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The Disconnect Between Extracorporeal Circulation and the Microcirculation: A Review

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Extracorporeal circulation (ECC) procedures, such as cardiopulmonary bypass (CPB) and extracorporeal membrane oxygenation (ECMO), take over the function of one or more organs, providing clinicians time to treat underlying pathophysiological conditions. ECMO and CPB carry significant mortality rates for patients, despite prior decades of research focused on the resulting failure of critical organs. Since the focus of these procedures is to support blood flow and provide oxygen-rich blood to tissues, a shift in research toward the effects of ECMO and CPB on the microcirculation is warranted. Along with provoking systemic responses, both procedures disrupt the integrity of red blood cells, causing release of hemoglobin (Hb) from excessive foreign surface contact and mechanical stresses. The effects of hemolysis are especially pronounced in the microcirculation, where plasma Hb leads to nitric oxide scavenging, oxidization, formation of reactive oxygen species, and inflammatory responses. A limited number of studies have investigated the implications of ECMO in the microcirculation, but more work is needed to minimize ECMO-induced reduction of microcirculatory perfusion and consequently oxygenation. The following review presents existing information on the implications of ECMO and CPB on microvascular function and proposes future studies to understand and leverage key mechanisms to improve patient outcomes. *ASAIO Journal* 2022; 68;881–889

Key Words: extracorporeal circulation, microcirculation, hemolysis, organ dysfunction, inflammation

According to the April 2021 Extracorporeal Life Support Organization (ELSO, Ann Arbor, MI) Registry International Summary, patients over the last three decades undergoing extracorporeal life support (ECLS) have a survival to discharge rate of 54%.[1] Extracorporeal life support involves extracorporeal circulation (ECC) of blood through an external membrane oxygenator and is vital in providing clinicians additional time to manage acute cardiac or respiratory failure patients. Fundamentally, ECC procedures temporarily replace the function of critical organs, namely the heart, lungs, or kidneys. Despite their extensive clinical utility, significant complications are known to develop, including thrombosis, thromboembolism, heparin-induced thrombocytopenia, cerebral hypoxia, pulmonary hemorrhage, and cerebrovascular compromise.[2–5] To date, most research has focused on effects within the context of organ-scale physiology and the macrocirculation. This review explores the microvascular changes during and post ECC to establish potential mechanisms that explain end-stage organ failure in ECC.

This review first introduces several types of ECC, with a specific emphasis on their setup and hemodynamic impacts. Due to the wide range of conditions encompassed under ECC, medication regiments provided during these procedures are out of the scope for this review. To grasp the extent of the potential damage resulting from ECC procedures, this review then presents the various organ injuries observed and key causes that have been proposed and researched. Since ECC entails the circulation of blood *ex vivo*, the review examines the causes of excessive shear stress and pressure differentials while considering the implications of hemolysis in the microcirculation. Finally, this review outlines the current research on ECLS's impact on the microcirculation and provide a call to action for microvascular ECLS research.

Hemodialysis and Hemofiltration

Dialysis, considered one of the earliest ECC procedures, is a form of renal replacement therapy (RRT) used in cases of chronic kidney disease, acute kidney injury (AKI), cardiorenal syndrome resistant to diuretic therapy, and end-stage renal failure secondary to glomerular disease in the absence of transplant.[6] For patients with end-stage renal disease (ESRD), there are three primary conduits for hemodialysis: an arteriovenous fistula (AVF), a synthetic arteriovenous graft (AVG), or central venous access. The most ideal is an AVF, which shunts blood from an artery to a vein, bypassing the distal microcirculation, providing increased flow[7] while reducing infection.[8] When hemodialysis and hemofiltration are used in tandem, the procedure is termed hemodiafiltration. In this section, we aim to provide a brief discussion of hemodialysis, followed by hemofiltration, and a comparison of the two methods.

According to the United States Renal Data System (USRDS), as of 2017 over 60% of patients with ESRD underwent dialysis treatment, with an adjusted mortality rate of 167 per 1000 patient-years.[9] Hemodialysis (**Figure 1A**) is a procedure to remove small metabolic waste products through diffusion and rectify electrolyte imbalance with a dialyzer. Blood is pulled from an artery and pumped into the lumen of a dialyzer's semipermeable membrane, while dialysate solution is pumped outside. Countercurrent flow allows for efficient transport of small molecules and water across the membrane. Used dialysate is discarded, while the blood re-enters the venous circulation.[10]

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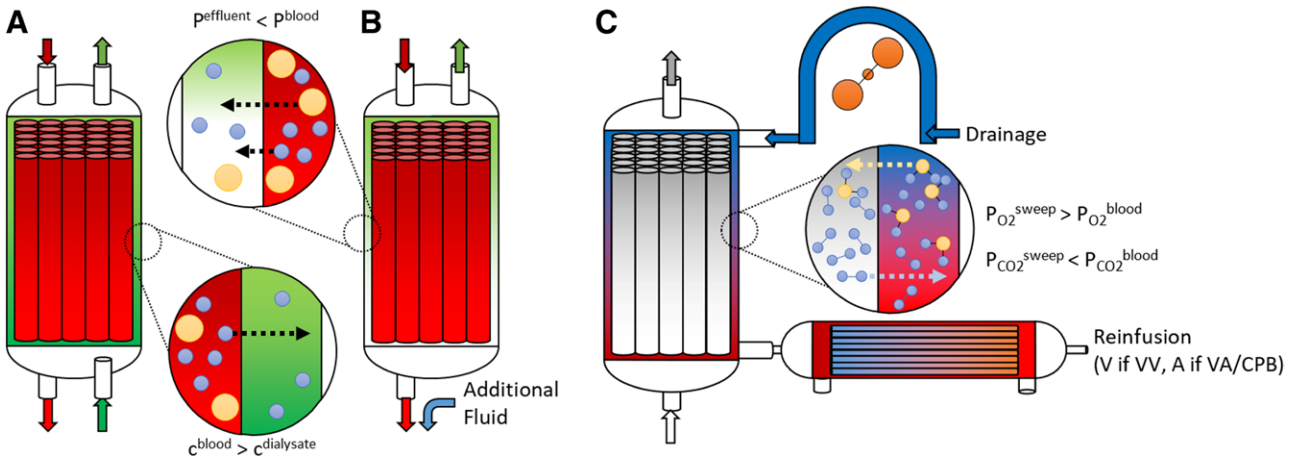

Figure 1. Various types of extracorporeal circulation. **A and B**: Renal replacement therapies. **C**: The basic components of ECMO. **A**: Hemodialysis: Blood (red) flows within the dialyzer lumen, while dialysate (green) flows outside. This leverages diffusion, allowing for small molecules (blue) to pass across the membrane from high to low concentrations. **B**: Hemofiltration: Blood (red) flows within the dialyzer as a pressure differential is applied transverse across the membrane. This leverages convection, allowing for larger molecules (blue) to pass across the membrane from high to low pressure. Additional fluids are added to account for volume loss. **C**: ECMO basics: Venous blood feeds a pump (top) that pushes the fluid into the oxygenator (left). Sweep gas (white) flows within the lumen of the hollow fiber membrane, while blood flows outside, allowing for diffusion of CO_2 and O_2. Blood then passes through a heat exchanger (bottom) before reinfusion. ECMO, extracorporeal membrane oxygenation. full color online

On the other hand, hemofiltration (**Figure 1B**) involves removal of excess medium to large sized metabolic waste products while reducing hypervolemia. Blood is passed through a dialyzer in a manner similar to hemodialysis; however, dialysate is not passed outside the semipermeable membrane. By adjusting the dialyzer flow and transverse pressure gradient across the membrane, fluid and molecules are forced through the pores of the membrane, forming an effluent, which drains out medium and large sized metabolic waste.[11] To maintain euvolemia, additional fluids are sometimes introduced before the reintroduction of blood back into the body.

The underlying physical mechanism by which hemodialysis and hemofiltration function accounts for many of their observed differences. Hemodialysis leverages diffusion, where concentration gradients between blood and dialysate across the membrane drive flux. On the other hand, hemofiltration leverages convective transport, in which the hydrostatic pressure differentials across the lumen of the membrane drives ultrafiltration.[12] As a result, transport in hemofiltration is much faster, although less specific. From a chemical perspective, hemodialysis ensures that electrolytes (small molecules) are equalized between blood and dialysate, although larger molecules may not be. Alternatively, hemofiltration allows for excretion of metabolic waste of variable size but risks hypovolemia, often requiring volume resuscitation before reinfusion due to the lack of dialysate solution used.[7]

Cardiopulmonary Bypass

One of the most well-known types of ECC is cardiopulmonary bypass (CPB), which allows for a bloodless field during cardiac surgeries such as heart transplant[13] or implantation of ventricular assist devices (VADs).[14] In several surgical procedures, a cardioplegic solution is passed through the chambers of the heart to induce hypothermia and prevent death of myocardial tissue. A heart-lung machine takes over the primary function of the heart to pump oxygenated blood throughout the body. Blood is drained *via* cannulas into a reservoir, which feeds an oxygenator to allow for gas exchange. Temperature is controlled with a heat exchanger before being pumped back into the body through a cannula into the aorta. Auxiliary pieces of the circuit include cardiotomy suction, a cell saver for return of red blood cells (RBCs), and devices for left ventricular venting to prevent excessive distention due to low contractility and high afterload.[15]

Extracorporeal Membrane Oxygenation

Some patients fail to wean off CPB after open heart surgery, requiring initiation of extracorporeal membrane oxygenation (ECMO), otherwise known as ECLS. In general, use of ECMO (**Figure 1C**) is reserved for patients with severe acute respiratory failure, although general guidelines are provided in detail by the ELSO.[15] Indications for ECMO include hypoxemic respiratory failure despite optimization of positive end-expiratory pressure and the inspiratory–to-expiratory ratio,[16] acute lung injury that may progress to severe acute respiratory distress syndrome,[17] hypercapnic respiratory failure (pH < 7.20),[18] cardiac failure/arrest, and cardiogenic shock.[19] There are two primary forms of ECMO, namely veno-arterial (VA-ECMO) and veno-venous (VV-ECMO). Importantly, VA-ECMO provides cardiac support, while VV-ECMO does not; several clinical situations merit the need of VA-ECMO *versus* VV-ECMO, although details are outside the scope of this review. For either technique, deoxygenated blood drains into a blood reservoir, which feeds a pump that imparts hydraulic energy to either supplement or take over cardiac function. Blood passes through an oxygenator to remove carbon dioxide (CO_2) and load oxygen (O_2) *via* gas diffusion across a semipermeable hollow fiber membrane. As with CPB, the heat exchanger controls temperature before reintroduction of newly oxygenated blood into the circulation. These components are connected by synthetic polymer tubing, usually comprised of silicone or PVC treated with DEHP, which can be connected to auxiliary elements such as bubble traps and filters to prevent emboli or coagulopathies.[15]

Several complications can result from ECMO, including bleeding, thromboembolism, neurologic compromise, vascular perforation, and heparin-induced thrombocytopenia. According to the ELSO International Summary for 2021, the survival rate over the past 30 years for VA-ECMO and VV-ECMO procedures is 65% and 77%, respectively.[1] Some of the major complications for VA-ECMO are estimated at 55.6% AKI with 46% requiring RRT, 41.9% requiring a thoracotomy, 40.8% hemorrhage, and 30.4% infection.[20] Despite ECMO's lifesaving potential, complications are significant, and each clinical situation should be considered carefully before introducing ECMO treatment. Due to their similarities, ECMO and CPB will be the focus of this review moving forward.

Clinical Complications in Vital Organs

Organ failure after ECLS leads to high postoperative hospital mortality.[21] The function of the disease is not well understood, although ECLS is known to induce a whole-body inflammatory reaction, postoperative complications, and complement activation. These symptoms are concurrent with conditions such as systemic inflammatory response syndrome and multiorgan dysfunction syndrome, which left untreated can be devastating to patient survival rate and recovery time.[22] The following section highlights the impact of ECMO and CPB on various critical organs (**Figure 2**).

Heart

Impacts on cardiac function can vary depending on the modality used; research in VV-ECMO shows limited impact on systemic hemodynamics,[23] while VA-ECMO and CPB have the potential to require left ventricular venting to preserve cardiac function. Due to high flow rates of blood returning from the circuit to the systemic circulation, supraphysiologic arterial pressures are often observed.[19] Normally, the myocardial tissue would compensate by increasing contractility since rise in afterload increases myocardial stretch, which heightens myocardial length-dependent activation and contractility *via* the Frank–Starling mechanism.[24] However, patients on VA-ECMO and CPB often have either acute fulminant or congestive heart failure, and both Frank–Starling and length-dependent activation at the sarcomere level are impaired. Some studies have suggested this is due to adrenergic downregulation, but the mechanisms remain unknown.[25] Nonetheless, in most cases, patients on VA-ECMO or CPB are unable to compensate without inotropic stimulation, which is often associated with poor outcomes. Even with partial compensation, mechanisms to increase contractility jointly increases metabolic rate and demand, further worsening cardiac function.[19] Furthermore, reduced right ventricular filling pressures with supraphysiologic arterial pressures in VA-ECMO or CPB can eventually result in decreased myocardial perfusion and ischemia.[26] When this is compounded by lack of oxygenated blood, ischemia may progress to myocardial infarction. Importantly, the progression of ischemia is more dependent on elevations in left ventricular end diastolic pressure, which directly decreases myocardial perfusion pressure, and not lack of oxygenated blood; the latter only worsens the observed pathophysiology. Without adequate relief or with increased VA-ECMO circuit flow rate, elevated LV filling pressures induce a dilated LV with systolic dysfunction, leading to flow stasis and LV apical thromboembolism, or even accumulation of neutrophils and platelets.[27] For these reasons, LV venting is preferred.

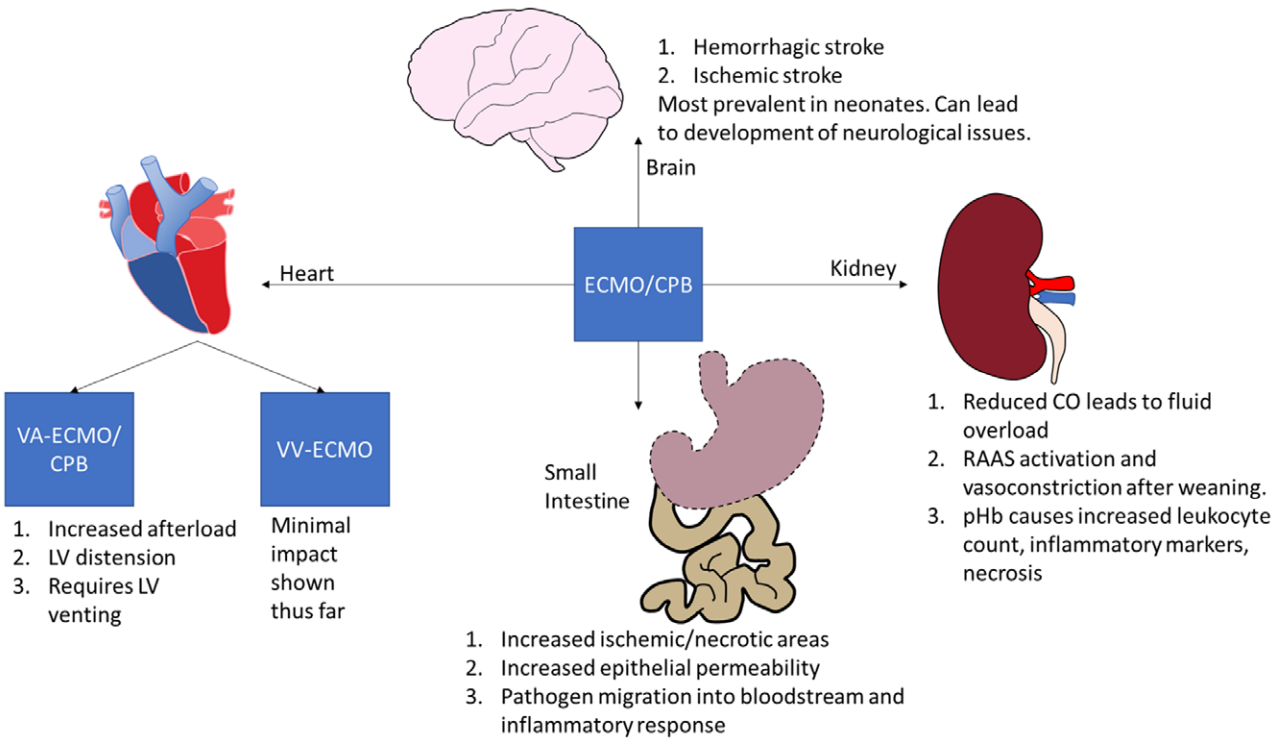

Figure 2. Impact of ECMO and CPB on various organs. CPB, cardiopulmonary bypass; ECMO, extracorporeal membrane oxygenation. full color online

The main strategy to offload the heart is decompression, which can be achieved by venting or unloading.[28] Venting passively diverts flow away without imparting energy to the fluid, either by atrial septostomy or fluid diversion by means of a split connector. In cases when venting on its own is not able to sufficiently depress ventricular filling pressures, one can unload the LV by means of a pump, which actively expends energy. Recent studies have shown that implantation of Impeller centrifugal pumps helps reduce myocardial infarction size by reducing metabolic demand and thus lowering the rate of LV remodeling.[29] However, there is a tradeoff, since pumps increase the rate of hemolysis *via* shear, excess pressure, and in failure cases, excess air entrainment.[30]

Brain

The brain is the most important organ in the body—given the immeasurable assignment of conscious and unconscious control over body functions, memory, and a multitude of other imperative tasks. Depending on age, the brain can account for 20–50% of total resting oxygen consumption, making cerebral blood flow and oxygenation critical for patient recovery.[31] Extracorporeal membrane oxygenation has been shown to induce neurologic injury beyond that secondary to cerebrovascular compromise and ischemic stroke, although the mechanism of acute neurologic injury remains unknown.[32] Specifically, encephalopathy, anoxic brain injury, stroke (both hemorrhagic and ischemic), myoclonus, brain death, and coma of uncertain cause have all been observed.[33] Importantly, the incidence of these is more associated with VA-ECMO. Risk factors for ECMO-induced neurologic injury include age, pre-existing coagulopathy, pre-existing cardiovascular disease, and pre-existing neurologic disease. With respect to age, the adult prevalence of ECMO induced neurologic injury is between 7% and 16%,[34,35] while neonatal prevalence is as high as 50%.[36] The pediatric population has recently been of clinical interest, as patients on ECMO have been observed to exhibit developmental deficiencies in adolescence in neuropsychologic areas such as visual-spatial memory and verbal skills later in life.[37] Of all neurologic sequelae observed post-ECMO, the most common is stroke, with hemorrhagic and ischemic stroke showing relatively equal incidence. Risk factors include thrombocytopenia, low plasma fibrinogen levels (indicative of a consumptive coagulopathy, *e.g.,* disseminated intravascular coagulation), and duration of ECMO.[34] VA-ECMO is often associated with reduced internal carotid artery diameter, due to carotid remodeling post decannulation.[36] Interestingly, peripheral cannulation is associated with lower middle cerebral artery velocity[38] and increased risk of stroke postdecannulation, suggesting that the site of cannulation is of clinical importance.[39] Furthermore, poor circulation leads to spikes in proinflammatory responses and metabolic shifts.[40] Elevated creatinine levels from low clearance of metabolic byproducts,[41] along with increased lactate and localized oxygen desaturation indicate the onset of cerebral hypoxia.[36] Increased counts of B and T cells during ECMO indicate upregulation of the adaptive immune system,[42] which persist post-ECMO, as reflected in elevated choline levels, although whether microglia are upregulated remains unknown, making the neurologic relevance questionable.[41] Increased inflammation has also been associated with decreased autoregulation of cerebral flow,[42] further increasing the risk of hypoperfusion and ischemic stroke.

Kidney

ECMO and CPB have been known to lead to acute kidney injury (AKI), with a prevalence in adults around 60%[43] and a prevalence in neonates and pediatrics ranging between 65% and 80%.[44,45] Severe AKI is often treated with RRT, which is only effective about 50% of the time in these two most vulnerable populations. Mortality in neonates and pediatric patients are around 20% and 30%[46] for those with less severe AKI, while those requiring RRT have an increased mortality of 40% and 60%,[46] respectively.

Systemically, there are various changes that occur during and after ECMO and CPB. First, it is extremely common for cardiac output (CO) to drop immediately after weaning due to lower arterial pressures. This induces activation of the renin-angiotensin-aldosterone (RAAS) system and subsequent systemic vasoconstriction, particularly of renal afferent arterioles. As a result, renal perfusion pressure is low, which induces a so-called prerenal AKI (prerenal because the mechanism of AKI is not due to glomerular or tubular disease).[47,48] The resulting oliguria along with reduced MAP have shown to be good predictors of mortality.[47] Additionally, reduced CO can lead to fluid overload, which limits oxygen transport in critical tissues, reduces blood volume, and further contributes to oliguria, all consistent with a prerenal AKI.[48] Various strategies to combat these issues have been used, including administration of inhaled nitric oxide (NO) before initiation of ECMO to help distribute blood flow.[44] Another strategy used are loop diuretics, whose aim is to prevent hyperkalemia and to decrease hypervolemia secondary to cardiogenic shock or congestive heart failure.

Besides urine output, there are also several markers which have been associated with AKI. Serum creatine and blood urea nitrogen are indices of renal function, the former being a proxy for glomerular filtration rate. Indications for RRT include acidosis, abnormal electrolytes, specifically hyperkalemia to prevent peaked T waves and sudden cardiac death, intoxication (*e.g.,* ethanol intoxication, ethylene glycol, or any compound that induces a high anion gap metabolic acidosis), fluid overload *(e.g.,* secondary to CHF), and uremia, which is known to cause several clinical sequala, including uremic pericarditis.[49] In neonates and pediatric patients, serum creatinine has been shown to double during CPB in 40% of patients,[50] with levels above 1.5 mg/dl during ECMO displaying an elevated mortality risk.[46] Additionally, elevated urinary neutrophil gelatinase-associated lipocalin (uNGAL) has been shown to be an early index of renal failure,[49,50] which may have implications for patient selection for RRT. The changes in these markers are driven by hemolysis, as evident by increased plasma hemoglobin (pHb) levels.[50] The downstream effects of increased pHb levels in the kidney are threefold. First, there are increased levels of neutrophils and platelets found in the kidney.[27] Second, inflammatory markers such as neutrophil elastase, tumor necrosis factor alpha (TNF-α), IL-6, and reduced A1M levels in renal tubules are increased due to oxidative stress and upregulation of heme clearance.[49] Last, tubular distension, epithelial cell detachment, and proximal tubular damage, all components of an ischemia mediated acute tubular necrosis have been observed.[49]

Intestines

Out of all the organs discussed regarding clinical sequala observed from ECMO or CPB, the least is known about the impact on the gastrointestinal tract, specifically the intestines.

ECMO has been shown to induce leukocyte extravasation into the basal layer of the small intestine mucosa, microhemorrhages, and apical microvilli swelling.[51] In addition, pHb increases spacing between epithelial cells, increasing epithelial layer permeability, as observed by migration of ileal lipid binding protein (FABP6)[52] and intestinal fatty acid binding protein (FABP2).[53] In cases of concordant infection, these changes allow for bacteria secreting proinflammatory pathogen associated molecular patterns, such as lipopolysaccharides, to pass into the bloodstream, inducing sepsis and a systemic inflammatory response. There are two primary mechanisms for this phenomenon. First, there is upregulation of the extrinsic apoptotic pathway by phosphorylation of p38 MAP kinase, which increases Fas ligand expression on small intestinal mast cells, leading to caspase 8 activation.[53] Second, local inflammation induces intestinal mast cells to release large granule stores of TNF-α and IL-8 cytokines, as measured by elevation of plasma tryptase levels.[51] High levels of inflammatory cytokines, such as TNF-α, have been shown to exacerbate permeability issues by leading to cytoskeleton contraction *via* increased MLC kinase activity.[54] The addition of pHb from intravascular hemolysis results in NO scavenging and a subsequent decrease in NO levels, resulting in decreased intestinal microvascular perfusion, further compromising the barrier.[52]

Excessive Shear Stress From Pumps Induce Hemolysis in Extracorporeal Circulation

As discussed above, ECC can result in several complications in key organs, which affect patient outcomes, the most common of which include bleeding and thromboembolism. As with many extracardiac devices, excessive mechanical shear and pressure gradients mainly contribute to a so-called macroangiopathic intravascular hemolysis in ECC. To understand the mechanism of hemolysis in ECC, a basic understanding of the engineering of these devices is required. There are two types of pumps used, namely peristaltic and centrifugal. Peristaltic (rotary) pumps utilize rollers to pinch tubing located in the pump boot, trapping a section of fluid, and forcing the fluid from the inlet to the outlet as the pump head rotates. These are classified as positive displacement pumps since they typically deliver a constant volume at a fixed rotational speed independent of system pressure. While setup can vary by number of rollers, occlusion level, and tubing size, studies have shown that excess negative pressure,[55] time with two consecutive rollers occluding the pump,[56] and pressure pulsation frequency[57] all increase the degree of hemolysis due to excess mechanical stress.

Centrifugal pumps are also used in both CPB and ECMO. These turbomachines impart energy to the fluid by transfer of kinetic energy generated by rotation of an impeller. A portion of the kinetic energy is then converted to pressure as the fluid passes through the volute. Design of these pumps are extremely complex, and the geometry of the pump is optimized to produce correct flow and pressure to meet system requirements. Computational fluid dynamic (CFD) analyses allow for optimal efficiency by adjusting key parameters, such as blade number and blade angle before impeller manufacture.[58] Furthermore, these simulations need to predict excessive red cell lysis to prevent macroangiopathic hemolytic anemia, as discussed above. From CFD, hemolysis indices are calculated based on shear stress and residence time for comparisons.[59] Verification using benchtop hydraulic testing with a blood-mimicking fluid such as glycerin and water solution[60] and xenograft blood show that friction points, especially at rotor bearings[61] create higher degrees of hemolysis. For this reason, it is more common for centrifugal blood pumps to have magnetically levitated rotors. Centrifugal pumps have also been used as VADs for heart failure patients, each with their own intended use and observed complications. For instance, pediatric heart transplant patients with an EXCOR implanted survived at a higher rate as compared with pediatric ECMO patients.[62] However, hemorrhage and stroke remain a big concern with these patients, with 29% of patients experience at least one neurologic event while the EXCOR was implanted.[63] Another device, the CentriMag, showed impact to leukocyte function,[64] increased levels of nonphysiologic shear stress leading to increased platelet activation and receptor shedding,[65] and decreases in von Willebrand factor collagen binding activity.[66] Recent investigation into off-label use of the CentriMag for pediatric ECMO has revealed increased platelet activation as compared with on-label use for adult ECMO; interestingly, the PediVAS, a device intended for pediatric ECMO showed retrograde flow during CFD analysis, highlighting that operation of blood pumps should be performed at their design point.[67] In summary, centrifugal pumps are generally regarded as producing more hemolysis as compared with roller pumps but require less circuit volume (and as a direct result, foreign surface area).[15] Clinically, there is no consensus as to which pump minimizes bleeding and thromboembolism and improves outcomes, and the choice of pump varies by institution.[15]

Beyond macroangiopathic hemolytic anemia, erythrocyte injury has several downstream consequences, including formation of systemic thromboemboli and several other systemic consequences secondary to cell-free hemoglobin release into the plasma (pHb).[68] Thrombus formation is driven primarily through activation of platelets, which is exacerbated from hemolysis *via* NO scavenging and ADP release from plasma free hemoglobin.[69] The Platelet Activation State has been used as a relative measure of platelet activation in both in-vitro[70] and computational settings.[71] Initial protein adsorption onto circuit surfaces over time eventually gets replaced by higher-affinity proteins for the surface such as fibrinogen, which is known as the Vroman effect. On the other hand, while adhesion with fibrinogen increases over time, research has also shown that adhesion due to von Willebrand factor decreases over time partly due to platelet receptor shedding,[65] which affects hemostasis. Bleeding is known to occur in between 30% and 50% of all patients who receive ECMO and is secondary to continuous anticoagulation and platelet dysfunction.[72,73] Furthermore, formation of antiplatelet factor 4 (anti-PF4) antibodies can result in heparin-induced thrombocytopenia, which has been associated with patients on ECMO,[5] the mechanism being unknown. Most commonly, systemic thromboemboli resulting from either direct formation within the ECMO circuit or secondary to the induced consumptive coagulopathy are known to occur. For this reason, new technologies are continuously developed to prevent serious complications and improve patient outcomes.

Extracorporeal Circulation and the Microcirculation

Hemolysis and the Microcirculation.

The effects of pHb have been well established and are particularly pronounced in the microcirculation. Once Hb is

released from lysed RBCs, pHb scavenges NO produced by vascular endothelial cells (ECs) and limits NO availability for smooth muscles, resulting in vasoconstriction.[74] NO oxidizes pHb into methemoglobin (metHb), which can dissociate and form hemin from heme.[75] Hemin contributes to low-density lipoprotein oxidation, resulting in proinflammatory upregulation of hemeoxygenase 1 and ferritin within ECs.[76] Heme itself causes a proinflammatory response by activating toll-like receptor 4 on the surface of macrophages, inducing release of TNF-α.[74,77] In addition, the depletion of NO by pHb can decrease the cGMP levels by decreasing soluble guanylyl cyclase (sGC) activity, leading to increased platelet aggregation.[78]

Blood contains several mechanisms to control pHb and its byproducts. pHb binds to haptoglobin (Hp) in plasma to form a Hb-Hp complex, which binds to CD163 receptors on the surface of macrophages to be internalized and metabolized, releasing heme and iron. However, when pHb levels exceed the Hp binding capacity, pHb oxidizes, causing methemoglobinemia and Hb dissociation, releasing heme.[79] Hemopexin (Hx) can bind heme, which then binds to CD91 receptors on hepatocytes, allowing for heme breakdown and clearance. Once oxidation occurs, Hx prevents excessive loss of iron and oxidative stress in critical tissues.[75] Hp is considered an acute response protein due to its high binding affinity for Hb, while Hx demonstrates more benefit in chronic hemolytic conditions to mitigate oxidative stress. One advantage of Hx is that it can be recycled, while Hp is ultimately consumed and needs to be remanufactured.[77] Innately, the levels of these molecules are miniscule when compared with concentrations of hemoglobin and can easily become overwhelmed in severe hemolytic conditions.[80]

As illustrated previously, ECC causes hemolysis of RBCs and thus increases pHb.[30,50] The resulting vasoconstriction can reduce perfusion in the microcirculation, leading to a hypoxic and eventually ischemic condition.[81] The generation of reactive oxygen species may contribute to endothelial dysfunction.[82] This increase in vascular permeability coupled with redistributed intravascular pressure may affect filtration and reabsorption across the lumen of capillaries and overall microcirculatory dysfunction. Therefore, the introduction of pHb into the circulation can ultimately lead to damage within multiple organ systems.[83]

Current State

While the main goal of ECMO is to increase oxygen blood content, whether this translates to improved oxygen delivery in the microcirculation is unknown. To investigate the effects of ECMO on the microcirculation, several techniques exist, including Laser Doppler, Near Infrared Spectroscopy and Imaging (NIRS), laser speckle imaging, orthogonal polarization spectral (OPS), and dark field microscopy (both incident and sidestream),[84] with the latter two used most commonly in ECMO research. Both OPS and dark field microscopy are relatively noninvasive imaging techniques, which allow for use in clinical settings. Additionally, dark field microscopy has become more popular due to better image quality and development of biomedical devices such as the CytoCAM devices (Braedius Medical).[85] Nonetheless, there are several limitations. Since both these imaging techniques are at a wavelength specific to hemoglobin, the cell-free layer (CFL) cannot be imaged. Consequently, in small arterioles[85] and hemodilution cases, where CFL thickness is increased relative to vessel size (Fahraues–Lindquvist effect[86]), vessel diameter measurements from these techniques may be underestimated. Further, both these imaging modalities require relatively translucent connective tissue for direct visualization.[85] Additionally, most of the parameters obtained are semiquantitative counts of vessel perfusion[87]; however, both OPS and dark field microscopy cannot address the mechanisms of flow changes, which may be important in assessing microcirculatory disorders secondary to septic shock or multiorgan dysfunction.

Recent research efforts to quantify changes in the microcirculation with ECC, specifically ECMO, have expanded. Studies have shown that administration of VA-ECMO in patients with severe respiratory failure improved functional capillary density (FCD) in the buccal mucosa (*i.e.,* cheek lining) as compared to patients on ventilation. However, FCD tends to be lower in VA-ECMO patients with respiratory disease, independent of blood pressure and heart rate.[88,89] Therefore, changes in the systemic circulation do not necessarily translate to the microcirculation, highlighting the relevance of microvascular perfusion. Furthermore, studies regarding type of ECMO support have shown little difference in the microcirculation, while patient age has been associated with changes in microcirculatory hemodynamics.[90] Microvascular measurements can also be used as predictive markers for cardiogenic (and septic) shock in patients,[84,89,91] especially in refractory cases.[92]

Mammalian models have been utilized to understand the effects of ECC on the microcirculation, which comes with advantages and disadvantages of its own. Animal research allows control over more variables (*e.g.*, priming fluids, pathophysiology of the animal, *etc.*), but it reduces translatability due to differences in underlying physiology as compared to humans. Some of the earliest models used were pigs, sheep, and dogs; these models were used in conjunction with OPS and other techniques such as microsphere deposition. Recently, researchers have combined conventional microcirculation techniques in small mammals (*e.g.*, cremaster, omentum, and dorsal skinfold preparations) with scaled down or simplified ECC circuits to allow for more precise assessments of the microhemodynamics. For instance, Kamler *et al.*[93] have done significant work regarding the inflammatory response in the microcirculation as a result of ECC in Golden Syrian hamsters. These techniques should be utilized more often and expanded upon to elucidate more of the underlying causes of microvascular dysfunction due to ECC.

CONCLUSIONS AND FUTURE WORK

The primary goal of ECC is to allow physicians time to treat underlying disease in patients with acute organ failure. ECC circuits can vary in their role, setup, and components. Due to the need to drive blood flow *ex vivo*, pumps are often used to impart energy to the fluid; however, this can induce macroangiopathic hemolytic anemia as well as downstream pHb release. Subsequent systemic vasoconstriction, organ dysfunction, and systemic immune system activation are observed, although not completely understood.

Importantly, however, the redistribution of flow in the microcirculation partly explains microvascular complications secondary to ECC. Thus, understanding the implications of ECC on microvascular hemodynamics is vital. Subsequent disruption

of metabolic waste exchange and nutrient/metabolite delivery can lead to ischemia–reperfusion injury, shock, and end-organ hypoperfusion, ultimately leading to organ failure. We therefore propose a call to action for research in ECMO microvascular hemodynamics and ECC in general to improve survival to discharge rates in an extremely vulnerable patient population.

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