Received: 12 August 2023 | Revised: 6 December 2023 | Accepted: 18 December 2023

DOI: 10.1111/aor.14704

Artificial Organs WILEY

MAIN TEXT

Sorbent functionalization with vancomycin enhances bacteria killing in extracorporeal hemoadsorption

Gonzalo Ramírez-Guerrero[1,2,3] | **Massimo de Cal**[1,4] | **Anna Lorenzin**[1,4] | **Dario Vigolo**[1] | **Anna Italia Toscano**[1] | **Marisel Araya-Rojas**[2] | **Monica Zanella**[1,4] | **Claudio Ronco**[1]

[1]International Renal Research Institute of Vicenza (IRRIV Foundation), Vicenza, Italy

[2]Nephrology and Dialysis Unit, Carlos Van Buren Hospital, Valparaíso, Chile

[3]Department of Medicine, Universidad de Valparaíso, Valparaíso, Chile

[4]Department of Nephrology, Dialysis and Kidney Transplantation, San Bortolo Hospital, Vicenza, Italy

Correspondence
Gonzalo Ramírez-Guerrero, Department of Nephrology and Dialysis Unit, Carlos Van Buren Hospital, San Ignacio #725, Valparaíso 2340000, Chile.
Email: ramirezguerrero.g@gmail.com

Abstract

Background: The level of bacteremia in patients with sepsis and septic shock is a predictor of complications and mortality, regardless of the type of bacteria. Devices for bacteria, endotoxin and cytokines removal by adsorption have been recently developed. Thus, extracorporeal blood purification therapies have been proposed as adjunctive therapy in sepsis in combination with drugs. Some potentially useful drugs, however, are precluded due to their organ or metabolic toxicity. The present study represents a preliminary report on the in vitro effect of a sorbent device (minimodule with HA380 beads, Jafron medical, Zhuhai, China) in which the particles have been functionalized with vancomycin on the surface. The impact of the surface-modified beads on circulating bacteria (*Staphylococcus aureus*) has been tested in a simulated in vitro circulation.

Methods: In vitro experiments were carried out with 800 mL of blood enriched with *S. aureus* species. Blood was circulated in the vancomycin-functionalized and non-functionalized mini-module cartridges in hemoadsorption setup (300 mL each) and the bactericidal effect was assessed. Also, 200 mL of blood was used as a control.

Results: A significant increase in the time to positivity of blood cultures was observed after 60 min and also after 120 min of therapy with the mini-module functionalized with vancomycin as opposed to the non-functionalized cartridge.

Conclusions: These results suggest a possible way of treating sepsis by using drug- or antibiotic-functionalized cartridges without worrying about pharmacological toxicity. The prolongation of the time to bacterial culture positivity to *S. aureus* after a passage through a column packed with beads functionalized with vancomycin represents a proof of concept.

KEYWORDS

HA380, hemadsorption, hemodialysis, sepsis, sorbent, vancomycin

1 | INTRODUCTION

Sepsis affects approximately 1.7 million adults in the United States and 3.4 million in Europe each year, causing almost 700 000 deaths yearly.[1,2] In patients with sepsis and septic shock, the total bacterial burden and the duration of bacteremia affect the outcome of patients, and the amount of bacterial DNA in blood correlates with mortality. The time to positivity of blood cultures is an indicator of pathogen load and a strong predictor of complications due to *Staphylococcus aureus* bacteremia.[3,4] *S. aureus* bacteremia remains a common condition in bloodstream infection with an increasing incidence due to the use of invasive procedures and central venous catheters. In these conditions, a mortality rate of 20%–60% is observed despite effective antibacterial therapies and source control strategies.[5,6] In some cases, specific drugs could represent a solution but their utilization is precluded due to their metabolic or organ toxicity.

Extracorporeal blood purification therapies have been proposed as adjunctive therapy to manage immune-dysregulation and complications in sepsis. They are mostly based on the principle of removal of endotoxin, inflammatory mediators or toxins (cytokines, chemokines, DAMPs, PAMPs), aiming to modulate the exaggerated inflammatory response. Removal of circulating pathogens with specific devices has also been proposed in the attempt to decrease the trigger of the immune cascade and the subsequent cytokine storm or to manage bacteremia due to drug-resistant pathogens.[7] The idea of removing pathogens with adsorption is not new and it is possible to find successful in vitro reports using activated charcoal or bendable nanowires.[8–10] New devices that focus on these targets in extracorporeal blood purification therapies in sepsis are being developed; some of them are

- The Seraph® 100 Microbind® Affinity Blood Filter (ExThera Medical, Martinez, CA, USA): a hemoperfusion device whose functional core utilizes heparin, mimics a naturally mammalian cell surface, and adsorbs bacteria and viruses[11]
- GARNET® Hemofilter (BOA™ Biomedical, Cambridge, MA, USA): a device of hollow polysulfone fibers coated with Fc-mannose-binding lectin with the properties of removing pathogens from the blood.[7]
- Hemopurifier® (Aethlon Medical, San Diego, CA, USA): a plasma filter with the ability to adsorb viruses in its extra capillary space via immobilized lectin proteins.[7]

Consequently, with the purpose of expanding the application of these therapies in sepsis, our group is now proposing the use of surface-modified and drug-functionalized adsorption cartridges in order to reduce the level of circulating bacteria. The concept consists in the possibility to immobilize a potentially toxic bactericidal drug on the surface of the sorbent beads and treat the patient while avoiding the systemic administration of the drug. This approach might contribute to antibiotic therapy and resuscitation of critically ill patients while reducing pharmacological toxicity and related complications. We recently demonstrated that HA380 cartridges (Jafron, Zhuhai City, China) adsorb significant amounts of vancomycin when perfused with a vancomycin-enriched solution.[12]

This study aims to test in vitro the HA380 cartridge's potential for removing and killing bacteria (*S. aureus*) through adsorption with functionalized beads with vancomycin.

2 | MATERIALS AND METHODS

The study used an in vitro hemoadsorption model to characterize the effect performed by an HA380 mini-module on a circulating bacterial load inoculated in blood.

2.1 | HA380 cartridge

HA380 contains neutro-meso/macroporous resin made of the styrene-divinylbenzene copolymer. The average diameter of the resin beads is 0.80 mm, ranging from 0.60 to 1.18 mm. The pore size distribution of the resin is 500 Da-60 kDa.[14]

2.2 | Functionalized the cartridge

A saline solution enriched with 600 mg of vancomycin was circulated in hemoperfusion in the mini-module HA380 cartridge at 250 mL/min for 30 min. After this time, the antibiotic was adsorbed in the beads. Vancomycin values at the end of the process were measured using the QMS assay (Thermo Fisher Scientific, Waltham, Massachusetts, USA) in ILab 650 platform (Instrumentation Laboratory, Milan, Italy) to report the concentration of vancomycin in the circuit reservoir.

2.3 | Determining the *S. aureus* concentration and reservoirs preparation

The *S. aureus* ATCC® 25923™ (MSSA) strain was chosen as a model for the experiment. Following the analysis of the growth on the blood agar plates (Tryptone Soy Agar +5% mutton blood, B19423 MEUS S.r.l.) for various concentrations between 3×10^2 and 3×10^8 CFU/mL, it was decided to use the concentration of 0.5×10^6

for inoculation in 800 mL of blood and incubated at 37°C. The choice to use the concentration of 0.5×10^6 CFU/mL for the experiments is in line with the study conducted by Jacobs et al.[15] This study shows that bacterial concentrations of 10^5 CFU/mL, especially for the *S. aureus* species, correspond to the most critical evolutions of the septic process, with moderately severe or severe shock reactions requiring extracorporeal therapies.

After 24 h, blood was partitioned into three reservoirs: 200 mL was maintained as a negative control (CTR); 300 mL was circulated in the functionalized cartridge (FC) and the remaining 300 mL was circulated in another cartridge without antibiotic (NFC).

2.4 | Preparation and testing of the circuit

The model involves the passage of the two reservoirs of 300 mL of heparinized (10000 IU heparin/L) blood with bacterial load in a functionalized cartridge (with vancomycin) and in a non-functionalized cartridge. The in vitro circulation was performed using a dedicated testing platform developed in our institute (Galileo), equipped with pressure sensors and peristaltic pumps. A scaled closed-loops HA circuits were setup as described in Figure 1. In order to evaluate the resin adsorption capacity towards bacteria, two customized cartridges were built assembling sorbent mini-modules (25% of the regular size HA380 cartridge) containing 75 g of wet resin (approximately 25 g of dry resin).

The devices were primed according to the instructions for use. Peristaltic pump was set at 250 mL/min and the circuits were operated in recirculation mode using a fixed volume of blood solution (300 mL each reservoir). The solutions were warmed, maintained at 37°C, and mixed with a magnetic hotplate stirrer during the experiments. The circulations were performed simultaneously for 2 h. Samples (from the two circuits reservoirs and the negative control reservoir) were drawn at baseline (T0), after one hour (T1) and two hours (T2) of circulation. All the samples were cultured on DB Bacter bottles to estimate the time after which the bacteria replication reached the predetermined growth threshold (time to positivity, TTP) and samples T2 were plated on blood agar plates with semi-quantitative techniques to assess bacterial growth, plates were incubated for 24 h at 37°C and 5% CO_2.

The experiments using FC and NFC were repeated in three opportunities.

2.5 | Statistical analysis

We used the ANOVA test with Bonferroni correction to compare the results of each time point at certain hemoadsorption times between the experiments with and without functionalized vancomycin cartridges. A *p*-value less than 0.05 was considered for significant differences. All analyses were conducted using the SPSS 25 program.

3 | RESULTS

3.1 | Functionalization of the cartridge

After passing a solution enriched with vancomycin into the mini-modules, a total of 540 mg of vancomycin on

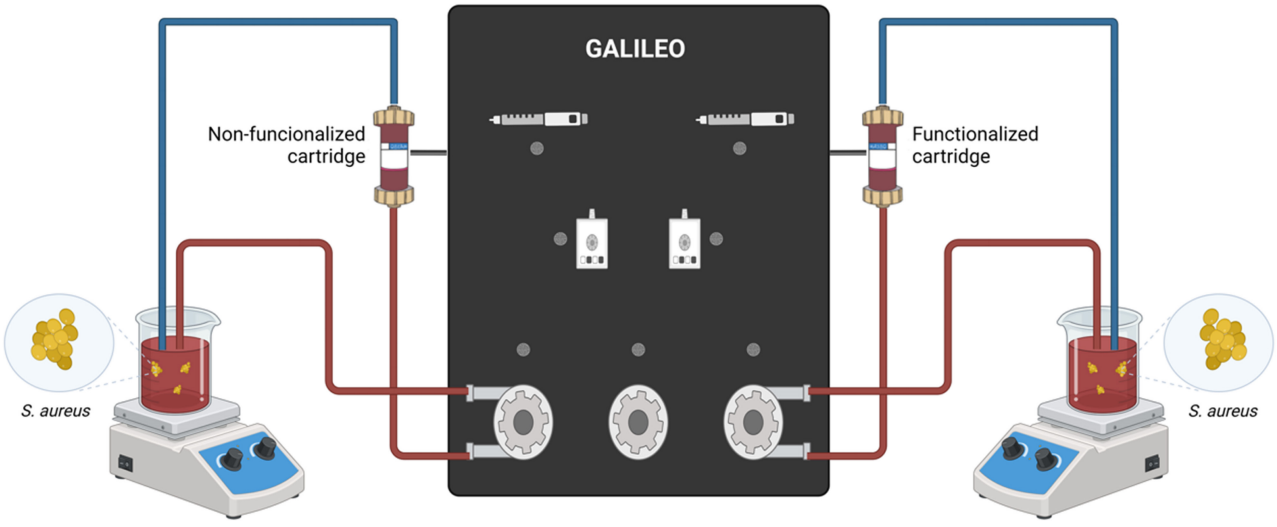

FIGURE 1 In vitro experimental setup. Customized extracorporeal circuits with HA380 mini-modules were applied to the Galileo testing platform. The reservoirs with blood containing *Staphylococcus aureus* were positioned on magnetic hotplate stirrers.

average has been adsorbed. As can be highlighted from the studies by Godi et al. and de Cal et al.,[12,16] this interval is sufficient for the cartridge to adsorb 99.2% ± 0.6% of the vancomycin present in the solution. We were able to adsorb 85% of vancomycin.

3.2 | Reduction capacity of circulating bacteria in the HA380 cartridge

At the basal time of the experiment, a mean blood culture positivity time of 85 ± 5 min was observed, representing the basal homogeneity of the reservoirs.

Subsequently, we evaluated the adsorption of circulating bacteria by the HA380 mini-module (FC vs. NFC). A significant increase in the TTP of blood cultures was observed in samples collected at two different times (T1 and T2) with the FC with vancomycin as opposed to the NFC and the CTR reservoirs. At T1, 232 ± 163 min in the FC vs 85 ± 5 min in the NFC vs 85 ± 5 min in the CTR were observed ($p = 0.034$). TTP at T2 251 ± 198 min in the FC vs 84 ± 5 min in the NFC vs 88 ± 7 min in the CTR were observed ($p = 0.037$). (Figure 2).

Regarding *S. aureus* in the blood agar plates, after 24 h of incubation a growth lower than 50% in the sample T2 was obtained in the FC versus the NFC and the CTR reservoirs (1×10^6 CFU vs 2×10^6 CFU vs 2×10^6 CFU) (Figure 3).

Vancomycin levels obtained at the end of each procedure in the FC reservoir were 5.9 ± 0.38 mg/L; no desorption phenomena were observed.

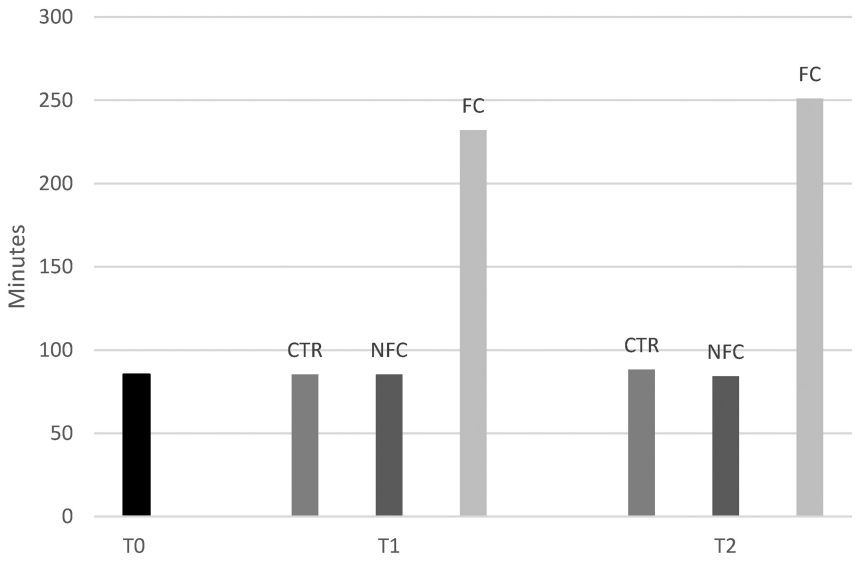

FIGURE 2 Time to bacterial culture positivity. T0 is the sample taken before blood was partitioned in the three reservoirs. CTR, control; FC, functionalized cartridge; NFC, non-functionalized cartridge.

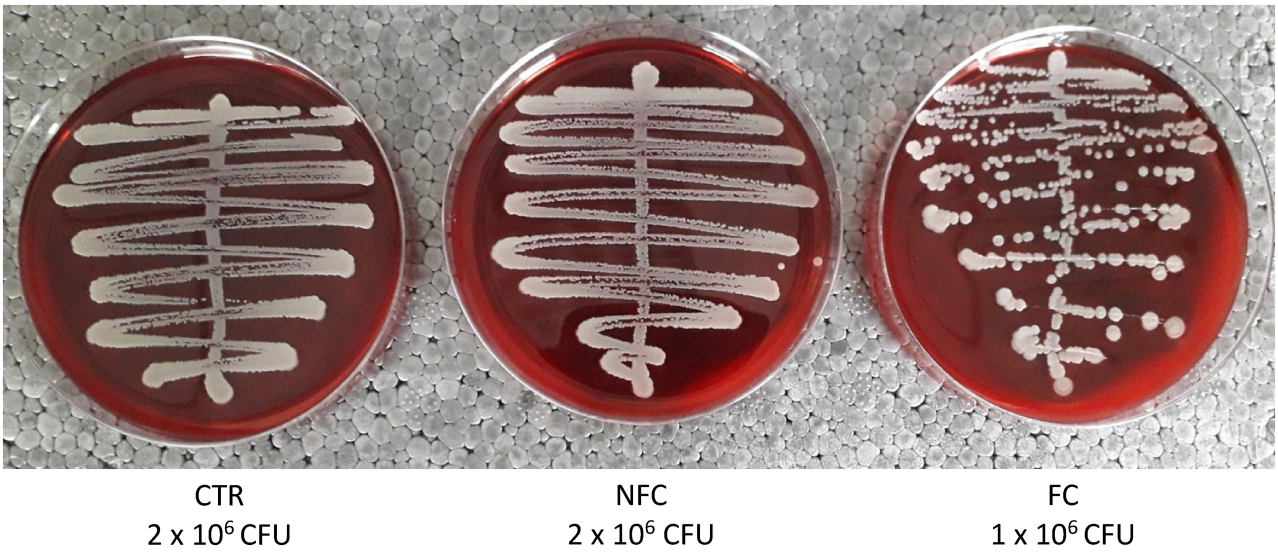

FIGURE 3 Colony-forming unit of samples T2 in plates after 24 h of incubation at 37°C and 5% CO_2. CFU, colony-forming unit; CTR, control; FC, functionalized cartridge; NFC, non-functionalized cartridge.

4 | DISCUSSION/CONCLUSION

In the present study, we demonstrate that a significant amount of *S. aureus* was removed from inoculated blood on passage through a column packed with beads functionalized with vancomycin, represented by changes in time to bacterial culture positivity and in the number of CFUs on the plates after 120 min. A functionalized cartridge could have an enhanced therapeutic capacity: in addition to its immunomodulated effect of adsorbing inflammation factors, it could support the immune system reducing the bacteremia thanks to the bound antibiotic.[17] The concept of an extracorporeal treatment for infections is relatively new and has been developed with cartridges such as Seraph® 100 and SARS-CoV-2, showing lower mortality compared to controls, as well as in the treatment of malaria and babesiosis with apheresis when the pathogen burden is high. However, different mass transfer or adsorption mechanisms are involved in the latter, as we mentioned previously.[18,19]

Time to bacterial culture positivity defined as the time from the start of incubation to the preliminary positive result of blood culture, provides indirect information about bacteremia load and the microbial growth rate in the blood sample and is helpful in predicting patient outcomes. Several studies have shown that shorter TTP is associated with significantly higher mortality risk in patients with bacteremia caused by several bacterial species, like *Escherichia coli*, *S. aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* and this corresponds to the hypothesis that short TTP might be correlated to higher bacterial load and greater disease severity.[20–22] In the present work, after 60 min and 120 min, we observed a prolongation in the TTP of the blood cultures. This TTP prolongation is reinforced by a significant decrease in the number of CFUs on Blood Agar plates after 120 min (similar results in a previous study of our group with saline instead of blood).[12] This is essential because this reduction in the mass of bacteria could potentially improve the prognosis of patients with sepsis and septic shock while waiting for the antibiotics to start their effect. In the case of vancomycin, the time-killing, dependent on time and concentration, confers a slow bactericidal effect.[23]

A possible explanation for the binding of vancomycin to bacteria via adsorption mechanisms is presented below. Vancomycin is active against a wide range of Gram-positive bacteria, particularly *staphylococci* and *enterococci*. Their activity arises from their ability to bind peptidoglycan precursors terminating in the sequence *-Lys-D-Ala-D-Ala (-KdAdA)*.[24] Several studies have shown the possibility of adsorbing antibiotics through cell-wall analogs immobilized on solid supports to form affinity adsorbents for the antibiotics, therefore, by immobilizing *D-Ala-D-Ala* on a surface, vancomycin was selectively adsorbed via electrostatic, hydrogen-bond and hydrophobic interactions with the cell-wall analogs.[25,26] Vancomycin has a molecular mass of 1448 Da and it carries positive charges; therefore, it may interact with the negatively charged surface of styrene copolymer, and its Einstein-Stoke radius could limit its passage through the pores of the sorbent. Resin adsorption is achieved through ionic bonds, van der Waal forces, and hydrophobic bonds.[16] In a previous work, we confirmed how the binding of bacteria to the beads occurs exclusively on their surface and not inside the pores, so functionalizing this surface accentuates this adsorption process.[12]

Recently, our group demonstrated the possibility of reducing the circulating bacterial load by adsorption using neutral meso/macropores styrene-divinylbenzene copolymer beads, functionalized and non-functionalized with vancomycin in an in vitro model with saline (0.9% NaCl) instead of blood. However, as it is carried out in saline, elements that could interfere with the bacterial and vancomycin adsorption such as the Vroman effect are not considered.[12] The Vroman effect is exhibited by protein adsorption to a surface by blood serum proteins. The small plasma proteins, which have the highest mobility, generally arrive first and are later replaced by larger, less mobile proteins with a higher affinity to the surface.[13] This phenomenon does not occur in a saline solution. A reason of using blood was to evaluate these interferences in the adsorption process with respect to various plasma proteins, which in the case of the functionalization of the beads, could maintain this specific adsorption effect, thus explaining the difference between the FC and the NFC with respect to the previous study with saline solution.

Vancomycin binds to the beads in an extremely strong way. In a similar experiment with functionalization of a cartridge with vancomycin, we evaluated the possibility of desorption.[12] Dosages performed on the supernatant after centrifugation of the bead showed a concentration equal to 0 μg/mL, so the possibility of desorption is negligible.[12] One potential concern is increased release of vancomycin from increased blood flow. Studies carried out by our team on the fluid dynamics of the cartridges confirmed that from 200 mL/min, the pressure drop increases,[27] being a point of greatest potential stress within the cartridge. Given the above, not observing deadsorption with 250 mL/min is a safety point to consider for future therapies with functionalized cartridges. The extremely low concentration of vancomycin in the functionalized cartridge's reservoir discards the option of a bolus antibiotic effect on the bacteria and demonstrates that the only active vancomycin was the one adsorbed by the functionalized cartridge.

In conclusion, these results suggest a possible way of treating sepsis by using antibiotic-functionalized cartridges. The effects are represented in a prolongation of the time to bacterial culture positivity and a reduction of the CFU of *S. aureus*, while awaiting the onset of antibiotic action. The study highlights the possible utilization of toxic drugs in sepsis immobilizing them on the sorbent matrix while maintaining their clinical action. One of the limitations of our work is the treatment time. Future studies should evaluate whether a greater prolongation of the TTP would be achieved by prolonging the adsorption time. This is a preliminary report that requires further validation and clinical studies to confirm the results obtained in vitro, its effectiveness and safety and further expand the knowledge on the use of extracorporeal therapies as a supplement to counteract the septic process.

AUTHOR CONTRIBUTIONS

Massimo de Cal, Anna Lorenzin, Claudio Ronco, and Gonzalo Ramírez-Guerrero designed the work. Massimo de Cal, Anna Lorenzin, Dario Vigolo, and Gonzalo Ramírez-Guerrero collected and analyzed the data. Gonzalo Ramírez-Guerrero, Massimo de Cal, Anna Lorenzin, and Claudio Ronco drafted the work or substantively revised it, and all authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

We thank Anita Zurita Poza for her technical assistance in the design of this article.

FUNDING INFORMATION

There was no funding for the study.

CONFLICT OF INTEREST STATEMENT

CR has received funding for lectures, been a consultant or advisory board member for Asahi, Astute, B. Braun, Baxter, bioM'erieux, Bioporto, CytoSorbents, Estor, Fresenius Medical Care, General Electric (GE), Jafron, Medtronic, and Toray.

DATA AVAILABILITY STATEMENT

Not applicable.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

STATEMENT OF ETHICS

Not applicable.

ORCID

Gonzalo Ramírez-Guerrero https://orcid.org/0000-0002-1696-9997
Anna Lorenzin https://orcid.org/0000-0001-5329-2359

REFERENCES

1. Rhee C, Jones T, Hamad Y, Pande A, Varon J, O'Brien C, et al. Prevalence, underlying causes, and preventability of sepsis-associated mortality in US acute care hospitals. JAMA Netw Open. 2019;2(2):e187571.
2. Mellhammar L, Wullt S, Lindberg A, Lanbeck P, Christensson B, Linder A. Sepsis incidence: a population-based study. Open Forum Infect Dis. 2016;3(3):ofw207.
3. Kirkbright S, Fatovich D, Kee C, Kay I, Flexman J, Pryce TM, et al. Quantitative rt-PCR holds promise as a screening tool for patients with severe sepsis. Emerg Med Australas. 2011;23(4):502–6.
4. Fowler VG Jr, Olsen MK, Corey GR, Woods CW, Cabell CH, Barth Rellera L, et al. Clinical identifiers of complicated *Staphylococcus aureus* bacteremia. Arch Intern Med. 2003;163(17):2066–72.
5. van Hal SJ, Jensen SO, Vaska VL, Espedido BA, Paterson DL, Gosbell IB. Predictors of mortality in *Staphylococcus aureus* bacteremia. Clin Microbiol Rev. 2012;25:362–86.
6. Bergin SP, Holland TL, Fowler VGJ. Bacteremia, sepsis, and infective endocarditis associated with *Staphylococcus aureus*. Curr Top Microbiol Immunol. 2017;409:263–96.
7. Monard C, Abraham P, Schneider A, Rimmelé T. New targets for extracorporeal blood purification therapies in sepsis. Blood Purif. 2023;52(1):1–7.
8. Marks D, Medina F, Lee S, Schuschereba ST. Removal of bacteria from blood by charcoal hemoperfusion. Biomat Art Cells Art Org. 1988;16(1–3):135–40.
9. Krishnamurti K, Soman SV. Studies in the adsorption of bacteria. Proc Indian Acad Sci. 1951;34:81–91.
10. Liu L, Chen S, Xue Z, Zhang Z, Qiao X, Nie Z, et al. Bacterial capture efficiency in fluid bloodstream improved by bendable nanowires. Nat Commun. 2018;9(1):444.
11. Seffer MT, Cottan D, Forni L, Kielstein JT. Heparin 2.0: a new approach to the infection crisis. Blood Purif. 2021;50(1):28–34.
12. de Cal M, Lorenzin A, Risino B, Zanetti F, Fanton G, Fallico L, et al. Extracorporeal therapy in the treatment of sepsis: in vitro assessment of the effect of an absorbent cartridge on the circulating bacterial concentration and its interaction with the antibiotic therapy. Int J Artif Organs. 2023;46(6):344–50.
13. Hirsh SL, Mckenzie DR, Nosworthy NJ, Denman JA, Sezerman OU, Bilek MMM, et al. The Vroman effect: competitive protein exchange with dynamic protein aggregates. Colloids Surf B Biointerfaces. 2013;103:395–404.
14. Ankawi G, Fan W, Pomarè Montin D, Lorenzin A, Neri M, Caprara C, et al. A new series of sorbent devices for multiple clinical purposes: current evidence and future directions. Blood Purif. 2019;47:94–100.
15. Jacobs M, Good C, Lazarus H, Yomtovian RA. Relationship between bacterial load, species virulence, and transfusion reaction with transfusion of bacterially contaminated platelets. Clin Infect Dis. 2008;46:1214–20.
16. Godi I, Lorenzin A, De Rosa S, Golino G, Knust M, Gaspar A, et al. Vancomycin adsorption during in vitro model of hemoperfusion with HA380 cartridge. Nephron. 2021;145:157–63.
17. Ronco C, Bellomo R. Hemoperfusion: technical aspects and state of the art. Crit Care. 2022 May 12;26(1):135. https://doi.org/10.1186/s13054-022-04009-w
18. Stussi G, Buser A, Holbro A. Red blood cells: exchange, transfuse, or deplete. Transfus Med Hemother. 2019;46:407–16.

19. Chitty S, Mobbs S, Rifkin B, Stogner SW, Lewis MS, Betancourt J, et al. A multicenter evaluation of the seraph 100 microbind affinity blood filter for the treatment of severe COVID-19. Crit Care Explor. 2022;4(4):e0662.
20. Lamy B. Blood culture time-to-positivity: making use of the hidden information. Clin Microbiol Infect. 2019;25(3):268–71.
21. Hsieh YC, Chen HL, Lin SY, Chen TC, Lu PL. Short time to positivity of blood culture predicts mortality and septic shock in bacteremic patients: a systematic review and meta-analysis. BMC Infect Dis. 2022;22(1):142.
22. Martín-Gutiérrez G, Martín-Pérez C, Gutiérrez-Pizarraya A, Lepe JA, Cisneros JM, Aznar J. Time to positivity of blood cultures in patients with bloodstream infections: a useful prognostic tool. Enferm Infecc Microbiol Clin. 2017;35(10):638–44.
23. Ackerman BH, Vannier AM, Eudy EB. Analysis of vancomycin time-kill studies with Staphylococcus species by using a curve stripping program to describe the relationship between concentration and pharmacodynamic response. Antimicrob Agents Chemother. 1992;36(8):1766–9.
24. Cooper MA, Fiorini MT, Abell C, Williams DH. Binding of vancomycin group antibiotics to D-alanine and D-lactate presenting self-assembled monolayers. Bioorg Med Chem. 2000;8(11):2609–16.
25. Corti A, Cassani G. Synthesis and characterization of D-alanyl-D-Alanine-agarose. Appl Biochem Biotechnol. 1985;11:101–9.
26. Yan H, Zhao Q, Yuan J, Cheng X, He B. Affinity adsorbents for the vancomycin group of antibiotics. Biotechnol Appl Biochem. 2000;31:15–20.
27. Lorenzin A, Neri M, de Cal M, Pajarin G, Mansi Montenegro G, Savastano S, et al. Fluid dynamics analysis by CT imaging technique of new sorbent cartridge for extracorporeal therapies. Blood Purif. 2019;48:18–24.

How to cite this article: Ramírez-Guerrero G, de Cal M, Lorenzin A, Vigolo D, Toscano AI, Araya-Rojas M, et al. Sorbent functionalization with vancomycin enhances bacteria killing in extracorporeal hemoadsorption. Artif. Organs. 2023;00:1–7. https://doi.org/10.1111/aor.14704