The inflammatory response to cardiopulmonary bypass: a therapeutic overview

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The demographic of cardiac surgery patients continues to evolve to include older, sicker candidates, all the while maintaining an expectation of excellent outcomes. These latter results can only be achieved by the parallel advancement and re-examination of the technology of cardiopulmonary bypass (CPB); the key tool used daily by surgical teams worldwide. In this review, we will provide an overview of integrated therapeutic strategies that can be utilized to minimize the complex and myriad changes related to inflammation after CPB with the understanding that this may abrogate the detrimental end-organ and systemic effects of blood activation. Therapeutic strategies specifically related to the technology can be classified into those targeting biomaterial dependent or independent processes. The former can be addressed by the utilization of currently available biocompatible surfaces such as with heparin-coated circuits, phosphorylcholine-coated circuits ('biomembrane mimicry') and circuits composed of copolymers containing surface-modifying additives. The most important strategies related to biomaterial independent activation include the modification of techniques related to cardiotomy blood management and blood filtration. Finally, all of these strategies must be integrated and tailored with complementary pharmacologic agents such as aprotinin and steroids to optimize anti-inflammatory synergism. Only if we are armed with a comprehensive knowledge of the molecular and cellular basis for these strategies will we be able to continue to evolve our treatment in parallel with our patients to achieve these goals. Perfusion (2004) 19, S5–S12.

The complex questions relating to the systemic and metabolic inflammatory responses to cardiopulmonary bypass (CPB) have been supplanted by scientific topics related to alternative cardiovascular technologies. Most notable amongst these is the universal trend (alternatively gaining and losing momentum around the world) of off-pump coronary artery bypass grafting (OPCAB). To the casual observer, this strategy is intuitively attractive; however, the limitations and the contraindications to OPCAB highlight the continuing need for ongoing research for the betterment of circulatory support. Now and in the future, we will be faced with a sicker population with unstable hemodynamics or diffuse small vessel disease precluding OPCAB techniques, as well as an expanding population needing open-heart procedures such as valve replacement. In fact, it is within this high-risk group that the greatest benefits of a scientific thrust in biocompatibility that addresses the inflammatory responses to CPB will be best realized.

Although, undoubtedly, the general inflammatory reaction to heart surgery is influenced by the very nature of the ‘controlled trauma’ of multiple incisions and the median sternotomy, as well as the systemic impact of general anesthetic agents, this review will concern itself primarily with the potentially modifiable aspects of surgical care, such as device choice and operative tactics.

Therapeutic strategies in this regard can be considered in two classes. First, we and others have provided evidence that blood–biomaterial interactions related to the composition of the synthetic surface of the circuit can play a significant role in the humoral systemic response to CPB (Biomaterial-dependent responses) and, thus, circuit choice may be an important contributor to outcome, particularly in high-risk patients. A classification of the spectrum of currently available surfaces will be presented with a view to integrate our understanding of the means by which they may ameliorate this response.

The alternative target (Biomaterial-independent responses) relates to aspects of surgical strategy, such as cardiotomy blood management and deployment of alternative pharmacologic and device technology (e.g., filtration), all of which may have been

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previously unrecognized as important approaches to limit inflammation in the adult cardiac surgical population.

The targeting of biomaterial-dependent processes during CPB

There is a complex myriad of events comprising the blood–biomaterial interaction and the reader is referred to alternative sources and reviews to gather perspective on this subject. However, it appears that the pivotal events within these cascades that may be modified in a positive manner to render improved biocompatibility may be categorized into three dynamic processes. The first involves the relative resistance to the activation of proteins within the contact activation pathway. The second relates to complement activation. Although this process may be secondary to the nature of protein (and particularly immunoglobulin) adsorption, it is complicated by the contribution from kallikrein activation and cytokine release. Finally, a sensitive predictor of biocompatibility relates to the dynamics of fibrinogen adsorption – not only the resulting absolute surface density, but also on the nature of the conformational changes that may occur after adsorption. There is a close inter-relationship between this event and the degree of platelet ‘accumulation’ on the surface. Despite the key role of platelets and fibrinogen in the process of thrombosis, it should be recognized that all of these steps are intimately entwined with the activation of inflammation and it becomes impossible to differentiate the processes of thrombosis and inflammation from an academic point of view.

Biomaterial options for CPB circuits

Economic factors have limited the evolution of novel materials for CPB circuits such that, at present, there remain only three primary types of ‘biocompatible’ surface modification commercially available for clinical use. The first of these is distinguished by the strategy of ‘biomembrane mimicry’, capitalizing on the recognition that the thromboresistance of the native nonactivated biological membrane (e.g., erythrocytes) is key to the maintenance of the fluidity of the circulation. Phosphorylcholine, the phospholipid component of interest on the outer or luminal surface, is electrically neutral (zwitterionic) unlike its polar thrombogenic counterpart on the abluminal surface (phosphorylserine). Phosphorylcholine has been incorporated into a copolymer (methacryloylphosphorylcholine/laurylmethacrylate, MPC:LM), which due to its hydrophilic properties, is referred to as a hydrogel. The hydrogel is relatively easily coated onto other polymer surfaces post-fabrication, in a manner which is stable and safe with minimal leaching. The surface has proven durable with maintained surface integrity over long periods. Coronary stents, coated using a similar process, have been explanted after up to six months with evidence of an intact MPC:LM layer in both animals and humans.

In in vitro testing, the MPC:LM coating has been proven to resist protein and, in particular, fibrinogen adsorption, likely related, in part, to the intrinsic hydrophilic properties of the surface (Figure 1). Platelet ‘binding’ and activation under static conditions is also inhibited as compared to control uncoated surfaces and there is evidence that contact activation, as determined by the degree of factor XII activation, is decreased. Interestingly, when solubilized, the MPC has an inhibitory effect on the activity of the enzyme thrombin, although the mechanism for this is not as yet clear.

This surface is in the early stages of clinical evaluation and, as such, human experience is minimal. Two prospective randomized trials from De Somer et al. have evaluated hematologic measures of biocompatibility related to MPC:LM coating. In the first, involving adult patients (n = 10/group), where the effects of cardiotomy blood were excluded, the MPC:LM coating was associated with decreased platelet activation as measured by levels of β-thromboglobulin (BTG). The test group was also found to have a decrease in the amount of early postoperative bleeding. In the second study, involving pediatric cases (n = 10/group), similar platelet preservation by the end of CPB was observed.

![Figure 1](image-url)

**Figure 1** Coating of polycarbonate and polyvinylchloride (PVC) with MPC:LM caused a significant reduction in fibrinogen adsorption. Fibrinogen measured by immunoassay, Peroxidase-mediated reaction with o-phenylenediamine dihydrochloride with absorbance change (Abs) read at 450 nm. Reproduced with permission from Campbell et al.10
(decreased BTG, decreased thromboxane B2) as well as a positive effect on complement generation as measured by levels of terminal complement complex (TCC).\textsuperscript{16}

The second group of biocompatible CPB surfaces is the heparin-coated circuits (HCC). Ionic binding of heparin to the surface (Duroflo\textsuperscript{TM}, Jostra, Germany) is an effective and relatively inexpensive process. However, there is evidence of heparin leaching, perhaps precluding this system’s benefit for long CPB cases. The Carmeda\textsuperscript{TM} (Medtronics, Minneapolis, MN) system is characterized by the use of covalent bonding of heparin through a polyethylene oxide spacer. Though more expensive, the permanence of the coating has made this an attractive option for ECMO. Trillium\textsuperscript{TM} (Medtronics) is an alternative product with a small quantity of heparin incorporated on the surface, but it is unclear as to whether the biocompatibility characteristics of the product are related to this admixture.\textsuperscript{17}

The intuitive understanding of the action of HCC, which was confirmed in \textit{in vitro} studies,\textsuperscript{18} is that the surface-bound heparin functioned effectively as a cofactor with anti-thrombin III in the inhibition of thrombin. Consequently, this supported the rationale to advance to clinical trials comparing HCC with decreased intensity heparinization (ACT 280 s) with standard circuits with standard heparinization. Many of these trials did demonstrate marked clinical benefit with this approach, with evidence of decreased bleeding and transfusion rates,\textsuperscript{19} shortened ICU and hospital stay,\textsuperscript{20} and decreased overall hospital costs of between US$1000 and US$3000.\textsuperscript{21}

What has remained unclear, however, is whether the positive outcomes seen in these studies are related to the surface as opposed to the differences in the heparin doses between the groups. It has not been demonstrated that this combined approach is associated with an amelioration of the generation of thrombin during cardiac cases as compared to control cases.\textsuperscript{22–24} Further, other trials have failed to show similar clinical benefits when identical heparinization regimes are used with HCC and control circuits\textsuperscript{25}, although there may be some advantages in high-risk patients.\textsuperscript{26}

Heparin has a proven anti-platelet effect when administered intravenously (as opposed to \textit{in vitro} platelet suspensions)\textsuperscript{27,28} and it is also associated with enhanced fibrinolysis.\textsuperscript{27} Therefore, one would have to assume that the increased heparin dose in the control populations in these studies may be associated with an augmentation of these effects, thus, biasing results.

This is not to say that HCC have no advantage in terms of biocompatibility. In fact, what has been consistently demonstrated in many clinical trials is the ability of HCC to inhibit the activation of complement,\textsuperscript{23} a potent contributor to the inflammatory impact of CPB.\textsuperscript{29} Although the mechanism has not been proven, it is anticipated that the effect of HCC is related to the inhibition of kallikrein by the formation of a complex of adsorbed high-molecular weight kininogen, kallikrein, anti-thrombin III and surface-bound heparin.\textsuperscript{30}

The final group of available biocompatible materials for CPB involves those utilizing a microdomain concept to minimize cell and protein interaction with the surface. Both the X-Coating\textsuperscript{TM} (PMEA, Terumo, Japan) and the SMAR\textsubscript{x}T\textsuperscript{TM} (Sorin Biomedica, Mirandola, Italy) surfaces are prepared as copolymers during circuit fabrication. With the SMAR\textsubscript{x}T surface, the copolymer (polycaprolactone, polysiloxane) migrates to the polymer surface to maximize surface concentration.

The conceptual advantage of this approach capitalizes upon the localization of alternating hydrophobic (polysiloxane) and hydrophilic (polycaprolactone) ‘domains’ on the blood-contacting surface. Protein adsorption, including fibrinogen, is more pronounced upon hydrophobic surfaces, whereas due to the polar groups and nucleophilic properties of hydrophilic surfaces, contact (negatively-charged surface) and complement activation are enhanced.\textsuperscript{31} By controlling the distance between the microdomains, it becomes possible to compete (having one microdomain inhibit the effect of the other), thus, limiting protein adsorption (Figure 2). Cell membranes of circulating leukocytes and platelets, although fluid in nature with facile but limited lateral motion of membrane

\textbf{Figure 2} Scheme of the local microenvironment of hydrophilic–hydrophobic surfaces in contact with blood components including cells and proteins. 

\textit{Adapted with permission from Deppisch R et al., Nephrol Dial Transplant}\textsuperscript{31}
constituents, undergo the same type of competitive interaction between the surface and their membrane constituents, minimizing cell binding. The distance between microdomains to optimize the success of this inhibitory effect has been demonstrated to range from 10–20 nm. In clinical trials, we have demonstrated a clear effect of this surface in the inhibition of thrombin generation and fibrinolysis during CPB as compared to control surfaces (Figure 3). There was also a consistent and significant platelet protective effect with better retention of platelet counts and minimization of platelet activation, as shown by measures of released BTG. Other trials have demonstrated clinical improvements in patients randomized to CPB with this surface. However, larger populations would need to be tested to qualify and confirm these changes.

In vitro studies of the PMEA surface have identified a decrease not only in the total protein adsorbed on the test surface, but a marked decrease specifically of adsorbed immunoglobulin. The impact of this can be demonstrated by close evaluation of the effects of complement activation. In the latter process, interaction through the classical pathway via artificial surfaces can be reflected by the number of activated leukocytes which bind complement factors. In fact, in these models, levels of CD 35+ leukocytes are significantly decreased in the presence of the test surface.

The targeting of Biomaterial-independent processes during CPB

Distinct from the choice of the biocompatible surface lie those strategies that primarily encompass a) modifications of surgical techniques or b) the use of devices or technologies to overcome the systemic changes related to CPB. For example, a key contributor to the initiation of the cytokine and leukocyte response is likely secondary to changes in gut submucosal blood flow, through the release of minute quantities of lipopolysaccharide (LPS) and the subsequent release of tumor necrosis factor. This is a process more related to the choice of perfusion pressure and perhaps the use or non-use of pulsatile flow. Assuming these latter two factors

Figure 3 Markers of thrombin generation in control and test (SMAR, T+) groups A) measurement of thrombin–antithrombin III complex (TAT); B) measurement of prothrombin fragment 1.2 (F1.2). Samples taken pre- and postheparin (hep), 10, 20, 40 and 60 min on CPB and 10 min, 24 hours (hr) and 48 hr post-protamine (PP). Values are mean ± standard error of the mean. * Represents significance with \( p < 0.05 \).

Reproduced with permission from Rubens et al. (1999)
have been optimized, LPS release can be minimized, but probably not eliminated entirely when CPB is used, and alternative means, such as pharmacologic suppression (see below), may be necessary. The presence or absence of an air–blood interface (as dictated by the use of an open or closed/collapsible venous reservoir) has been assessed in clinical studies with small sample sizes, with a suggested effect of enhanced fibrinolysis in the open group although larger trials will be necessary to resolve this issue. The use or non-use of filters for CPB or for cardioplegia will also be addressed at this forum by numerous contributors with significantly greater experience than the author.

Many of the trials designed to assess these latter technologies have been complicated by the failure to consider the impact of cardiotomy blood on the hematologic and inflammatory response to CPB. It is now well accepted that this blood is likely the primary source for thrombin generation during CPB, despite the maintenance of adequate heparin levels. The volume of cardiotomy blood may range from hundreds to thousands of milliliters during a standard case. Mechanical damage may be a consequence of the injurious effects of the sucker or the marked air–blood interface. There are also a variety of reasons why heparin is ineffectual in cardiotomy blood, thus, promoting thrombin generation and, ultimately, contributing to inflammation due to the close connection between these two processes. These include the lower concentration of heparin in cardiotomy blood secondary to leaching of heparin onto mesothelial surfaces, its inhibition by platelet factor four released from activated platelets and heparin’s inability to inhibit clot-bound thrombin. Mechanisms of thrombin generation on the surface of macrophage/macrophages, such as those related to tissue factor and cathepsin release, are likely also not inhibited by heparin.

The other major consequence of particular current interest is the role of cardiotomy blood in microemboli formation that may ultimately result in neurologic dysfunction. It would appear, in particular, that fat microglobules from the mediastinal fat or the marrow are poorly cleared by current filtration devices in the cardiotomy reservoir and are, thus, culprits in the formation of small capillary and arteriolar dilatations (SCADs), which are the histologic landmark for post-CPB neurologic injury. We are currently evaluating the role of processing of cardiotomy blood through the combined action of fat filtration and centrifugal washing as a means of ameliorating the hematologic and neurologic consequences of this blood, in a formal randomized clinical trial with anticipated results within two years.

**Pharmacologic agents to suppress inflammation of CPB**

**a) Steroids.** It is debatable whether glucocorticoids given pre-CPB increase or decrease the systemic endotoxemia during CPB. However, steroids blunt the resulting complement activation and the release of TNF, IL-6, and IL-8 and CD 11b upregulation and neutrophil elastase release are also inhibited. Finally, glucocorticoids reduce airway NO concentrations during CPB, compatible with inhibition of bronchial epithelial iNOS expression.

The inhibition of these aspects of inflammation by steroids may explain the stabilizing effect of these drugs on hemodynamics after CPB. Teoh et al. demonstrated that methylprednisolone sodium succcinate (MPSS) given pre-CPB results in higher arterial pressures and higher vascular resistance after normothermic CPB. Others have demonstrated improved postoperative cardiac performance after standard CPB and a reduced incidence of hemodynamic instability that correlated with a drop in cytokine release after steroid administration. MPSS has also been demonstrated to be associated with decreased measured CK-MB as a measure of myocardial injury.

Although they are inexpensive, steroids are immunosuppressive and it has been suggested that they contribute to the pathogenesis of post-transfusion graft-versus-host disease. Their use has not been conclusively demonstrated to be associated with postoperative wound infection, although Mayumi et al. demonstrated that high-dose steroid administration pre-CPB suppressed T-cell function. Further, hyperglycemia was more frequent in the patients receiving steroids, which may also contribute to the risk of infection. In other trials, no detrimental effects of steroid administration were encountered. Larger clinical trials to specifically address the potential clinical benefits as opposed to these surrogate markers have not been completed.

**b) Aprotinin.** Although the primary indication for the use of the serine protease inhibitor aprotinin is to reduce blood loss during and after CPB, a number of studies have shown that aprotinin is to reduce cytokine levels and cytokine-induced events when used during CPB. However, this drug is very expensive when compared to steroid therapy. Further, work by Diego et al. demonstrated that the anti-inflammatory effect of MPSS exceeded that of
aprotinin as reflected by a greater inhibition of IL-6 at all time points after CPB. Steroids may augment the anti-inflammatory action of aprotinin. Tassani et al. demonstrated that high-dose MPSS attenuates the systemic inflammatory response during coronary bypass grafting in aprotinin-treated patients. IL-6 and IL-8 were significantly less in the MPSS group, whereas the anti-inflammatory IL-10 was greater. These changes corresponded to augmented oxygenation, improved lung compliance as well as improved cardiac index in the steroid-treated group.

Combining approaches to target the inflammatory cascade

It is evident that advances in biomaterial design and technical refinements in cardiac surgical strategy alone will only partially address the multitude of systemic changes that contribute to disseminated inflammation. Alternatively, the beneficial effects of the various strategies could be applied in an additive manner or, indeed there may be synergistic effects such that the combined effect exceeds the additive effect. For example, surgical strategy for high-risk patients in the future may be tailored to involve the use of anti-inflammatory HCC with pharmacologic agents to augment the anti-thrombotic effects of heparin. Alternatively, surfaces with intrinsic anti-thrombotic effects (e.g., SMA) may be suited to lower heparin dosing coupled with anti-inflammatory agents.

We have recently completed a clinical trial with the concept of combined strategies in which patients were randomized within a factorial design model to undergo CPB with or without a biocompatible surface (SMARx™) and with or without concomitant treatment with the anti-inflammatory MPSS (1 g IV at induction). Steroids were significantly effective in the suppression of the cytokine response (IL-6, IL-8), whereas there was a significant surface effect in the suppression of neutrophil activation ($p < 0.05$). Finally, there was a combined effect of the surface and steroids in the suppression of complement activation on bypass (unpublished data). The significance and broader safety of this approach will only be determined by the application of larger clinical trials powered to detect important clinical benefits.

In summary, this review has presented an overview that serves as the foundation for the development of current clinical trials in CPB research. Based upon the compelling nature of the clinical severity faced in current and future cardiac surgery cases, we believe that the research mandate of biocompatibility will once again rank prominently as a thesis in academic cardiovascular surgery. An improved understanding of the exact mechanisms by which these various anti-inflammatory strategies bring benefit will also help tailor approaches such that we can continue to offer excellent outcomes.

References

12 Murphy EF, Lu JR, Brewer J, Russell J, Penfold J. The reduced adsorption of proteins at the phosphoryl Inflammation and cardiopulmonary bypass

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