Intraoperative Vein Graft Preservation: What Is the Solution?

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Saphenous vein graft (SVG) disease and subsequent vein graft failure remain a major problem after coronary artery bypass graft operations. In an effort to mitigate loss of endothelial viability, the vein is stored, intraoperatively, in a preservation solution. However, human SVG samples demonstrate endothelial denudation and dysfunction after such storage, the severity of which varies, depending on the medium. The paucity of clinical data evaluating preservation solutions is illustrated by the absence of optimal procedural protocol. This review evaluates the potential efficacy of different storage solutions in preserving vein grafts, in relation to a mechanistic understanding of SVG pathophysiology.

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REVIEW

Coronary artery disease is the leading cause of death worldwide [1], and the single largest contributor to the global burden of disease [2]. Importantly, coronary artery bypass graft (CABG) operation is an effective approach for improving prognosis, symptoms, or both in selected patients with advanced coronary artery disease [3]. However, the long-term efficacy of CABG operations is hampered by vein graft failure (VGF), defined as complete graft occlusion, greater than 70% stenosis, or extensive conduit narrowing on angiography [4]. Indeed, 10% to 15% of saphenous vein grafts (SVGs) occlude within 1 year of operation, and almost one-half of the conduits fail at 10 years [4, 5], increasing the patients' risk of major adverse cardiac-related events [6] and often necessitating repeat revascularization. Correspondingly, vein graft disease is temporally correlated with reoperation and mortality [7]. As such, there is a critical need for strategies that improve long-term vein graft patency and clinical outcomes in patients undergoing CABG operation.

VGF is largely attributable to three temporally distinct processes, with interlinked underlying pathophysiology: acute thrombosis, subacute intimal hyperplasia, and long-term atherosclerosis [4]. Pharmacologic interventions, lifestyle modifications, and molecular therapies have been extensively investigated for the prevention of VGF [4]. However, it is clear that intraoperative measures are crucial in avoiding graft failure. These include use of a no-touch technique, minimizing graft trauma, avoidance of distension [8], and, more recently, use of expandable external vein graft supports [9]. Notwithstanding these important advances, much controversy remains as to the ideal preservation solution for short-term intraoperative storage of the SVG after harvesting. Indeed, evidence from ex vivo studies and animal models is contradictory [10–30], and there is a severe lack of clinical studies investigating graft storage solutions. Accordingly, considerable variation exists in the preservation solutions currently used, and which preservation solution depends largely on surgeon choice, rather than any firm evidence basis.

In this review, we use current understanding of the mechanisms underlying VGF, together with critical appraisal of available evidence, as a rational framework for discussing the characteristics of an optimal preservation solution.

Mechanisms of VGF

Thrombosis is a major cause of early VGF [31], with up to 12% of SVGs occluding within the first month after CABG operation [7]. Vein graft thrombosis results from a failure of local hemostatic balance, through a combination of vessel wall damage, hypercoagulability, and altered flow dynamics, classically defined in Virchow's triad. In short, focal endothelial disruption, a universal feature of SVG harvesting, results in loss of protective antithrombotic pathways, exposure of the thrombogenic basement membrane, and the expression of procoagulant and inflammatory mediators [31]. Subsequent local fibrin accumulation and platelet adherence can lead to thrombus formation and occlusion of the venous conduit.

Although thrombosis is the principle cause of VGF in the first 30 days after CABG operation, intimal hyperplasia, the accumulation of smooth muscle cells (SMCs), and extracellular matrix in the vein intima, are major contributors to SVG disease 1 month to 1 year after

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Abbreviations and Acronyms

AHB = autologous heparinized blood
CABG = coronary artery bypass graft
EDR = endothelial-dependent relaxation
eNOS = endothelial nitric oxide synthase
NO = nitric oxide
NOS = nitric oxide synthase
NS = normal saline
SEM = scanning electron microscopy
SMC = smooth muscle cell
SVG = saphenous vein graft
UWS = University of Wisconsin Solution
VGF = vein graft failure

implantation [31]. Such intimal thickening leads not only to luminal narrowing but also to forming a diffuse atherosclerosis-prone region within the conduit, as also occurs in native arteries [32]. This adverse vascular remodelling of an SVG after surgical manipulation and introduction into the high-pressure arterial circulation is complex and incompletely understood [33]. Nonetheless, as with thrombogenesis, endothelial cell loss is centrally implicated in the pathogenesis of intimal hyperplasia [33, 34]. This is unsurprising, given the key role of endothelial cells in modulating SMC proliferation and ingress and the deposition of extracellular matrix by a number of tonic inhibitory pathways [31]. Moreover, endothelial activation and denudation precipitate the infiltration of inflammatory cells, which secrete cytokines and growth factors, promoting SMC proliferation and chemotaxis [34]. Finally, mitogens, such as platelet-derived growth factor, released from platelets activated at the site of endothelial injury, further stimulate subendothelial fibroproliferation [35]. In addition to endothelial cell loss, transient ischemia followed by reperfusion during vein harvesting and grafting reduces endothelial production of antiproliferative mediators, such as prostacyclin and nitric oxide (NO) [31].

Necropsy studies have identified extensive atherosclerotic lesions in SVGs as early as 1 year after coronary bypass operation [36]. Correspondingly, atherosclerosis is the main cause of VGF beyond the first year of graft implantation [37]. As described above, both inflammatory cell infiltration through the damaged endothelium and intimal hyperplasia contribute to conduit atherogenesis. Hence, loss of endothelial integrity, adhesion molecule expression, reduced prostacyclin and NO formation, and generation of SMC mitogens also contribute to vein graft atherosclerosis [38]. Although conceptually sound, it is important to note that there is no evidence for an advantageous effect of endothelial structural integrity on graft patency or clinical outcomes after CABG operation.

To date, there are no double-blinded, randomized controlled clinical trials assessing the relative efficacy of different intraoperative storage solutions. However, this understanding of the pathophysiology underpinning the three predominant mechanisms of VGF allows for critical evaluation of available evidence from ex vivo studies of human SVGs and animal models (Table 1).

Search Strategy

In March 2015, the PubMed database was searched using the terms "CABG," "saphenous vein graft," "storage solution," and "preservation." Reference lists of identified articles were searched for further articles, and the "similar articles" function was used on all included articles.

Evaluation of the Evidence

Although technical failure and a multitude of factors may contribute to VGF, endothelial damage during vein harvesting and implantation is directly and indirectly implicated in acute, intermediate, and long-term vein graft disease [31, 34, 35, 38]. The structural and functional viability of the SVG endothelium may be impaired through trauma during harvesting, excessive manipulation and distension during preparation for grafting, and through exposure to high arterial pressures and turbulent flow. Nevertheless, the choice of intraoperative storage solution has been shown to significantly influence the preservation of the endothelial structural characteristics [11, 12, 15, 17–20, 22, 23, 25] and vascular function [11–13, 16–19, 23–28, 30]. Thus, the question of "which storage solution best preserves SVGs?" may ultimately be restated as "which solution best preserves endothelial integrity?"

Preservation of Endothelial Structure

Maintenance of a structurally intact endothelial barrier at the luminal surface of SVGs is imperative to graft patency, particularly through avoidance of acute thrombosis. However, ex vivo studies investigating the effect of various intraoperative solutions on endothelial structural integrity have yielded conflicting results.

In 1980, Gundry and colleagues [10] challenged the widespread use of saline for SVG storage after harvesting. With the use of scanning electron microscopy (SEM), the group revealed superior preservation of endothelial structural characteristics in human saphenous vein segments stored in autologous heparinized blood (AHB), compared with storage in normal saline (NS) at the same temperature [10]. Similarly, Lamm and colleagues [22], also using SEM, showed that continuous perfusion with autologous blood minimized endothelial damage, compared with storage in a crystalloid solution. However, these results potentially reflected not only the influence of solution composition but also the effect of continuous graft perfusion versus conventional storage, thereby hampering interpretation of the findings.

In contrast, other studies have failed to consistently substantiate the superiority of storage with autologous blood over crystalloids. Indeed, Catinella and colleagues [11] reported reduced endothelial desquamation and formation of fibrin-platelet aggregates in ex vivo human saphenous vein segments stored in buffered heparinized

Table 1. Summary of Studies Evaluating Vein Graft Preservation Solutions

Study (first author, year)	Nature of Study	Solutions	Measures and End Points	Outcome Summary	Conclusions
Gundry, 1980 [10]	Ex vivo human SVG segments 30 patients	NS AHB	Endothelial morphologic structure under SEM	Warm blood: moderate endothelial damage Warm saline: massive endothelial loss Cold blood: fully preserved endothelium Cold saline: mural oedema	Superior preservation of endothelial morphologic structure after storage in blood compared with saline
Catinella, 1982 [11]	Prospective, nonrandomized clinical study 40 patients Ex vivo human SVG segments	Buffered, heparinized saline + papaverine AHB	VGF rates at 10 days after CABG operation Morphologic studies under SEM	Significantly higher VGF rates for grafts stored in blood than buffered saline solution (20% vs. 7%, $p < 0.01$) Endothelial desquamation and separation, fibrin-platelet aggregates, and severe venous smooth muscle spasm in SVG segments stored in blood, but not in those stored in saline solution	Superior preservation of endothelial integrity and reduced thrombogenicity, corresponding with improved short-term graft patency in SVGs stored in buffered saline solution, compared with blood
Lawrie, 1990 [12]	Ex vivo human SVG ring segments	Heparinized NS Plasma-Lyte solution Heparinized blood	EDRF-induced relaxation, as a measure of vascular smooth muscle function	Lowest EDRF response in the saline group Response closest to control in the blood group	Blood is superior to saline and Plasma-Lyte solution in maintaining EDRF activity
Chester, 1993 [13]	Ex vivo human SVG ring segments	AHB Heparinized NS 199-TC solution St Thomas' cardioplegic solution Plasma-Lyte solution	Vessel constriction in response to noradrenaline, dopamine, histamine, 5-hydroxytryptamine, and ACh Vessel relaxation in response to ACh or SNP after preconstriction with histamine	Constrictor response was greatest after storage in St Thomas' cardioplegic solution and lowest in the Plasma-Lyte group No difference in relaxation response between solutions AHB neither enhanced nor depressed vascular reactivity	Storage in AHB neither enhances nor depresses vascular reactivity. Potentiation of constriction with saline does not appear to be due to depression of vasodilatation
Santoli, 1993 [14]	Ex vivo human distal SVG segments	AHB HSSP UWS 30 minutes or 5 hours	Endothelial morphologic structure under SEM	AHB: marked endothelial cell detachment and loss, medial edema and necrosis at both time points HSSP: endothelium well-preserved in 12 samples, partial detachment and edema in 3 samples after 30 min Few remnants of endothelial cells at 5 hours UWS: no significant endothelial changes at 30 minutes. Some partial endothelial detachment and subendothelial edema at 5 hours	Potential advantages of specific preservation solutions (eg, UWS) over saline and blood

(*Continued*)

Table 1. Continued

Study (first author, year)	Nature of Study	Solutions	Measures and End Points	Outcome Summary	Conclusions
Sanchez, 1994 [15]	Ex vivo human SV fragments Ex vivo canine SVs	NS Plasma-Lyte	Dilatation of noradrenaline-activated saphenous veins	Plasma-Lyte solution effectively dilated NAd-activated human saphenous vein fragments Storage in Plasma-Lyte solution completely relaxed NAd-activated canine saphenous veins within 20 minutes Veins stored in saline remained partially constricted Venodilation in the Plasma-Lyte solution was reversed by the addition of 1.6 mmol/L $CaCl_2$	Plasma-Lyte solution effectively relaxes canine and human saphenous veins Implication: lower distension pressure required during graft preparation Low calcium is critical to the venodilatory activity of Plasma-Lyte solution
Roubos, 1995 [16]	Ex vivo human SVG rings	Heparinized Ringer's solution Heparinized Ringer's solution + papaverine Heparinized Ringer's solution + glyceryl trinitrate + verapamil (GV)	Percentage endothelial coverage Peak and mean pressures required to distend the SVG ATP levels in the vein wall	Highest endothelial cover in the GV group Lowest peak and mean pressures required for distension in the GV group Highest ATP content in the GV group	GV solution improves endothelial coverage, allows for use of lower distension pressures, and reduces breakdown of high-energy phosphates
Cavallari, 1995 [17]	In vivo canine model: autogenous jugular and femoral veins grafted onto carotid or femoral arteries	NS AHB UWS	Dogs killed after 6 weeks Light microscopy, SEM, and isometric tension studies performed on the explanted vein grafts Control: vein segments immediately implanted, without storage in preservation solution	Intact endothelium in veins stored in all 3 solutions Intimal thickening was similar in veins stored in UWS to controls; significantly increased in veins preserved in NS or AHB Maximum contraction and sensitivities to NAd were significantly reduced in the AHB group but not NS or UWS groups	Long-term (24 hours) storage of vein grafts in UWS has beneficial effects on smooth muscle and endothelial structure and function after grafting, compared with storage in NS or AHB
Cavallari, 1997 [18]	In vivo canine model: autogenous jugular and femoral veins grafted onto carotid or femoral arteries	NS AHB UWS 45 minutes	Dogs killed after 6 weeks Light microscopy, SEM, and isometric tension studies performed on the explanted vein grafts Control: vein segments immediately implanted, without storage in preservation solution	Intact endothelium in veins stored in all 3 solutions Significant increase in intimal thickness in veins stored in AHB and NS, but not in veins stored in UWS Sensitivity and maximum contraction to NAd not altered in the UWS group; significantly reduced in veins stored in AHB and NS, compared with controls	Superior prevention of intimal thickening and preservation of smooth muscle cell function in vein grafts preserved in UWS than NS of AHB in this canine vein graft model

(*Continued*)

Table 1. Continued

Study (first author, year)	Nature of Study	Solutions	Measures and End Points	Outcome Summary	Conclusions
Cavallari, 1997 [19]	Ex vivo canine external jugular and common femoral vein segments	NS AHB UWS 45 minutes and 24 hours a	Morphologic studies using SEM and TEM Isometric tension studies	Extensive separation and desquamation of endothelial cells and marked neutrophil migration in veins stored in NS and AHB, compared with UWS Maximum contractile responses were reduced after storage in NS and AHB, but not UWS	Superiority of UWS, compared with NS and AHB, in terms of minimizing structural and functional endothelial impairment
Hickethier, 1999 [20]	Ex vivo human SVG segments	NS Buffered solution (M 199) + 5% albumin 45 minutes	Endothelial integrity assessed by TEM and SEM; immunohistochemistry	Greater destruction of endothelial cells in the NS group than for veins stored in buffered solution + 5% albumin (55% vs. 26% cell destruction on SEM)	Greater endothelial cell preservation after storage in buffered solution with albumin than NS
Kown, 2001 [21]	In vivo rabbit model: external jugular veins grafted onto carotid artery Ex vivo rabbit external jugular veins	PBS solution L-arginine polymer 5, 7, or 9, at 10 or 100 μmol/L 15 minutes	Rabbits killed after 28 days Calculated the intima:media ratio to assess neointimal hyperplasia In vitro NO production	Lowest intima:media ratio in grafted vein segments treated with the longest length (R9) and highest concentration (100 μmol/L) of L-arginine polymer Greatest neointimal hyperplasia in the PBS group NO levels were significantly higher in vein segments treated with 100 μmol/L L-arginine polymer 9 compared with controls	L-arginine polymers of sufficient length and concentration were effective at reducing neointimal hyperplasia and increasing NO levels in a rabbit vein graft model
Lamm, 2002 [22]	Ex vivo human SVG samples	Conventional or endoscopic vein harvest with storage in crystalloid solution Conventional or endoscopic vein harvest under continuous perfusion with autologous blood	Endothelial integrity under SEM	Greater endothelial integrity in SVGs continuously perfused with blood, compared with stored in crystalloid solution Less endothelial damage in veins endoscopically harvested than conventionally harvested in the crystalloid group. No difference between the harvesting techniques when continuously perfused with blood	Perfusion with autologous blood minimizes endothelial damage, compared with storage in a crystalloid solution

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Table 1. Continued

Study (first author, year)	Nature of Study	Solutions	Measures and End Points	Outcome Summary	Conclusions
Thatte, 2003 [23]	Ex vivo human SVG segments	HLS AHB HBSS TCM GALA 1 to 24 hours	Endothelial cell structural viability Calcium mobilization NO generation	Endothelial cell viability compromised within 1 hour in HLS, AHB, and TCM groups Cellular integrity lost after 2 hours in HBSS 76% to100% cell viability maintained in the GALA group over 24 hours Calcium mobilization and NO production impaired at 1 hour, and undetectable by 3 hours in the HLS, AHB, and TCM groups. Reduced but detectable at 5 hours in the HBSS group. Increased at 5 hours in the GALA group	GALA solution maintains endothelial structure, calcium handling, and NO production over extended periods of time
Dumanski, 2007 [24]	Ex vivo human SVG fragments	NS AHB 60 minutes Control vein fragments were not exposed to high-pressure flushing	Expression of adhesion molecules VCAM-1, ICAM-1, and P-selectin	Increased expression of all 3 adhesion molecules after storage in either solution, compared with control Slightly higher, although not statistically significant, expression in saline group compared with blood group	Mechanical stress caused by exposure to high pressure during graft preparation damages the endothelium, irrespective of the storage solution used
Weiss, 2009 [25]	Ex vivo human distal SVG segments	NS NS + 5% albumin HTK solution AHB Plasma preparation freed of isoagglutinins and coagulation factors (PP) 45 minutes to 5 hours	Endothelial morphologic structure under SEM Veins perfused in vitro under arterial conditions of pulsatile pressure and flow Assessed factor Xa formation and platelet adhesion	NS: immediate disintegration of the endothelium, and >40% of the cells were dead at 2 hours NS + 5% albumin: slightly slower decline in endothelial structure; large proportion of cells dead by 2 to 5 hours HTK solution: endothelial separation at 20 minutes and loss of tissue integrity by 2 hours AHB: contracted endothelial cells PP: supported long-term stability and survival of the endothelial cells Lowest factor Xa formation rates in veins perfused with PP Extremely high levels of platelet adhesion after exposure to all the solution, except PP	Improved endothelial integrity and cell survival, and less prothrombotic state in veins stored in PP compared with storage in a range of crystalloid solutions

(*Continued*)

Table 1. Continued

Study (first author, year)	Nature of Study	Solutions	Measures and End Points	Outcome Summary	Conclusions
Wilbring, 2011 [26]	Ex vivo human SVG ring segments	NS TiProtec 90 minutes	Vessel wall tension Endothelium-dependent vasodilation Endothelium-independent vasodilation	Maximum vessel wall tension was significantly impaired in the saline group Endothelium-dependent vasodilation was significantly reduced in the saline group Endothelium-independent vasodilation was maintained equally well in both groups	Saline has a detrimental effect on wall tension and endothelium-dependent vasodilatation TiProtec is superior at maintaining endothelial vasoactive function
Wilbring, 2013 [27]	Ex vivo human SVG segments	NS TiProtec 24 to 96 hours	Endothelium- and smooth muscle cell-dependent vasodilatation Concentration-relaxation curves for bradykinin and SNP	Endothelium-dependent vasodilatory function nearly abolished at 96 hours in saline group; largely preserved in TiProtec group No difference in endothelium-independent vasodilatation across the two groups Maximum vessel wall tension better preserved TiProtec group than saline group	Vessel wall contraction and endothelium-dependent vasodilatation is less impaired after storage in TiProtec than saline
Wilbring, 2013 [28]	Ex vivo human SVG segments	NS AHB 30 minutes	Endothelium- and smooth muscle cell-dependent vasodilatation Concentration-relaxation curves for bradykinin and SNP ATP levels (energy charge)	Superior endothelium-dependent vasodilatation in response to bradykinin in AHB group Energy charge significantly better preserved after blood storage than saline Maximum vessel wall tension significantly reduced in saline group	AHB better preserves vascular contractile, endothelial functions, and cellular energy, compared with saline
Harskamp, 2014 [29]	Retrospective, non-randomized, observational analysis of clinical trial data	NS Buffered saline AHB Time in solution not documented	1-year angiographic VGF (≥75% stenosis) 5-year rates of death, myocardial infarction, and subsequent revascularisation	1-year VGF rates lowest in buffered saline group, highest in saline group Long-term clinical outcomes: all-cause mortality, MI, and additional revascularisation at 5-years lowest in buffered saline group	Lower VGF and better long-term clinical outcomes with vein grafts stored in buffered saline than in NS or blood
Wise, 2014 [30]	Ex vivo human SVG samples	Heparinised NS UWS Low-potassium UWS Celsior Plasma-Lyte 1 hour Control: un-manipulated SVG segment	KCl-dependent contractility Endothelial-dependent and -independent relaxation	NS: reduced KCl-induced contractility compared with UWS-stored SVGs Reduced endothelial-dependent relaxation after storage in NS, compared with UWS No differences in endothelial-independent relaxation between groups	NS is detrimental to the physiologic functions assessed Buffered, balanced solutions such as UWS are superior for graft preservation

ACh = acetylcholine; AHB = autologous heparinised blood; ATP = adenosine triphosphate; CABG = coronary artery bypass graft; EDRF = endothelium-dependent relaxation factor; GALA = glutathione, ascorbic acid, L-arginine; GV = glyceryl trinitrate + verapamil; HBSS = Hank's balanced salt solution; HLS = heparin, lidocaine, sodium chloride; HSSP = heparinized saline solution + papaverine; HTK = histidine-tryptophan-ketoglutarate; ICAM-1 = intercellular adhesion molecule 1; MI = myocardial infarction; NAd = nicotinamide adenine dinucleotide; NS = normal saline; PBS = phosphate-buffered saline; PP = plasma preparation; SEM = scanning electron microscopy; SNP = sodium nitroprusside; SV = saphenous vein; SVG = saphenous vein graft; TCM = tissue culture medium; TEM = transmission electron microscopy; UWS = University of Wisconsin solution; VCAM-1 = vascular cell adhesion protein 1; VGF = vein graft failure.

saline with added papaverine, compared with saphenous vein segments preserved in AHB. More importantly, this corresponded with significantly lower VGF rates at 10 days after CABG operation in 40 patients in a prospective, nonrandomized clinical study [11].

Although the blood versus saline debate continues, there is mounting evidence demonstrating the protective value of alternative storage solutions on endothelial structure. This includes University of Wisconsin solution (UWS), a preservation medium used widely in organ transplantation, which was shown in 1993 by Santoli and colleagues [14] to mitigate the endothelial cell detachment observed after storage of human saphenous vein segments in heparinized saline with papaverine and AHB. Likewise, Cavallari and colleagues [17, 18] described the maintenance of a completely intact endothelium (>95% uniform endothelial coverage at three standardized locations under SEM) in a canine model of autogenous jugular or femoral veins grafted onto carotid or femoral arteries for 6 weeks. This remained true after both 45-minute [18] and 24-hour [17] storage in UWS before implantation. Interestingly, SEM analysis of ex vivo unmanipulated canine external jugular and common femoral vein segments after 24 hours of storage in UWS showed spindle-shaped, shrunken endothelial cells that had separated, exposing the underlying matrix [19], questioning the validity of the in vivo findings. Importantly, short-term (45 minute) preservation in UWS maintained intact endothelial coverage, comparable with control specimens [19]. Taken together, these studies support the favorable preservation of endothelial structural integrity after vein graft storage in UWS, compared with widely used AHB and saline.

In addition to UWS, new solutions have been specifically formulated in an effort to circumvent the profound endothelial cell damage associated with storage conditions in common clinical use today. Of arguably the greatest clinical significance is GALA, a buffered, heparinized physiologic salt solution that combines L-arginine, a NO synthase (NOS) substrate, and the antioxidants ascorbic acid and glutathione [39]. Compared with storage in a range of solutions, including AHB and Hank's balanced salt solution, human saphenous vein segments stored in GALA showed enhanced preservation of endothelial structural integrity under light microscopy [23]. Moreover, multiphoton imaging of the endothelium, using an array of fluorescent markers, confirmed a high level of structural viability and few dead cells after 1 hour and 24 hours in storage. Whether this protective influence translates into reductions in VGF has yet to be clinically validated. Nonetheless, GALA has been patented as a saphenous vein preservation solution [39].

Preservation of Endothelial Function

Far from being an inert physical barrier, endothelial cells are a unique multifunctional cell type, with a wide range of crucial basal and inducible synthetic and metabolic functions. Of prime importance to vein graft longevity, the endothelium regulates coagulation, vasomotor tone, and inflammatory responses. Hence, an effective intraoperative storage solution must support these roles. However, as with structural maintenance, studies assessing the impact of solution composition on endothelial cell function are far from unanimous.

The endothelium has a fundamental role in coordinating procoagulant and anticoagulant mechanisms [31]; preservation of this function is essential in mitigating graft thrombosis. Despite the clinical burden of acute conduit occlusion, few studies have investigated the influence of different storage solutions on thrombogenesis. Furthermore, no studies have attempted to delineate the relative contribution of loss of endothelial structural integrity, with exposure of the prothrombotic basement membrane, from an imbalance in endothelial production of thrombogenic and antithrombotic factors. Nonetheless, as described earlier, the higher VGF rates with AHB compared with buffered saline plus papaverine in the clinical study of Catinella and colleagues [11] described earlier was associated with formation of fibrin-platelet aggregates in the blood-stored veins. More recently, Weiss and colleagues [25] attempted to mirror the conditions faced by the saphenous vein after grafting, exposing ex vivo human saphenous vein segments to arterial conditions of pulsatile pressure and flow. Preincubation for 60 minutes with plasma preparation free from isoagglutinins and coagulation factors resulted in lower factor X activation than preincubation with saline or AHB. However, the small sample size ($n = 2$ in each group) and large variability in the data calls into question the relevance of these findings. Clearly, further studies are required to explicitly evaluate endothelial antithrombic properties after SVG storage in different solutions. Quantification of endothelial-derived anticoagulant factors, such as thrombomodulin and heparin sulphate, and procoagulant tissue factor and endothelin should be included.

A second crucial endothelial function is endothelium-dependent vasomotion. In 2011, Wilbring and colleagues [26] directly assessed endothelial-dependent relaxation (EDR) after storage in two different solutions. Specifically, bradykinin was used to stimulate endothelial NO production and, thus, elicit endothelial-dependent vasodilation in human SVG segments after 90-minute storage in physiologic saline, or an *N*-acetylhistidine–buffered, potassium chloride-enriched, and amino acid-fortified solution, TiProtec. Endothelium-dependent vasodilatation was far greater in the TiProtec group than with saline (32.5% versus 15.2%, $p = 0.048$, $n = 19$). Blunting of bradykinin-induced relaxation using NMMA, a NOS inhibitor, corroborated the NO dependency of this response. However, use of an endothelial NOS (eNOS)-specific inhibitor would have attested the endothelial dependency of this finding. Meanwhile, there was no difference in endothelial-independent vasodilatation between the groups. Subsequent studies demonstrated superior EDR after long-term (24 to 96 hours) SVG storage in TiProtec compared with saline [27] and after 30-minute preservation in AHB compared with NS [28]. A recent abstract by Wise and colleagues [30] supports the inferiority of saline for preserving EDR. Rings of human SVG showed significantly greater endothelial-dependent

vasodilatation after storage in UWS and Plasma-Lyte (a crystalloid solution with electrolyte composition, osmolality, and pH closely mimicking plasma) than after storage in NS. This is of ostensible clinical implication, given the widespread use of saline for short-term saphenous vein storage during CABG operations.

In addition to being a potent vasodilator, and thus marker of endothelium-dependent vasomotor activity, NO inhibits platelet adhesion and activation and SMC proliferation [23, 31]. Accordingly, the reduction in NO production that occurs during SVG preparation [40] likely contributes to both thrombosis and intimal hyperplasia. In addition to assessing endothelial structure, as described above, Thatte and colleagues [23] used multiphoton fluorescence microscopy to demonstrate severe attenuation of NO production during storage for 60 minutes in several preservation media, including AHB. In contrast, NO generation actually increased in GALA-stored grafts over an extended 5-hour storage period. The importance of NO production in preventing vein graft disease has been verified in vivo in a rabbit model, whereby external jugular veins were grafted onto the carotid artery [21]. Veins were treated for 15 minutes with L-arginine polymers of various lengths and concentrations or were stored in phosphate-buffered saline. Twenty-eight days after grafting, veins treated with the longest length (R9) and highest concentration (100 μmol/L) of L-arginine polymer had the lowest intima-to-media ratio (smallest degree of intimal hyperplasia), which corresponded with increased venous NO production. Given the fundamental contribution of NO to vascular homeostasis, evaluation of endothelial NO production is core to the appraisal of any storage solution. Of note, maintenance of NO production not only necessitates the provision of an eNOS substrate, but also binding of the cofactor tetrahydrobiopterin (BH4), known as eNOS "coupling" [41]. This requires the availability of BH4 and protection of BH4 from oxidation, for example, using 5-methyltetrahydrofolate [42].

The Optimal Intraoperative Storage Solution

Evidence as to which currently available storage solution best maintains SVG integrity is equivocal. Nonetheless, the studies discussed above, together with a mechanistic understanding of the factors contributing to VGF, provide a firm basis for postulating the features of ideal intraoperative preservation solution.

Warranting detailed discussion, the solution should be pH buffered. Deviation from physiologic pH is highly detrimental to the structural and functional viability of the vein segments [40], associated with loss of endothelial structural integrity under acidic and alkaline conditions [23]. Unfortunately, neither of the commonly used NS or AHB are pH controlled. Unsurprisingly, buffered solutions such as GALA [23], buffered saline [11, 21, 23, 29], TiProtec [26, 27], and M 199 [20] have proved superior at preserving various aspects of graft structural characteristics and function, compared with NS or blood. The importance of pH buffering in graft storage has been verified in a recent retrospective, nonrandomized, observational analysis of data from the PREVENT IV (The Project of Ex-vivo Vein Graft Engineering via Transfection) trial [29]. Harskamp and colleagues [29] compared rates of VGF and long-term clinical outcomes in 3,014 CABG operation patients whose SVGs had been stored in NS, buffered saline, or AHB. One-year VGF rates were far lower in the buffered saline than in the saline group (graft-level odds ratio 0.63, $p < 0.001$] or the blood group (graft-level odds ratio 0.62, $p < 0.001$). Likewise, storage in buffered saline solution was associated with lower 5-year risk of death, myocardial infarction, or subsequent revascularisation than with saline or blood, although these trends did not reach statistical significance (hazard ratio 0.81, $p = 0.08$, 0.81, 0.09, respectively). Although these findings are consistent with the ex vivo data, this study, to our own admission, has several important weaknesses. Principally, its retrospective nature means that potentially confounding variables were not controlled for; distension pressure during flushing, duration in storage solution, and solution temperature were not documented. Controlling such factors in future trials will be a considerable challenge. Further, the pressure-mediated delivery system used may reduce the generalizability of these results. Despite these limitations, this is the first large-scale clinical study, to our knowledge, assessing intraoperative SVG storage solutions, and represents an important step toward establishing the nature of the optimal preservation medium.

Conclusions

Data from ex vivo studies and animal models have repeatedly demonstrated that the composition of preservation solutions influences vein graft endothelial structural and functional integrity. Given the mechanistic basis of VGF, it is reasonable to assume that this translates into differences in graft patency and, consequently, clinical outcomes after CABG operations. Although current evidence is insufficient to definitely conclude what constitutes the optimal storage solution, valuable insights have been gained. Particularly, the solution must maintain a physiologic pH and support endothelial NO production by provision of an eNOS substrate. Recent findings by Harskamp and colleagues [29] represent important progress toward determining which commonly used preservation solution is superior. However, further basic mechanistic studies on human saphenous vein samples, using innovative experimental techniques, are required to establish how different solutions can influence various endothelial attributes. The next crucial step will be the direct comparison of diverse intraoperative storage solutions on vein graft patency and long-term clinical outcomes in thousands of CABG operation patients in a double-blinded, randomized controlled trial.

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