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A Guide to the Implementation and Design of Ex Vivo Perfusion Machines for Vascularized Composite Allotransplantation

Tessa E. Muss, MD*
Amanda H. Loftin, DVM*†
Zachary H. Zamore, BA*
Eleni M. Drivas, MD*
Yi-Nan Guo, MD*
Yichuan Zhang, MS*
John Brassil, MS‡
Byoung Chol Oh, DVM, PhD*
Gerald Brandacher, MD, FAST*§

Background: Ex vivo machine perfusion (EVMP) is a versatile platform utilized in vascularized composite allotransplantation (VCA) to prolong preservation, salvage tissue, and evaluate graft viability. However, there is no consensus on best practices for VCA. This article discusses the common components, modifications, and considerations necessary for a successful VCA perfusion.

Methods: A systematic literature review was performed in several databases (PubMed, Scopus, Embase, Web of Science, Cochrane Library, and ClinicalTrials.gov) to identify articles published on VCA EVMP (face, limb, abdominal wall, uterus, penis, and free flaps) before August 2022. Graft type and animal model, general perfusion parameters, core components of the circuit, and optional components for enhanced monitoring were extracted from the articles.

Results: A total of 1370 articles were screened, and 46 articles met inclusion criteria. Most articles (84.8%) were published in the last 10 years. Pigs were the main model used, but 10 protocols used human grafts. Free flaps were the most common graft type (41.3%), then upper extremities/forelimbs (28.3%), uteruses (17.4%), and hindlimbs (13.0%). Postperfusion replantation occurred in 15.2% of studies. Normothermic perfusion predominated (54.1%), followed by hypothermic (24.3%), and subnormothermic (21.6%). The majority of studies (87.0%) oxygenated their systems, most commonly with carbogen.

Conclusions: EVMP is a rapidly growing area of research. Leveraging EVMP in VCA can optimize VCA procedures and allow for expansion into replantation, flap salvage, and other areas of plastic surgery. Currently, VCA EVMP is achieved through a variety of approaches, but standardization is necessary to advance this technology and attain clinical translation. *(Plast Reconstr Surg Glob Open 2024; 12:e6271; doi: 10.1097/GOX.0000000000006271; Published online 12 November 2024.)*

INTRODUCTION

Ex vivo machine perfusion (EVMP) has evolved into a powerful platform in both solid organ and vascularized composite allotransplantation (VCA) to preserve, salvage, and assess grafts for research or transplantation.[1] However, there is currently no Food and Drug Administration approved machine specifically for VCA EVMP, leading many groups to create their own respective perfusion devices.[2] Because of these individualized circuits, there is a lack of consensus on best practices and delineation of which components are necessary for a successful perfusion circuit in VCA.

VCA EVMP has been rapidly gaining traction due to the many successes of EVMP in solid organ transplantation.[3] Many protocols using this platform have already made their way to the clinic in the solid organ field due to their efficacy in increased duration of preservation times, the repair of organs donated after circulatory death, or

*From the *Department of Plastic and Reconstructive Surgery, Vascularized Composite Allotransplantation Laboratory, Johns Hopkins University School of Medicine, Baltimore, Md.; †Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Md.; ‡Functional Circulation, LLC, Lake Mills, Wis.; and §Department of Visceral, Transplant and Thoracic Surgery, Medical University Innsbruck, Innsbruck, Austria.*

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for quality assessment metrics of transplant suitability.[4–6] Although VCA circuits share many of the same principles and device components, VCA perfusion circuits must be adapted to face the number of unique challenges such as multiple tissue types and narrow ischemic window.[7] The vast majority of the literature on these circuits has been published over the last 5–10 years.

This rapid uptick in interest is also due to these circuits' potential for expansion into a variety of clinical scenarios.[7] As VCA circuits are designed to optimize conditions for multiple different tissue types, these same setups can be easily adjusted to meet the needs of various indications, including replantation in cases of traumatic amputations or free flap salvage in irradiated or vessel-depleted tissue.[7,8]

This article aims to review the literature on the design and implementation of VCA EVMP. Secondary objectives of this review are to determine which components are critical for a successful VCA perfusion and which modifications can be made for different experimental designs. Another purpose of this article is to standardize methodology across groups to improve reproducibility and validity.

Takeaways

Question: This article explores the standard elements, modifications, and considerations for successful ex vivo machine perfusion (EVMP) of vascularized composite allotransplants (VCA).

Findings: A systematic review was performed on VCA EVMP articles published pre-August 2022. Human tissue was used in 21.7% of circuits. Graft types included free flaps, upper extremity/forelimbs, uteruses, and hindlimbs. A portion of the studies (15.2%) replanted the grafts after perfusion. Approximately half of the studies (54.1%) used normothermic temperatures and 87.0% oxygenated their system, typically with carbogen.

Meaning: Standardizing EVMP practices is critical for clinical translation, and these advancements may allow for expansion from VCA optimization to areas like replantation and flap salvage.

METHODS

A comprehensive literature search of articles listed in PubMed, Scopus, Embase, Web of Science, Cochrane Library, and ClinicalTrials.gov databases was conducted in August 2022 in compliance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[9] The databases were queried using search terms designed to capture EVMP across a broad range of VCA grafts and flaps (Table 1). The following filters were applied in each database to capture full-length articles: PubMed: "Full text"; Scopus: "Article"; Embase: "Article," "Article in Press"; Web of Science: "Article"; Cochrane Library: "Trials" tab; ClinicalTrials.gov: n/a.

Predetermined inclusion criteria for selecting studies were (1) preclinical articles studying normothermic, near-normothermic, and hypothermic perfusion; (2) perfusion of any tissues within VCA (face, limb, abdominal wall, uterus, and penis) and free flaps with at least 2 tissue types (3) randomized control trials, prospective and retrospective case-control and cohort studies, cross-sectional cohort studies, case reports, and technique papers. Exclusion criteria were (1) reviews without presentation of new data, abstracts/conference papers, editorials, comments; (2) articles about solid organ perfusion or perfusion of flaps with less than 2 tissue types; (3) articles reporting little data on perfusion technique and details on circuit components; and (4) articles without a diagram, schematic, or image of the perfusion circuit.

Papers meeting exclusion criteria, duplicate publications, and articles unrelated to VCA perfusion were eliminated. Remaining works were sought for retrieval as full texts, and their reference lists screened for additional relevant articles meeting inclusion criteria that were missed in the electronic search. Two independent authors (T.E.M. and A.H.L.) conducted the search, screening, and eligibility assessment to agree upon a comprehensive list of included articles. Controversies were resolved by discussion with a third reviewer (Y.Z. or Y.G.).

Table 1. Database Search Terms for Comprehensive Literature Search

Search Terms		
Vascularized composite allotransplantation	AND	Machine perfusion
Vascularized composite allotransplant		Machine preservation
Vascularized composite allograft		Ex vivo perfusion
Vascularized allograft		Extracorporeal perfusion
Vascularized allogeneic tissue		Extracorporeal circulation
Vascularized composite tissue transplantation		
Vascularized composite tissue transplant		
Composite tissue allotransplantation		
Composite tissue allotransplant		
Composite tissue allograft		
Composite tissue allografting		
Composite tissue transplantation		
Composite tissue transplant		
Uterus		
Reconstructive transplant		
Flap		

Table 2. Overview of Perfusion Studies in VCA by Graft Type and Species

	No. Studies	Percentage
VCA type		
Free flap	19	41.3
Forelimb/UE	13	28.3
Hindlimb	6	13.0
Uterus	8	17.4
Model used		
Pig	24	52.2
Human	10	21.7
Rat	8	17.4
Rabbit	2	4.4
Sheep	2	4.4

General Parameters, Outcomes, and Discussion

The literature search yielded 1370 articles, of which 46 articles met inclusion criteria.[10–55] All included studies were published between 1970 and 2022. Notably, 84.8% of included articles were published in the last 10 years, with 73.7% of those published in the last 5 years. Overall, these 46 articles described EVMP circuits for 46 VCA grafts, of which 10 were human and 36 were animals. [**See Table, Supplemental Digital Content 1**, which displays the overview of machine perfusion studies in VCA (1970–2022), **http://links.lww.com/PRSGO/D588**.] Among the animal models, pigs were the most utilized (24 of 36), followed by rats (8 of 36), sheep (2 of 36), and rabbits (2 of 36). Nineteen of 46 (41.3%) of grafts were free flaps, followed by upper extremity (UE)/forelimb (13 of 46, 28.3%), uteruses (8 of 46, 17.4%), and hindlimb grafts (6 of 46, 13.0%) (Table 2). Sixteen of 46 (34.8%) studies incorporated replantation/autotransplantation or transplantation into their study designs for in vivo assessment after perfusion. A general VCA EVMP circuit noting both core and optional components is included in Figure 1.

Temperature

Perfusate temperature during graft perfusion was used to classify perfusions as hypothermic (0°C–15°C), subnormothermic (20°C–33°C), or normothermic (34°C–40°C). Of the 37 (n = 46) perfused grafts that reported perfusate temperature, 54.1% were normothermic, 21.6% were subnormothermic, and 24.3% were hypothermic (Fig. 2). This trend toward normothermia was seen regardless of the graft type or animal model. Achieving these thermal conditions was done most often through heat exchangers (21.7%). Nineteen studies specified the frequency of temperature measurement, with hourly (8 of 19 or 42.1%)

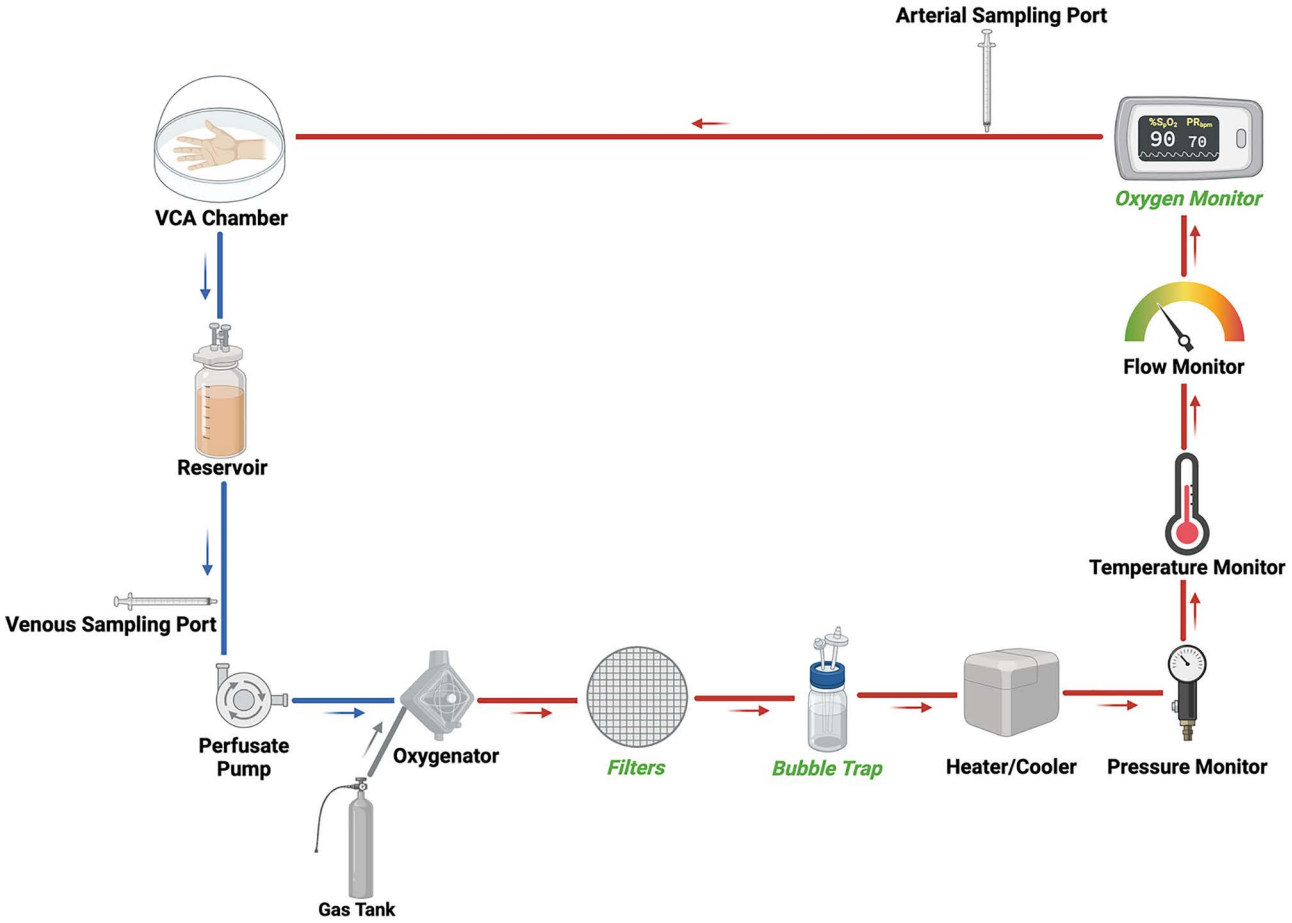

Fig. 1. Diagram of the core components and modifications in a VCA EVMP circuit. Core components are labeled with black text. Modifications are labeled with italicized green text. Created with BioRender.com.

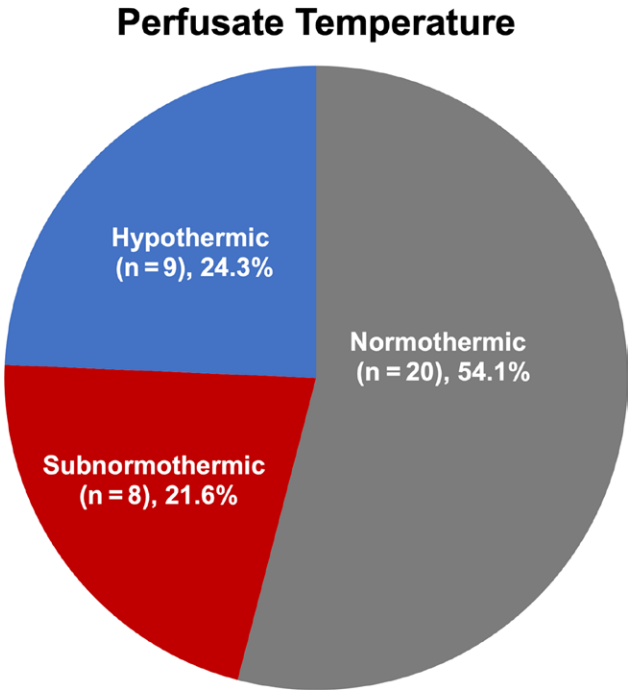

Fig. 2. Characterization of perfusate temperature in included studies. Classifications were as follows: hypothermic (0°C–15°C), subnormothermic (20°C–33°C), or normothermic (34°C–40°C).

and continuous (7 of 19 or 36.8%) monitoring being the predominant choices.

One reason for this trend toward normothermic conditions may be that normothermic temperatures provide a near physiologic and protective environment for the organ, thus lessening tissue damage, especially for marginal grafts.[7] By contrast, the evidence for lowering temperature to subnormothermic conditions is mixed. Some studies found that it offers improvements in biochemical parameters, improvement in oxygen consumption, and tissue ATP content.[7,56] Adams et al[57] found that kidneys perfused at subnormothermic temperatures (32°C) had lower creatinine clearance and urine output than kidneys perfused at normothermic temperatures (37°C). However, there is currently no consensus on the role of subnormothermic machine perfusion in VCA. Finally, hypothermic temperatures rely on the basic principle of hypothermia to maintain cellular viability, but the benefits of hypothermia may be compromised during graft rewarming, where temperature shifts can cause tissue stress and cellular injury.[58]

Pressure and Flow

Within all graft types, the uterus had the highest perfusion pressures on average (106.3 ± 30.8 mm Hg), followed by the forelimb/UEs (70.5 ± 28.4 mm Hg), free flaps (65 ± 54.4 mm Hg), and hindlimb/lower extremities (46.1 ± 30.6 mm Hg). Large animal or human extremities showed slightly higher pressure ranges compared with small animal models (67.4 ± 29.4 versus 50.1 ± 33.8 mm Hg).

The absolute flow rate varied by species and graft type, with large animal and human models having higher average flow rates than small animal models of the same graft type. As flow and pressure dynamically change with each other, either flow or pressure can be set to physiologic rates specific to the species type and graft area. Pressure can be measured using a pressure sensor or transducer, which is commonly placed right before the graft. Flow rate can be measured via built-in panels in the pump or with a flowmeter, usually placed on the arterial portion of the circuit. Continuous monitoring of pressure and flow is advisable because aberrations in these measurements can injure the microvasculature or lead to insufficient or uneven perfusion.

Core Components

VCA Chamber

All circuits utilized a chamber to house the VCA grafts regardless of indication or experimental condition. The most simplistic design featured a dish large enough to support the size and weight of the graft. Fitted, transparent lids were used 34.8% of the time. Additionally, 4.4% of groups included a humidity modulator, whereas 69.6% utilized a heating or cooling element.

Transparent lids are practical elements to help protect the graft and preserve aseptic conditions while still allowing for clear visualization and monitoring of the graft during perfusion. Moreover, they also provide a closed, moist environment to maintain tissue hydration, although a few groups included a formal humidity modulator. Heating and cooling elements were a more popular addition, as they offered another point of thermoregulation in addition to controlling the temperature of the perfusate.

Reservoir

Every circuit was equipped with at least 1 venous reservoir to collect the perfusate. Almost all circuits were designed with an open venous outflow that would allow the perfusate to collect at the bottom of the VCA chamber and flow into the reservoir via gravity. The size of the reservoir was adjusted to the size of the graft, with a total circulating volume range of 25 mL for a rat hindlimb up to 5600 mL for a pig hindlimb.

Continuous replacement of the reservoir volume may be necessary when recirculation is not optimal. The reservoir volume is replaced in some protocols that utilize blood-based perfusate because volume replacement can help minimize fluctuations in electrolyte values and ensure there are sufficient red blood cells for oxygen delivery.

Pump

Generally, the number of pumps correlated with the number of arterial supplies to the graft. Except for the uterus, grafts were typically perfused through a single artery and used only one pump. By contrast, due to the multiple arterial supplies of the uterus, 62.5% of these studies had 2 separate pumps to independently supply each uterine artery. The remaining uterine studies were equipped with only 1 pump, where the main branch later split into 2 separate branches to supply the 2 arteries. Additional pumps were occasionally added along the venous outflow route to prompt recirculation. Finally, auxiliary pumps were rarely used to include additives to

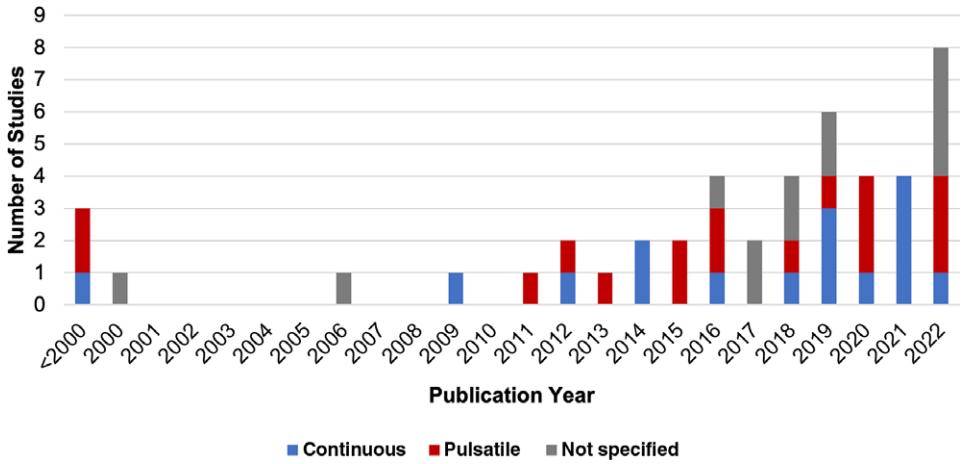

Fig. 3. Flow type used in publications over time. A marked uptick in VCA EVMP publications has occurred in the past 5–10 years, but neither continuous nor pulsatile flow type has shown predominance.

the perfusate, such as one uterus perfusion study[19] which added another pump before arterial branches to incorporate hormonal components.

Once the number of pumps was established, there were then 2 main types of pumps utilized: roller and centrifugal. Among all perfused grafts, 60.9% studies utilized roller pumps, 19.6% used centrifugal pumps, and 19.6% used other types of pumps or did not specify. Regarding flow conditions, 37.0% set a pulsatile flow, 23.9% set a continuous flow, and 39.1% did not specify. Within groups that used a roller pump, 46.4% used a pulsatile flow, 14.3% continuous, and 39.3% not specified. Neither the type of pump nor the type of flow used correlated with any specific graft type or indication. No small animal studies applied a centrifugal pump.

Although most of these circuits utilize 1 pump to generate arterial flow, it is important to consider the impact of additional pumps. In the case of grafts with more than 1 arterial supply, 1 pump that splits into multiple branches offers the advantage of mimicking physiologic conditions, in that the heart is the sole pump of the body. However, if one of the branches of the perfusion system becomes more resistant to flow, the perfusate will divert flow away from this branch, causing uneven conditions between arterial supplies. By contrast, having the number of pumps equal to the number of arteries differs from physiologic conditions but offers more control of each individual arterial inflow, better ensuring consistent conditions for experimentation.

As exemplified by this dataset, both continuous and pulsatile flow have been used at similar frequencies since 2016 (Fig. 3). Continuous flow may be less destructive to blood components,[59] but pulsatile flow offers the physiologic benefit of mirroring the arterial pulse generated by the heart. Thus, the final decision of pump choice should also consider perfusate components, accessibility, and overall experimental goal.

Gas

Overall, 87% of groups oxygenated their systems with a variety of combinations. The most popular combination was oxygen (O_2) and carbon dioxide (CO_2) (41.3%), of which the vast majority used carbogen (95% O_2, 5% CO_2) (Table 3). Additionally, 15.2% of groups used only oxygen, with 4.4% using 21% O_2, 2.1% using 100% O_2, and 8.7% not specifying the concentration of oxygen. A few groups (2.2%) added nitrogen (N_2) into the mixture, yielding a gas composition of 40%–60% O_2, 5%–10% CO_2, and the remaining balance of nitrogen. Some groups (8.7%) diverged from these groups and created custom mixtures, such as 100% O_2 at 1.35 L/min, 7% CO_2/93% N_2 at 5 L/min. Finally, 19.6% of groups indicated an oxygenated system but did not specify the gas composition used.

Table 3. Gas Composition of Included Articles

Gas Composition	No. Studies	Percentage
None	6	13.0
O_2 (Overall)	7	15.2
21% O_2	2	4.4
100% O_2	1	2.2
Unspecified O_2	4	8.7
O_2, CO_2 (Overall)	19	41.3
95% O_2, 5% CO_2	17	37.0
Variable or unspecified O_2, CO_2	2	4.4
40%–60% O_2, 5%–10% CO_2, balance N_2	1	2.2
Unspecified gas composition	9	19.6
Other	4	8.7

Of the studies evaluated, 40 of 46 (87.0%) oxygenated their EVMP circuits. Oxygen (O_2) and carbon dioxide (CO_2) was the most utilized gas combination, of which 95% O_2, 5% CO_2 (carbogen) was the most frequently utilized. Less commonly used gases include solely O_2 or addition of nitrogen (N_2).

By contrast, there were 6 circuits (13.0%) that did not include any additional gasses. Two of these studies were decellularization protocols. One study was specifically focused on optimizing circuit hemodynamic parameters and establishing stable circulation. Another study quantified the oxygenation levels with different perfusates to determine if a formal oxygenation unit would be necessary for future perfusions. One group designed a bioreactor system with access to ambient air, but no additional gas. Finally, 1 group used fresh arterialized blood taken from patients' central venous catheters to perfuse the circuit.

The oxygen component is crucial for machine perfusion, as it is essential in maintaining cellular respiration and production of ATP. Providing 100% oxygen could potentially cause oxidative stress, formation of reactive oxygen species, and eventual damage to the tissue.[60] Carbogen is a frequently chosen gas mixture among all studies. Given that carbon dioxide helps maintain the pH balance, the inclusion of carbon dioxide has become customary to prevent alkalemia.[17] Nonoxygenated gases, such as nitrogen or argon, can also be used in the oxygenation system to remove any residual oxygen from the perfusate.[36] These are particularly important when tissue is being preserved for transplantation, as the absence of oxygen can reduce the metabolic activity of cells. However, it is important to note that prolonged exposure to nonoxygenated gas can also be detrimental to tissue viability.[61,62] Ultimately, the choice of gas used must be carefully considered and optimized to ensure the viability and preservation of the transplanted tissue.

Sampling Ports

Most studies integrated sampling ports into the arterial or venous system. Nearly half of the groups (45.7%) included at least 1 arterial and 1 venous port, 67.4% included at least 1 port, 10.9% had 0 ports, and 10.9% referenced perfusate sampling from the circuit, but did not explicitly state the number of ports or include it in their circuit diagram. The frequency of sampling was variable amongst groups, but most circuits were sampled at least hourly to monitor gas content, glucose levels, and electrolyte balance. Biomarker assays, viability assessments, complete blood counts, and comprehensive metabolic panels were also regularly checked for a more in-depth analysis.

Sampling ports are an important feature to build into circuits to easily check the status of the perfusate and monitor the status of the graft. Arterial ports should be placed after the oxygenator and before the graft, whereas venous ports should be placed after the graft and before the oxygenator. Additionally, these sampling ports can be used to administer medication or other additives to the perfusate while the perfusion is running.

Modifications

Bubble Trap

Bubble traps are an important safety component to mitigate the risk of air thrombi, which can cause significant microvascular damage. Both formal bubble traps and filters can be used to rid air from the circuit. Approximately one-third of groups (34.8%) used only a bubble trap, 15.2% used only filters, and 8.7% used filters and bubble traps together.

The venous collection reservoir and the sampling ports are a few areas at risk for air infiltration. As such, it is advisable to incorporate bubble traps into the arterial portion of the circuit or directly into the venous reservoir.

Filters

Filters are used to prepare the perfusate for downstream use. The type, size, and placement of the filter are important considerations to minimize graft damage. Eleven studies assessed (23.9%) utilized a filter. The types of filters used included cell strainers (n = 1), leukocyte filters (n = 3), bacterial filters only (n = 2), prefilters and bacterial filters combined (n = 3), hemofilters (n = 1), and arterial line filters (n = 1).

A variety of filters may be incorporated and can act as powerful modifications to modulate the perfusate before it reaches the graft. Cell strainers in various mesh sizes are useful for the rapid removal of cell aggregates and tissue fragments. Leukocyte filters decrease the residual leukocytes and microaggregates in red blood cells, platelets, and plasma-based perfusate solutions. Bacterial filters are used for the removal of microorganisms, with the 0.2-μm filter being the most common one used in the included studies.[26,53,54] Prefilters can also be used in series with a bacterial filter to further improve filtration. Hemofilters may offer some tighter electrolyte control. Finally, arterial line filters protect the graft from gaseous macro- and microemboli that originate in the perfusion circuit.

Oxygen Monitoring

Knowing the partial pressure of oxygen (pO_2) or oxygen saturation of the perfusate or graft can be a powerful tool to assess the viability and status of the graft. Many circuits (19.6%) incorporated a direct pO_2 sensor, 21.7% of circuits included a tissue pulse oximeter, 30.4% calculated oxygenation status via perfusate sampling, and the remaining studies did not specify.

A pO_2 sensor can provide a more accurate measurement of the overall oxygenation status of the graft, as it assesses the perfusate as it passes through the circuit rather than being limited to a specific area of tissue. However, the accuracy of the measurement can varies by its placement and presence of bubbles in the perfusate. Pulse oximeters measure the oxygen saturation level of hemoglobin in the tissue using a sensor placed on the surface of the tissue. Its accuracy can be affected by its position, tissue thickness, and presence of certain pigments in the tissue, and may not be representative of the overall oxygenation status of the graft. Perfusate sampling is another method whereby a small amount of perfusate is taken from the circuit and then analyzed for oxygen content. This does not allow for real-time or continuous monitoring.

CONCLUSIONS

VCA EVMP circuits are multifunctional platforms that can be used to extend preservation times, salvage damaged tissue, and assess graft viability. As there is no consensus on best practices for assembly, this article offers a summary of the common components and modifications of current VCA EVMP circuits to help standardize practices. Furthermore, the discussion of the indications and implications for each component, might help open the door for expansion of this platform to other clinical areas, such as replantation and free flap salvage.

Gerald Brandacher, MD, FAST
Department of Visceral, Transplant and Thoracic Surgery
Anichstrasse 35
6020 Innsbruck, Austria
E-mail: gerald.brandacher@i-med.ac.at;
brandacher@jhmi.edu

DISCLOSURE

The authors have no financial interest to declare in relation to the content of this article.

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