# Heparin 2.0: A New Approach to the Infection Crisis

Malin-Theres Seffer[a, b]    Daniel Cottam[c]    Lui G. Forni[c, d]    Jan T. Kielstein[a]

[a]Medical Clinic V, Nephrology | Rheumatology | Blood Purification, Academic Teaching Hospital Braunschweig, Braunschweig, Germany; [b]Microbial Proteomics, Helmholtz Centre for Infection Research, Braunschweig, Germany; [c]Intensive Care Unit, Royal Surrey Hospital NHS Foundation Trust, Guildford, UK; [d]Department of Clinical & Experimental Medicine, School of Biosciences & Medicine, University of Surrey, Guildford, UK

**Abstract**

In April 2020, the US Food and Drug Administration granted emergency use authorization for certain medical devices to be used in patients with coronavirus disease 2019 (COVID-19). This included extracorporeal blood purification devices. This narrative review will give a brief overview regarding some of the extracorporeal devices that could be used to treat COVID-19 patients, including the Seraph® 100 Microbind® Affinity Blood Filter, produced by ExThera Medical (Martinez, CA, USA), first licensed in the European Economic Area in 2019. The Seraph® 100 contains ultrahigh molecular weight polyethylene beads with end point-attached heparin and is approved for the reduction of pathogens from the bloodstream either as a single agent or as an adjunct to conventional anti-infective agents. Bacteria, viruses, fungi, and toxins have been shown to bind to the immobilized heparin in a similar way to the interaction with heparan sulfate on the cell surface. This binding is nonreversible and as such, the pathogens are removed from the bloodstream. In this review, we describe the pathophysiological basis and rationale for using heparin for pathogen removal from the blood as well as exploring the technology behind the adaptation of heparin to deprive it of its systemic anticoagulant activity. In addition, we summarize the in vitro data as well as the available preclinical testing and published clinical reports. Finally, we discuss the enormous potential of this technology in an era of increasing antibiotic resistance and high mortality associated with sepsis and consider the application of this as a possible treatment option for COVID-19.

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## Introduction

*Extracorporeal Therapies in Patients with COVID-19*

By April 30, 2020, the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) had infected more than 3 million people globally and had claimed the life of some 217,769 individuals. The ongoing pandemic continues to stretch healthcare systems around the world to the limit and will continue to do so for the months to come.

Currently, there is no established drug therapy available for coronavirus disease 2019 (COVID-19). Promising therapies including remdesivir await further confirmatory evidence [1], have been shown to be unsuccessful
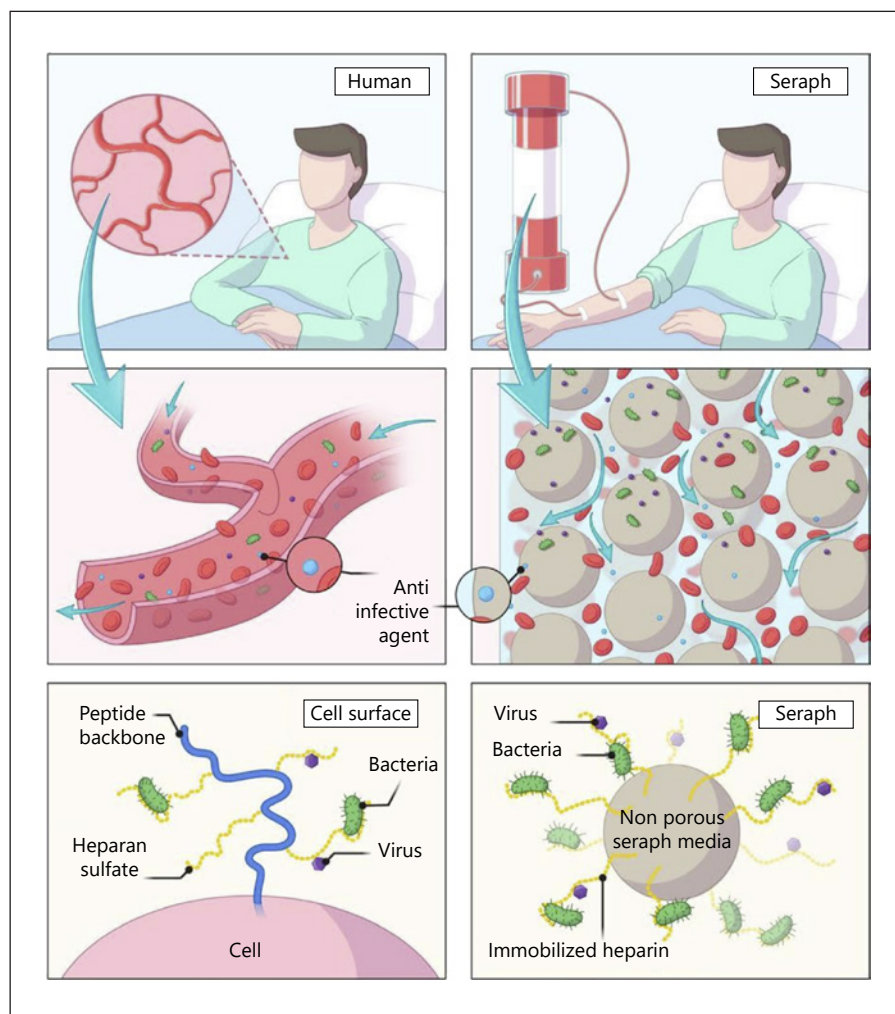
Jan T. Kielstein
Academic Teaching Hospital Braunschweig
Medical Clinic V Nephrology | Rheumatology | Blood Purification
Salzdahlumer Straße 90, DE–38126 Braunschweig (Germany)
j.kielstein@klinikum-braunschweig.de

**Fig. 1.** Structural similarities between the HS on cell surfaces and heparin bound to polyethylene beads in the Seraph® 100 cause infective agents in the blood that is pumped through the Seraph® 100 to adhere to the heparin-coated filter media and not recirculated back systemically, thereby being removed (for better visibility, sizes are relative but not true to scale). HS, heparan sulfate.

(lopinavir-ritonavir) [2], or may even have undesirable effects (chloroquine and hydroxychloroquine) [3]. Thus, the search for alternative treatment strategies in critically ill patients with COVID-19 continues. Aside from pharmacological interventions, several extracorporeal strategies have been discussed and indeed utilized [4]. As AKI is commonly observed in critically ill patients with COVID-19, with approaching 46% of all ARDS patients, renal replacement therapy is frequently necessary [5, 6]. Other extracorporeal treatment options have recently been reviewed in the context of COVID-19 [4, 7]. In brief, the intervention deemed most promising is the extracorporeal treatment of cytokine release syndrome, with IL-6 being considered to be the most important causative cytokine. Interestingly detectable serum SARS-Cov-2 RNA in the blood of COVID-19 patients has been shown to be associated with elevated IL-6 concentration and poor prognosis [8]. High-volume hemofiltration [9] and thera-peutic plasma exchange decrease inflammatory cytokine levels including, but not limited to, IL-6 in patients with sepsis [10]. In patients with sepsis, therapeutic plasma exchange using fresh frozen plasma has been shown to improve the disequilibrium of coagulation factors by removing pro- and replacing anticoagulant factors [11], an intervention that might be of promise in the hypercoagulative state of COVID-19 patients. This procedure could also be coupled with the administration of convalescent plasma [12]. A simpler approach to decreasing the level of proinflammatory cytokines includes use of the Cyto-Sorb® cartridge, which consists of a highly porous high-tech polymer that can bind to a wide range of inflammatory mediators, including cytokines [13]. In patients with septic shock and high endotoxin activity due to superimposed bacterial infections, polymyxin B hemoperfusion may also be considered despite disappointing clinical trials [14].

## Heparin: An Essential Medicine

The first WHO essential drugs list, published in 1977, started a process of objective selection of drugs that should be made readily available to everybody due to their efficacy, safety, cost-effectiveness, and relevance for the care of patients. Heparin was on the original list through its use as an anticoagulant employed for prophylaxis of thrombosis through the treatment of myocardial infarction and remains the most widely used anticoagulant for extracorporeal treatments ranging from hemodialysis to extracorporeal membrane oxygenation. Despite its almost ubiquitous usage, its molecular structure is ill-defined, being a negatively charged biopolymer with wide variation in molecular weight composed principally of repeating trisulfated disaccharide units [15]. The anticoagulant activity of heparin is based on a dual action, inhibition of thrombin generation and inhibition of thrombin activity through the protease inhibitor antithrombin III (AT-III). Moreover, heparin has many other pharmacological properties, including anti-inflammatory, antiviral, antiangiogenesis, antineoplastic, and antimetastatic effects through high affinity interactions with a variety of mediators, including proteases, protease inhibitors, chemokines, cytokines, growth factors, and their respective receptors [15].

## Heparin Binding of Bacteria and Viruses

Heparan sulfate (HS) sequences are highly negatively charged, partially sulfated, carbohydrate portions of proteoglycans that are present on the surface of almost all mammalian cells. HS chains are built through alternating D-glucosamine and glucuronic acids (L-iduronic and D-glucuronic acids) and as such are structurally related to heparin varying only in the saccharide chains [16]. Aside from covering the cell surface HS can be found in the intracellular milieu and extracellular matrix and interact with numerous soluble and insoluble ligands, including cytokines and growth factors [17]. Moreover, many bacteria, viruses, and toxins adhere to human cells via the HS as well as surface proteins. Charge, or electrostatic interaction, is the main mechanism behind this binding with the negatively charged HS attracting the basic amino acids of surface proteins [18]. Examples of this include the herpes simplex virus HSV-1, which attaches to cell surface proteoglycans through the glycoprotein complex (gB and gC [19]) and hepatitis B virus which uses a large envelope protein to bind to cells [20]. Of note, it has recently been demonstrated that SARS-CoV-2 attaches to heparin through its surface protein Spike 1 receptor-binding domain [21]. Given that many bacteria bind to HS or heparin, it follows that potentially exploiting such adhesion



**Fig. 2.** Seraph® 100 is measuring 22 × 7 cm. After priming with a volume of 160 mL, the entire assembly weighs about 400 g. Electron microscopic image (magnification 144×) from the heparin-coated beads – the active ingredient of the Seraph® 100.

pathways to "trap" bacteria and viruses and remove them from the circulation through an extracorporeal treatment may be a promising approach. Additionally, heparin also has a direct effect on bacteria and viruses. For example, heparin, released from mast cells and basophils through tissue damage, reduces hepcidin expression and interrupts the iron availability of *Mycobacterium tuberculosum* [22] and also the cytopathogenicity of the human immunodeficiency viruses has been shown to be reduced by 50% by heparin at a concentration of 4.7 μg/mL [23]. There is also an observed inhibitory effect of heparin on herpes simplex virus that can bind to cell surface HS as well as to heparin [24]. It follows that manipulation of this binding affinity of microorganisms to HS and equivalents could lead to potential therapeutic interventions. The Seraph® 100 Microbind® Affinity Blood Filter is an extracorporeal hemoperfusion device whose functional core, that is, polyethylene beads (diameter of 0.3 mm) with immobilized heparin bound to it, mimics a naturally mammalian cell surface (Fig. 1). The structure of this adsorber replicates to some degree the HS on the cell surface and, therefore, may fulfill the requirements necessary to bind microorganisms. The attachment of the heparin to the polyethylene beads (approximately 2 mg heparin/g

**Fig. 3.** In vitro binding of bacteria to the Seraph® 100 shown a reduction of CFU (average of 3). Mini cartridges were conditioned with 2.0 mL of PBS, then 2.0 mL of FBS, then 2.0 mL of PBS prior to inoculation. Cultures were diluted in defibrinated horse blood to ~2–3 × 105 CFU/ml. A sample of each test inoculum was immediately harvested and enumerated to find the initial bacterial concentration. 2.0 mL of dilute test inoculum was repeatedly filtered through the microcolumns, with enumerations of remaining bacteria on the third filtrate.
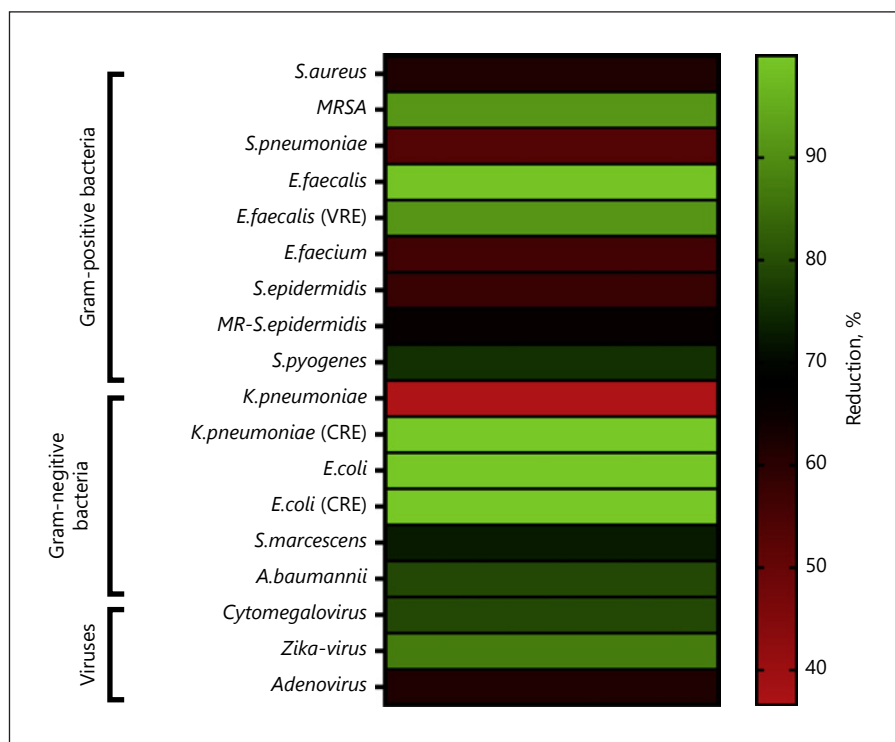
beads) is such that only insignificant amounts of heparin are released systemically [25, 26]. This is shown using electron microscopy in Figure 2.

*In vitro Data*
Bacteria
As indicated, the possession of heparin-binding proteins is common in bacteria and as such, one could envisage similar binding properties with the Seraph® 100 filter through charge interactions [27]. Indeed, *Staphylococcus aureus* and the highly resistant strain, MRSA, have been demonstrated to adhere to the heparinized beads [28]. This is summarized in Figure 3.

Viruses
HS binds certain viruses and, in some cases, mediates target cell infection. Therefore, neutralizing infection with viruses that use HS for cellular attachment by competitive inhibition for binding with the Seraph® 100 can be envisaged. Indeed, such principles are already employed for diagnostic purposes where heparin is bound to carbon nanotubes and used as a biorecognition element for dengue virus instead of an antibody assay [29].
The theory that such an extracorporeal device can remove viruses from blood has been seen in preclinical tests that showed a reduction of viral load: up to 87% for Zika

virus, 79% for CMV, and 62% for adenoviruses. Furthermore, recent in vitro data have also shown that SARS-CoV-2 can be removed by the Seraph® 100.

Cytokines
Given the role of cytokines in the inflammatory cascade associated with sepsis, much attention has focused on mitigating the "cytokine storm." Axelsson et al. [26] have investigated the adhesion of proinflammatory cytokines to heparinized beads. Vascular cell adhesion molecule, IL-6, TNF-alpha, RANTES, interferon-gamma, and antithrombin, suspended in donated blood, were studied. There was a significant reduction, especially for TNF-alpha, which was reduced by approximately 59% (Fig. 4). The magnitude of this effect in vivo has been debated but not been clinically studied yet [30].

Drug Clearance
Antibiotics and related therapeutics play a pivotal role in the treatment of bloodstream infections and sepsis. It follows that if any extracorporeal device removes antimicrobial agents, this may negate any potential benefit. The removal characteristics for 18 anti-infective drugs was tested in a life size in vitro approach using human plasma, which circulated with a flow rate of 250 mL/min for 1 h through the Seraph® 100. Samples were taken after 5′, 15′,

**Fig. 4.** In vitro clearance of proinflammatory cytokines from septic patients by Seraph microcolumns. Significant reduction of proinflammatory cytokines versus controls have been taken from a publication of Axelsson et al. [26].
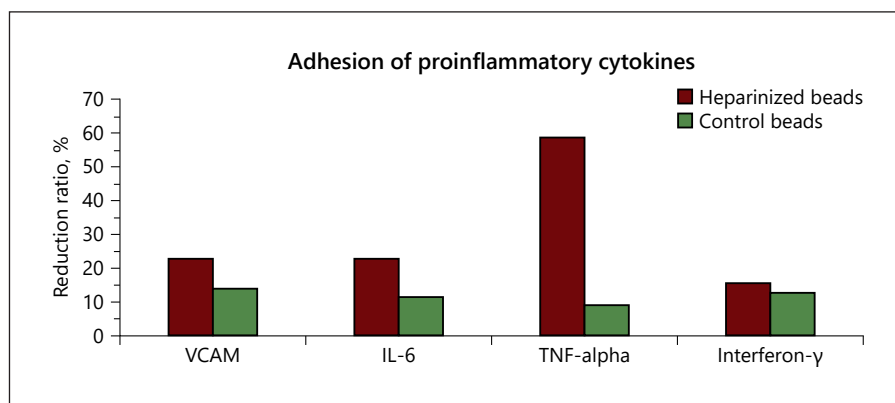
30′, and 60′ min. There was no clinical relevant reduction of the anti-infective agents that included antibiotics, antiviral, and antifungal medication [31].

*Clinical Use of the Seraph® 100*

The CE mark study consisted of 15 patients undergoing hemodialysis. The primary outcome measure was to demonstrate the safety of the Seraph® 100 Microbind® Affinity Blood Filter in a hemodialysis circuit assessed by rate of adverse events during the procedure and 14 days thereafter. The secondary outcome measure was the reduction of bacteria in blood passed through the Seraph® 100 Microbind® Affinity Blood Filter over the 4-h treatment. The number of bacteria was assessed by colony-forming units/mL or time to positivity of blood cultures. All treatments were well tolerated with no significant changes in vital signs, including blood pressure or heart rate during the 4-h treatment but, of note, a significant increase in oxygen saturation ($p = 0.02$) was noted. This observation may reflect the removal of sepsis mediators that influence endothelial function, including heparin-binding protein, histones, and ultra-large von Willebrand factor leading to changes in the pulmonary vasculature manifest as an increase in $SpO_2$. For those patients with bacteremia (4 out of 15), a significant reduction of bacterial load by the Seraph® 100 was demonstrated with a significant increase in time to positivity ($p = 0.03$). The documented clinical applications demonstrate that the Seraph® 100 can be used in different modes of renal replacement therapy from intermittent hemodialysis to prolonged intermittent hemodialysis as well as in a CRRT machine for up to 24 h.

Coronavirus Disease 2019

The Seraph® 100 has been used in Europe since 2019 for the reduction of pathogens from the blood. Authori-

zation for emergency use in patients with COVID-19 admitted to the ICU with confirmed or imminent respiratory failure was granted by the US FDA on April 17, 2020. An online registry was recently established to evaluate the clinical effect of this intervention (ClinicalTrials.gov Identifier: NCT04361500). What is the rationale to use the Seraph® 100 in COVID-19 patients? First, there is elimination of the virus from the blood as it had been shown in vitro for several viruses (Fig. 3). In support of this theory is the observation that SARS-CoV-2, through the surface protein Spike 1 receptor-binding domain, attaches to heparin [21]. Viremia has been shown to be present in 41% [32] of patients in general and in up to 50% of critically ill patients with SARS-CoV-2 [6]. Moreover, detectable serum SARS-Cov-2 RNA (RNAaemia) in CO-VID-19 patients has been shown to be associated with elevated IL-6 concentration and poor prognosis [8], so decreasing RNAaemia might also help to blunt the (overwhelming) inflammatory response. Second, the development of a secondary hemophagocytic lymphohistiocytosis manifest by a cytokine storm may play an important role in determining outcome, so mediating this response may be of use [33]. A significant reduction of proinflammatory cytokines has been shown for the Seraph® 100 in vitro [26], highlighting further potential therapeutic benefit. Also, of note is the observation within the initial safety study and subsequent clinical reports improvement in oxygen saturation is demonstrated, although the pathophysiology behind this is, as yet, undetermined. One relevant component might be the improvement of pulmonary microcirculation in COVID-19 patient that exhibit a deranged coagulation function [34]. Last but not least, in patients on renal replacement therapy, the use of drugs like Remdesivir may be prohibitive in terms of side effect profile and, hence extracorporeal therapies may be the only option.

*Future Directions*

Sepsis remains a leading cause for mortality in critically ill patients. This applies in particular for infections with multidrug-resistant bacteria given the limitations in available treatments. Development of new antibiotic drugs as well as the increased prescribing of "older" antibiotics may preserve contemporary antibiotics and avoid the spread of resistance in order to overcome this growing medical and cost issue. Although there are several other approaches to remove infectious agents from the bloodstream, the Seraph® 100 is the first licensed device in the EU but also the only one that takes a WHO essential medicine, heparin, to the next functional level.

## Conclusions

The role of extracorporeal techniques in the management of the critically ill is an area of great expansion. The routine use of renal replacement therapy within intensive care units shows how these have been adopted and the recent expansion in the provision of extracorporeal membrane oxygenation both venous-venous and venous-arterial underlines the move toward increased extracorporeal technologies. Development of new membranes and filters with tailored, personalized approaches as part of these circuits is almost certainly the next direction of travel. The application of columns with a specific binding profile and the direct targeting of infectious agents may well be the next step.

## Author Contributions

All authors were involved in extracting and collating references and writing the script. J.T.K. and L.F. had the final say on the manuscript's content.

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