) or IV—plasmalyte at room temperature for 1 h. Analysing the contractile action of noradrenaline, 5-hydroxytryptamine, dopamine, histamine and Ach	I—AWB II—NS III—199-TC solution IV—STCS V—Plasmalyte	*Tension generated*: AWB 37.8 mN; heparinized saline 38.4 mN; 199-TC solution 47.1 mN; STCS 56.5 mN; plasmalyte solution 28.9 mN. The response seen after storage in cardioplegic solution was significantly greater than those seen in either blood (P < 0.005) or heparinized saline (P < 0.005)	Storage in AWB neither enhances nor depresses vascular reactivity. No significant difference in the use of saline. The potentiating effect is not shown to be due to depression of vasodilator mechanisms
Lamm *et al.* [12]	*Sample*: influence of continuous perfusion of veins with normal AWB on their endothelial integrity according to four groups (total 80 patients): (1) Conventional vein harvest, storage in NS (2) Endoscopic vein harvest and storage in NS (3) Conventional harvest under continuous perfusion with 100 ml of AWB (4) Endoscopic vein harvest under continuous perfusion with AWB	I—NS II—AWB	*Scanning electron microscopy*: the endothelial integrity was rated in five categories [from 'completely confluent endothelium' (1) to 'no endothelium' (5)] *Results*: Group 1: 2.7 ± 1.13, Group 2: 2.2 ± 1.06, Group 3: 1.6 ± 0.68, Group 4: 1.6 ± 0.69	The authors conclude that, with regard to endothelial integrity, endoscopic vein harvesting is superior to conventional vein harvesting. If the grafts are harvested while continuously perfused with AWB, there is not much of difference between the groups

AWB: autologous whole blood; NS: normal saline = sodium chloride; STCS: St Thomas' cardioplegic solution; SVG: saphenous vein graft.

independently of the substances used and may be a pure result of the pressure. However, all vein segments were initially flushed with saline to remove residual blood in this study and this may be an interfering factor [5].

Unal *et al.* [6] reported a novel approach with the use of lidocaine as a preservation agent. SVGs from 11 patients were divided into three segments. Segments were separated into three groups: controls (Group C), PS group (Group PS) and lidocaine group (Group L). Nitric oxide synthase (NOS), nitric oxide (NO) pool, superoxide dismutase (SOD) and thiobarbituric acid reactive substance levels were measured. Histological examinations of the Groups L and C were similar, but histological scoring of Group PS was statistically higher than Group C (P = 0.008). NOS activity and NO pool were higher in Groups L and PS than in Group C (P = 0.010). SOD activity was higher in Group L than in Group PS (P = 0.008). SOD activity was lower in Group PS than in Group C (P = 0.047). The authors concluded therefore that the primary damage might occur during surgery due to traumatic handling of the graft. The following injuries occur due to ischaemia–reperfusion injury during the waiting period. Adding lidocaine to the preservation solution can protect from later injury, but the sample size was small and no similar study was reported up to now [6].

In summary, higher distension pressures or overdistension outbalances any benefit of whole blood or a specific storage solution over saline. Saline worsens the effect of high distension pressures, especially when at room temperature or above.

INFLUENCE OF AUTOLOGOUS WHOLE BLOOD

The work by Lawrie *et al.* analysed the preparation of vein grafts in 85 patients undergoing CABG (Table 2). A total of 139 vein segments were examined. Grafts were stored at room temperature in either saline or AWB and additionally in a plasmalyte solution. Effects of pressure up to 400 mmHg and temperature changes (room temperature/2–4°C) were measured in relation to the endothelium-dependent relaxation factor (EDRF). This study showed clearly that saline solution is inferior to AWB and plasmalyte in terms of the EDRF capacity, meaning that the capacity of the endothelium was severely compromised. High pressure and cold temperature storage were additionally identified as negative factors [7].

Zerkowski *et al.* [8] reported on human grafts stored in different solutions in 1993. Thirty patients undergoing CABG were included and *in vitro* tests on macroscopically intact SVG were performed in the organ bath using isolated SVG rings incubated for 60 min in AWB, Bretschneider's cardioplegic solution (histidine–tryptophan–ketoglutarate, HTK), human albumin solution (HAS) or Ringer's solution (RS). Samples were compared with untreated control

Table 4: Grouped summary of available literature analysing special storage solutions

Study	Publications analysing special storage solutions			Conclusion
	Specimens/method	Storage solution	Key results	
Santoli *et al.* [13]	*Sample*: SVGs of 15 patients *Storage*: portions of the distal saphenous vein were then either immediately fixed (control), immersed in I—AWB, II—UWS or III—HSSP	I—AWB II—UWS III—HSSP	Electron microscopy of endothelial structure *AWB*: endothelial cell loss, medial oedema and necrosis *HSSP*: well-preserved endothelium in 12 cases and partial detachment and oedema in 3 cases after 30 min, but very few endothelial cells survived after 5 h of immersion *UWS*: after 30 min, no severe alteration in the cells and partial oedema after 5 h	Autologous blood is not without its pitfalls. This study identifies that AWB can cause significant damage to the endothelium. The authors conclude that the development of other solutions such as UWS may be more sufficient
Cavallari *et al.* [14]	*Sample*: canine external jugular and common femoral vein segments *Storage*: I—UWS, II—AWB or III—NS at 4°C for 45 min and 24 h	I—UWS II—AWB III—NS	*Scanning electron microscopy*: marked neutrophil migration and separation of endothelial cells were noted for veins stored in AWB and NS compared with UWS *Isometric tension studies*: maximum contractile responses were significantly reduced ($P < 0.05$) after storage in AWB (0.09 g/mm^2) or NS (0.12 g/mm^2), but not in UWS (0.36 g/mm^2)	There is little difference between the use of NS and AWB in terms of structural and functional damage to the endothelium. UWS is shown to be superior in both these aspects
Thatte *et al.* [15]	*Sample*: human SVG *Storage*: I—NS and II—GALAI. Multiphoton microscopy was used to assess the structural and functional integrity of SVG stored in multiple preservation solutions, and to design a superior storage solution (GALA)	I—NS II—GALA	*Standard preservation solutions*: after 60 min of harvest and storage, calcium mobilization and nitric oxide generation were markedly diminished with more than 90% of endothelial cells no longer viable in the vein *GALA*: veins could be stored for 24 h without substantial loss in cell viability	Standard solutions led to decline in saphenous vein endothelial cell viability. The physiological solution (GALA) maintained endothelial function and structural viability for up to 24 h
Alexander *et al.* [16] (PREVENT IV)	*Sample*: human SVG *Storage*: I—NS (placebo) and II—edifoligide. A phase 3 randomized, double-blind, placebo-controlled trial of 3014 patients. Vein grafts were treated *ex vivo* with either edifoligide or placebo in a pressure-mediated delivery system	I—NS II—edifoligide	Edifoligide had no effect on the primary endpoint of per patient vein graft failure (436 [45.2%] of 965 patients in the edifoligide group vs 442 [46.3%] of 955 patients in the placebo group)	Edifoligide is no more effective than placebo (NS) in preventing vein graft failure
Weiss *et al.* [17]	*Sample*: morphology on scanning electron microscopy of SVG (293 patients) *Storage*: SVGs were stored in I—NS, II—+5% albumin, III—HTK solution or IV—plasma preparation (PP) freed of isoagglutinins and coagulation factors	I—NS II—+5% albumin III—HTK IV—PP	*NS*: endothelium disintegrates almost immediately and after 2 h. More than 40% cells are dead *Saline + 5% albumin*: slightly slower process of decline but still significant numbers dead after 2–5 h *HTK*: tissue failed to maintain integrity *PP*: long-term stability and survival were observed	Only a very specific PP appears to preserve function of vein grafts
Weiss *et al.* [18]	*Sample*: human SVG *Storage*: I—NS and II—customized plasma. Analyses of the effect of storage solutions and the intravasal pressure on the degree of endothelialization	I—NS II—customized plasma	*Intravasal exposure to Alcian blue at pH <3*: highly specific staining of intimal regions with functionally or structurally damaged endothelium *Saline*: rinsing and as intraoperative storage medium resulted in the loss of more than 50% of the endothelium at intravasal pressures of 0–100 mmHg *Customized plasma*: pressures of up to 200 mmHg were tolerated with no significant endothelial loss. After exposure to 1000 mmHg, more than 70% of the endothelium was intact and vital	Quality of venous bypass grafts can be improved substantially by the use of a plasma derivative solutions for intraoperative preservation

Continued

Table 4: (*Continued*)

Study	Publications analysing special storage solutions			Conclusion
	Specimens/method	Storage solution	Key results	
Wilbring *et al.* [19]	*Sample*: human SVGs of 99 patients were stored in two solutions: I—NS and II—TiProtec for an average of 1.5 h before examination: (i) vessel wall tension constriction kinetics (ii) endothelial-dependent vasodilatation (iii) endothelial-independent vasodilatation	I—NS II—TiProtec	(i) *Vessel wall tension constriction kinetics*: mean tension 3.08 mN mm^{-1}, TiProtec: 8.85 mN mm^{-1} ($P = 0.01$) Constriction kinetics delayed by 100 ms in NS compared with TiProtec ($P = 0.02$) (ii) *Endothelial-dependent vasodilatation*: bradykinin response at maximum concentration was 15.2 vs 32.5% for NS vs TiProtec ($P = 0.048$) (iii) *Endothelial-independent vasodilatation*: response curves did not differ significantly: 77.4% in NS vs 90.2% in TiProtec ($P = 0.12$)	*Detrimental effect of saline on several specific features of vessel function*: wall tension and endothelium-derived vasodilatation. This suggests that TiProtec is a superior storage solution to saline
Wilbring *et al.* [20]	*Sample*: human SVGs ($n = 19$) *Storage*: I—NS, II—potassium chloride (KCl) or III—*N*-acetylhistidine-enriched storage solution (TiProtec) for 24 and 96 h. Preconstriction with norepinephrine, concentration–relaxation curves was assessed for bradykinin and sodium nitroprusside. The maximum wall tension and endothelial cell and smooth muscle cell (SMC)-dependent vasodilatory function were compared	I—NS II—KCl III—TiProtec	Maximum vessel wall tension was significantly better preserved in TiProtec-stored vessels in comparison with NS, after 24 h (5.11 ± 4.79 vs 2.48 ± 2.43 mN/mm; $P = 0.033$) and after 96 h (4.94 ± 2.82 vs 2.80 ± 1.76 mN/mm; $P = 0.042$). Endothelium-derived vasodilatory function was significantly better after 24 h in TiProtec-stored vessels (36.9 ± 2.6 vs 11.8 ± 30.9%; $P = 0.005$). After 96 h, endothelium-dependent vascular function was nearly abolished in NS-stored vessels, but largely preserved in TiProtec-stored segments (20.6 ± 2.9 vs 1.9 ± 4.3% in NaCl; $P = 0.015$)	TiProtec is able to largely reduce the loss of endothelium-dependent vascular function during cold storage. Feasible option for longer-term storage of saphenous vein grafts in CABG and transplant surgery
Harskamp *et al.* JAMA. [21] (PREVENT IV Follow-up)	*Sample*: human SVG *PREVENT IV protocol*: first vein grafts were treated *ex vivo* with either edifoligide or placebo in a pressure-mediated delivery system *Storage*: I—NS, II—AWB or III—buffered saline	I—NS II—AWB III—buffered saline	Most patients had grafts preserved in saline (1339 [44.4%]), followed by blood (971 [32.2%]) and buffered saline (507 [16.8%]). One-year VGF rates were much lower in the buffered saline group than in the saline group	The use of buffered saline solution also tended to be associated with lower VGF rates and trends towards better long-term clinical outcomes compared with saline- or blood-based solutions
Wise *et al.* [22]	*Sample*: human SVGs were characterized after 2-h storage in different solutions: I—Plasmalyte, II—NS, III—UWS, IV—Celsior solution, V—AWB and VI—GALA. Vascular smooth muscle contractility was measured after exposure to depolarizing KCl and phenylephrine	I—Plasmalyte II—NS III—UWS IV—Celsior V—AWB VI—GALA	Preservation in NS and AWB impaired contractile, whereas preservation in UWS and Celsior improved contractile responses. Storage in NS impaired endothelial-independent relaxation. Preservation in Plasma-Lyte A, NS and UWS impaired endothelial-dependent relaxation	Preservation in NS causes impaired physiological function and decreased viability. Tissue injury is decreased by the use of buffered salt solutions. The use of balanced, buffered salt solution, with as arginine, P2X7 receptor antagonists, can maintain smooth muscle function and the endothelial monolayer

ACh: acetylcholine; AS: autologous serum; AWB: autologous whole blood; CABG: coronary artery bypass grafting; EDRF: endothelium-dependent relaxation factor; GALA: heparinized physiological buffered salt solution containing glutathione, ascorbic acid and L-arginine; HAS: human albumin solution; HSSP: heparinized saline solution with papaverine; HTK: histidine–tryptophan–ketoglutarate; ICAM1: intercellular adhesion molecule 1; IH: intimal hyperplasia; KCl: potassium chloride; mN: milli-newton (1 mN = 0.001 N); NO: nitric oxide; NOS: nitric oxide synthase; NS: normal saline; PGI: prostacyclin; PGF: prostaglandin F; RS: Ringer's solution; SNP: sodium nitroprusside; SOD: superoxide dismutase; STCS: St Thomas' cardioplegic solution; SVG: saphenous vein graft; TBARS: thiobarbituric acid reactive substances; UWS: University of Wisconsin solution; VCAM1: vascular cell adhesion molecule 1; VGF: vein graft failure.

samples (C) harvested from the same patients. Samples stored in AWB (13.4 ± 0.4 mN) showed similar maximal contractions with noradrenaline to those in the control group (14.4 ± 0.5 mN). Relaxation due to acetylcholine (Ach) was found in 72.4% of the samples after AWB, in 44% of the HTK samples, but in none of the HAS and in only 1 RS sample. In 76.9% of the HAS and 83.3% of the RS samples, paradoxical contractions in response to ACh were observed. The authors concluded that AWB is significantly better for maintaining a functionally intact endothelium [8].

The latest study by Wilbring *et al.* [9] in 2013 stated that saline should no longer be recommended as a storage solution. This study used human vein segments stored in saline or heparinized

blood for ~30 min at room temperature. Analysis was carried out using the Mulvany myograph. After preconstriction with norepinephrine, the concentration–relaxation curves were assessed for bradykinin and sodium nitroprusside to investigate endothelium- and smooth muscle cell-dependent vasorelaxation. Even the adenosine triphosphate energy charge was determined based on liquid chromatographic measurements of nucleotide tissue levels. After the incubation time, the receptor-dependent and receptor-independent maximum of developed vessel wall tension was significantly reduced in the saline group ($P = 0.05$) and the energy charge was significantly ($P = 0.046$) better preserved after blood storage [9, 20].

All three studies showed a clear benefit of AWB and a negative effect of saline on the endothelium.

STUDIES SHOWING NO DIFFERENCE BETWEEN SALINE AND AUTOLOGOUS WHOLE BLOOD

The initial search for this section found three original papers and one review article by Tsakok *et al.* in 2012 that reports similar findings in this section. The review paper is not included in the table, but the following findings are in part cited and updated with our conclusions [27].

Bush *et al.* reported on an animal model of mongrel dogs ($n = 75$), in which bilateral external jugular veins were dissected and stored in either tissue culture medium 199 at 37°C (Group I), heparinized AWB at 37°C (Group II) or HNS at 4°C and distended with a pressure of 200 mmHg (Group III). Vein grafts were then used as a reconstruction material in the carotid circulation and studied for biochemical and morphological changes before or after arterialization.

After arterialization, Group I developed abnormal-surfaced endothelial linings after 24 h. Group II showed significant damage after 24 h. The healing process took about 4 weeks in Group I and 6 weeks in Group II. Despite the abnormal surface before arterialization in Group III, the morphological changes and repair process were similar to that of Group II [10]. This study highlighted that there are some advantages of AWB in the early period after vein harvesting, but that this difference does not persist post-arterialization.

Chester *et al.* analysed the contractile action of noradrenaline, 5-hydroxytryptamine, dopamine, histamine and Ach on 210 ring segments of human SVG comparing AWB (I), HNS (II), 199-TC solution (III), St Thomas' cardioplegic solution (IV) and plasmalyte (V) solution. All solutions were tested at room temperature for an 1-h duration in terms of contractile tension forces. The response seen after storage in cardioplegic solution was significantly greater than those seen in either blood ($P < 0.005$) or HNS ($P < 0.005$).

Therefore, storage in AWB neither enhances nor depresses the reactivity of the vessel in this study [11].

Lamm *et al.* reported on the influence of continuous perfusion with AWB and compared endoscopically harvested veins with those harvested by the conventional open approach. As is known, these methods differ in several aspects and should be discussed separately (Table 3). A clear benefit or negative impact of AWB during endoscopic harvesting might be influenced by the method itself [12].

Further information can be found in the review article by Tsakok *et al.* [27].

ALTERNATIVE OR SPECIALIZED STORAGE SOLUTIONS

Santoli *et al.* published a study in 1993 focusing on morphological changes in the intima assessed by electron microscopy. Fifteen patients undergoing CABG were included and portions of distal saphenous veins were then either immediately fixed (control) or immersed in AWB, University of Wisconsin Solution (UWS) and HNS solution with papaverine (HSSP).

Interestingly, this study revealed that autologous blood is not without pitfalls and identified AWB as a risk factor for significant damage to the endothelium, as segments treated by AWB showed enhanced cell loss, oedema and even necrotic areas when compared with veins stored in UWS or HSSP.

The authors concluded that the development of other solutions such as UWS may be promising and therefore called for the development of alternative solutions [13].

Cavallari *et al.* investigated in a canine model the use of UWS compared with saline and AWB at 4°C. They concluded that there was little difference between the use of saline and AWB in terms of structural and functional damage to the endothelium, but UWS was shown to be superior in both aspects [14].

In 2009, Weiss *et al.* investigated the morphology of stored human vein samples by scanning electron microscopy in 293 patients. SVGs were stored in saline, saline + 5% albumin, HTK (histidine ketoglutarate) solution or a plasma preparation (PP). The study reported that vein preservation using saline was associated with almost immediate and extensive disintegration of the endothelium.

After 2 h, >40% cells were not alive; saline + 5% albumin slowed the process of decline but still significant numbers showed endothelial destruction after 2–5 h. With the HTK solution, the tissue failed to preserve its integrity and only with PPs long-term stability and survival were observed [17].

The 2010 study from the same group confirmed the initial findings from 2009 and concluded that the quality of venous bypass grafts can be improved substantially using a plasma derivative solution for intraoperative preservation. In both studies, preservation in saline was clearly inferior [17, 18].

The call for an individual storage solution termed GALA™ was answered in 2003 by Thatte *et al.* Multiphoton microscopy was used to assess the structural and functional integrity of SVGs stored in multiple preservation solutions in order to produce the new solution GALA™ [15].

The new physiological solution (GALA™) based on Hank's balanced saline solution maintained endothelial function and structural viability for up to 24 h.

Another article that followed from the same group in 2011 reported on endoscopic vein harvest and GALA™ [15, 28]. While the first paper showed a benefit of the new solution, the second study focused on the harvesting method and not the solution; both groups (open harvest versus endoscopic) were immersed in GALA™ solution.

The PREVENT IV trial in 2005 reported results from a phase 3 randomized, double-blind, placebo-controlled trial of 3014 patients who underwent CABG surgery with the use of SVGs [16]. Edifoligide, an oligonucleotide decoy, that inhibits E2F transcription factors (eukaryotic transcription factor 2) to prevent neointimal hyperplasia and vein graft failure was used in one of the implanted vein segments. Vein grafts were treated *ex vivo* with either edifoligide or placebo and patients were examined at a 12- to 18-month

follow-up angiography. Edifoligide was found to be no more effective than the placebo, but a longer-term follow-up was needed to determine whether edifoligide has delayed beneficial effects.

A follow-up of the PREVENT IV trial in 2014 reported data regarding preservation of vein grafts in saline, blood or buffered saline solutions. In the same collective, 1-year angiographic vein graft failure and 5-year rates of death, myocardial infarction and subsequent revascularization were analysed. Patients had grafts preserved in saline solution (44.4%), blood (32.2%) and buffered saline (16.8%). Baseline characteristics were similar among the groups.

One-year vein graft failure rates were reported to be lower in the buffered saline group than in the saline group or the blood group. The use of buffered saline also tended to be associated with a lower 5-year risk of death, myocardial infarction or subsequent revascularization when compared with saline. Therefore, the patients whose vein grafts were preserved by the buffered saline solution had lower rates of vein graft failure and a better long-term clinical outcome [16, 21].

TiProtec™ was introduced in 2011, a custom-made storage solution for vein grafts [19]. The study investigated the influence of TiProtec™ versus saline in terms of preservation in 99 human vein rings by the use of vessel wall tension constriction kinetics. Saline showed severe endothelial damage, while the alternative solution preserved the endothelial function to a greater degree [19]. TiProtec™ is a potassium chloride and *N*-acetylhistidine-enriched storage solution by Köhler Chemie (Alsbach-Hähnlein, Germany).

Wilbring concluded in 2013 again very clearly that normal saline should no longer be recommended as a storage solution and stated that TiProtec™ is able to largely reduce the loss of endothelium-dependent vascular function during cold storage [20]. Although the sample size of the second study was small with only 19 human saphenous veins, incubation times were of great interest in this paper as veins were stored for 24 and 96 h in the storage solution.

Again saline showed severe endothelial damage, while the alternative solution TiProtec™ preserved the endothelial function as already reported in 2011 [9, 19, 20].

A recent study by Wise *et al.* [22] described that preservation in saline causes graft injury and stated that this effect is mitigated by the use of buffered salt solutions as well as preservation media. This article had compared human saphenous veins in six different storage solutions: Plasma-Lyte A, 0.9% saline, University of Wisconsin solution, Celsior solution, AWB or glutathione–ascorbic acid L-arginine (GALA™) solution [22]. Table 4 gives and compares the most important details of the different solutions.

Again, saline had devastating effects on the endothelium, buffered salt solutions decreased the level of injury and balanced buffered solutions maintained physiological functions.

NOVEL ALTERNATIVE SOLUTIONS

The issue of a custom-made vein storage solution is addressed from time to time, e.g. GALA™ or TiProtec™. Results seem to be promising, but acceptance and clinical use are yet underdeveloped.

A short time ago, DuraGraft®, a specially designed tissue preservation solution for storage of harvested vein segments had a broad introduction on the market in European countries. The solution is a so-called endothelial damage inhibitor that protects the vascular endothelium. Three key ingredients in it were chosen because of their putative effect on endothelial cell function (Table 5) [15].

Glutathione, a cellular reducing agent, has been found to increase L-arginine transport in endothelial cells and may lead to the stimulation of eNOS activity. Ascorbic acid is an antioxidant known to scavenge reactive oxygen species. L-arginine is the known substrate of NOS and has been shown to decrease neutrophil–endothelial cell interactions in inflamed vessels.

Currently, the product is under evaluation with clinical trials in the set-up phase and the US market introduction is planned for late 2016. The need for a novel solution seems to be unbowed, but one could criticize the costs compared with that of blood-based mixtures. Once outside the circulatory system, blood seems to lose its protective effects [19]. Due to decrease in pCO_2 *ex vivo*, there is a rapid loss of CO_2 from the blood, leading to increase in pH to as high as 8.0. Alkaline pH affects the endothelial and smooth muscle cell function due to a loss of ionic balance [15, 29].

In summary, the need for a specific storage solution seems to be necessary, but large trials that are mandatory in the forefront will be demanding and cost-intensive to execute.

CONCLUSION

The saphenous vein is still the most frequently used graft in CABG, despite its known limitations on long-term patency [30–32]. It has distinctive native properties and a degree of intrinsic degeneration, which can impact on its subsequent performance. Vein grafts are constantly evolving conduits that adapt to the arterial circulation with the development of intimal hyperplasia [33].

Nowadays, intimal hyperplasia is the principal obstacle to more durable grafts. *In vitro* data suggest that intraoperative preservation solutions influence endothelial function. There is conflicting evidence in the available current literature regarding the use of saline or blood-based products calls for comparable large randomized trials. A comprehensive review article by Tsakok *et al.* compared various studies and stated already that the University of Wisconsin solution, for example, may be advantageous when compared with both blood and crystalloid solutions. Saline solution was found to be harmful to the endothelium, but AWB also had its shortcomings [27].

All three studies in the above-described section 'influence of autologous blood' showed by different well-established means and in human grafts, a clear benefit for AWB and a negative effect of saline on the endothelium. To summarize studies on alternative storage solutions, saline had devastating effects on the endothelium, buffered salt solutions decreased the level of injury and balanced buffered solutions maintained physiological functions.

AWB was found to lose its protective properties once outside the circulatory system. Data showing no difference between saline and AWB are limited to basically three relevant papers. The first study carried out in mongrel dogs and with arterialization of jugular veins could not show a long-term benefit of AWB after arterialization. The second available study by Lamm *et al.* [12] combined the influence of continuous perfusion with endoscopic vein harvesting: the endoscopic approach can itself be of great influence on the endothelium. Only Chester *et al.* [11] state that AWB neither enhances nor depresses vascular reactivity and is not superior to saline.

Of great importance is that higher distension pressures or overdistension outbalances any benefit or negative effect of whole

Table 5: Overview of key ingredients of storage solutions in use

Ingredients	Vascular protection storage solutions									
	NS	RS	Plasmalyte	HTK	UWS	TiProtec	KCL	GALA	AWB	Celsior
Electrolytes										
Acetate (mmol/l)			27							
Calcium (mmol/l)		2.3				0.05			2.20–2.65	
Calcium chloride (g/l))								0.14		
Calcium chloride (mmol/l)				.015						0.25
Chloride (mmol/l)	154	156	98			103	194		95–110	
Hydrogen carbonate (mmol/l)										
Magnesium (mmol/l)			1.5			8			0.7–1.1	
Magnesium chloride (g/l)								0.1		13
Magnesium chloride (mmol/l)				4						
Magnesium sulphate (mmol/l)					5					
Phosphate (mmol/l)									0.84–1.45	
Dihydrogen phosphate (mmol/l)						1				
Potassium (mmol/l)		4	5			93	40		3.6–5.2	
Potassium chloride (g/l)								0.4		
Potassium chloride (mmol/l)				9						15
Potassium phosphate (g/l)									0.06	
Potassium phosphate (mmol/l)					25					
Sodium (mmol/l)	154	147	140			16	154		135–148	100
Sodium chloride (g/l)								8		
Sodium chloride (mmol/l)				15						
Sodium bicarbonate (g/l)								0.35		
Sodium phosphate (g/l)								0.048		
Carbohydrates										
Glucose (mmol/l)						10			3.6–5.6	
Mannitol (mmol/l)				30						60
Lactobionate acid (mmol/l)					100					80
Raffinose (mmol/l)					30					
Sucrose (mmol/l)						20				
Aminoacids and proteins										
Aspartate (mmol/l)						5				
L-arginine (mmol/l)								0.5		
Gluconate (mmol/l)			23							
Glutamic acid (mmol/l)										20
Glutathione (mmol/l)					3			1		3
Glycine (mmol/l)						10				
Histidine (mmol/l)				198						30
Tryptophan (mmol/l)				2		2				
Amino acid derivatives										
α-Ketoglutarate (mmol/l)				1		2				
N-Acetylhistidine (mmol/l)						30				
Medication										
Adenosine (mmol/l)					5					
Allopurinol (mmol/l)					1					
Dexamethasone (mg/l)					16					
Heparin (units/ml)								50		
HES (g/l)					50					
Insulin (U/l)					40					
Penicillin G (U)					200000					
Vitamins										
Ascorbic acid (mmol/l)								0.5		
pH	4.5–7.0	6.5	7.4	7.4	7.4	7.0	4.5–7.5	7.4	7.4	7.3

AWB: autologous whole blood; GALA: heparinized physiological buffered salt solution containing glutathione, ascorbic acid and L-arginine; HTK: histidine–tryptophan–ketoglutarate; KCl: potassium chloride 0.3% and sodium chloride 0.9%; NS: normal saline = sodium chloride; RS: Ringer's solution; TiProtec: custom storage solution by Köhler Chemie; UWS: University of Wisconsin solution.
UWS: Cavallari *et al.* [14]; HTK: Dr Franz Kohler Chemie GmbH, PO Box 1117, D-64659 Alsbach-Hähnlein, Germany; TiProtec: Dr Franz Kohler Chemie GmbH, PO Box 1117, D-64659 Alsbach-Hähnlein, Germany; GALA: Thatte *et al.* [15]; Celsior: Wise *et al.* [22].

blood or specific storage solutions over saline. Saline worsens the effect of high distension pressures, especially when at room temperature or above.

Novel or alternative solutions, such as GALA™, TiProtec™ or DuraGraft®, show promising results and were designed with the intention to overcome the above-described shortcomings, but the literature on these is sparse and their clinical use underdeveloped. The custom-made storage solution seems to be the next logical step, but large trials that are mandatory will be challenging and cost-intensive to perform.

Conflict of interest: none declared.

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