

HYPOTHESIS

Open Access



# A potential hypothesis for 2019-nCoV infection therapy through delivery of recombinant ACE2 by red blood cell-hitchhiking

Zahra Sadat Aghili<sup>1</sup>, Seyed Abbas Mirzaei<sup>2,3</sup> and Mehdi Banitalebi-Dehkordi<sup>1,3\*</sup>

## Abstract

A novel infectious disease, caused by 2019 Novel Coronavirus (2019-nCoV) is responsible for the recent outbreak of severe respiratory disease. The 2019-nCoV spread rapidly and reaching epidemic proportions in many countries of the world. ACE2 was identified as a key receptor for 2019-nCoV infections. Excessive form of soluble ACE2 rescues cellular ACE2 activity which has a protective role in acute lung failure and neutralizes the virus. The short half-life of ACE2 is a major limitation to its practical application. Nanoparticle-based drug delivery systems are one of the most widely investigated approaches for developing novel therapies for a variety of diseases. Nevertheless, nanoparticles suffer from the rapid removal from the bloodstream by the reticuloendothelial system (RES). A noncovalent attachment of nanoparticles to RBCs increases their half-life in blood and allows transient accumulation in the lungs, while decreases their uptake by the liver and spleen. Connecting the recombinant ACE2 into the surface of nanoparticles that were attached to RBCs can be a potential therapeutic approach for 2019-nCoV infection through increasing their lung targeting to naturalize the virus and also acting as a bioreactor in the blood circulation to decrease serum level of Angiotensin II and protects lungs from injury/ARDS.

**Keywords:** ACE2, Drug delivery, Nanoparticles, 2019-nCoV, RBC hitchhiking

## Introduction

The 2019 Novel Coronavirus (2019-nCoV) or SARS-CoV-2 is an enveloped RNA virus that it is spreading rapidly and scientists are trying to discover drugs for an effective treatment [1]. This coronavirus induces excessive and abnormal non-effective host immune responses that are associated with acute respiratory distress syndrome (ARDS) and severe lung pathology, leading to death [2]. Spike (S) proteins of coronaviruses, including SARS-CoV and the 2019-nCoV, correlate with cellular receptors to mediate infection of their target cells

[3]. It has been reported that the angiotensin-converting enzyme II (ACE2) plays an important role in the entry of the virus into cells, thus the ACE2-expressing cells may act as target cells and are susceptible to 2019-nCoV infection [4]. ACE2 is mainly expressed in alveolar epithelial type II cells. In most 2019-nCoV infected patients, lungs are heavily infected by the virus and alveolar epithelial cells serve as a viral reservoir [5]. ACE2 is also expressed in multiple extrapulmonary tissues including the human heart, kidneys, blood vessels, and intestine that might reveal the multiorgan dysfunction observed in patients [6, 7]. Researchers showed that 2019-nCoV directly infects and multiplies within the human blood vessel and kidney organoids [8]. Overexpression of human ACE2 enhanced disease severity in a mouse model of

\*Correspondence: banitalebi.m@skums.ac.ir; mehdiбанitalebi@gmail.com

<sup>3</sup> Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran  
Full list of author information is available at the end of the article



© The Author(s) 2020. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

SARS-CoV infection, demonstrating that ACE2-dependent viral entry into cells is a critical step [9].

### **Renin–angiotensin system in human coronavirus pathogenesis**

There are two forms of ACE2. The full-length ACE2 is a type I integral membrane protein and the soluble form of ACE2 lacks the anchoring site and circulates in small amounts in the blood [10]; it has been shown to block the binding of the SARS-CoV spike protein to its receptor. ACE2 is a carboxypeptidase and is an ACE homologous. These receptors play critical functions in the renin-angiotensin system (RAS). ACE cleaves angiotensin I (Ang I) to form angiotensin II (Ang II); Ang II, the main active RAAS component, exerts its effects mainly via angiotensin II type 1 receptors (AT1R). Major effects of Ang II include vasoconstriction, renal sodium reabsorption and potassium excretion, aldosterone synthesis, blood pressure elevation, and induction of inflammatory and pro-fibrotic pathways [11, 12], while, ACE2 negatively regulates the RAS and cleaves Ang II to formation of angiotensin 1–7 (Ang 1–7) which opposes the actions of Ang II [3, 12, 13]. Ang 1–7 exerts vasodilating, anti-inflammatory, and anti-fibrotic effects through binding to the Mas receptor [14]. Imai et al. showed that the RAS has a crucial role in severe acute lung injury and excessive ACE2 rescues cellular ACE2 activity which has a protective role in acute lung failure [13]. Indeed, Kuba et al. demonstrated that SARS CoV and the Spike protein of the SARS-CoV reduce ACE2 expression (but not ACE) in mice, contributing to severe lung failure [15].

### **Recombinant ACE2 as a decoy receptor for 2019-nCoV**

In vitro studies in monkey kidney cells, Vero E6, showed that a soluble form of ACE2 blocked association of the S1 domain with Vero E6 cells thus SARS-CoV replication was blocked [16]. Moreover, ACE2 fused to the Fc portion of immunoglobulin has just been described for high-affinity binding to the receptor-binding domain (RBD) of SARS-CoV and 2019-nCoV and potently neutralized SARS-CoV and 2019-nCoV in vitro [3]. Therefore, the rACE2 protein may be a potential therapeutic approach in the management of emerging lung disorders that suffer from ARDS [15, 17]. Nevertheless, the short half-life of rACE2 is the main limitation to its practical application [3].

### **Nanoparticle-based targeted drug delivery**

Successfully, in recent decades, nanoparticle-based drug delivery systems have been employed as experimentally and clinically to improve the efficacy of many drugs and

therapeutic molecules. The field of nanomedicine provides distinct advantages over free drugs including targeting any organs while avoiding off-targets, and sustained release for improving the current treatment strategies for cancer and other diseases. But nanoparticles (NPs) suffer from the rapid clearance from the bloodstream by the reticuloendothelial system (RES) principally located in the liver and spleen, thereby limiting the dose available for the disease site. Due to this reason and other unfavorable factors, nanocarriers are not able to accumulate in many sites of therapeutic interest [18–20]. Erythrocytes are the particularly attractive vascular carrier for drug delivery of wide varieties of therapeutic agents to promote their biodistribution, pharmacokinetics, pharmacodynamics, and controlled release. RBC hitchhiking is a universal solution for dominant liver uptake and limited target organ deposition of nanocarriers as a drug delivery system. No damaging coupling to RBCs may prolong the lifetime of NPs in the circulation [21].

### **Hypothesis and evaluation**

Recently, it has been reported that the serum level of Ang II is significantly raised in 2019-nCoV infected patients and shows a linear positive correlation to viral load and lung injury [22]. Activation of the RAS causes extensive endothelial dysfunction and differing degrees of multiple organ (heart, kidney, and lung) injuries [23]. ACE2 has two different functions, both as the entry receptor of SARS-CoV causes to worsen the disease, and also as a negative regulator of the RAS, protect the lung from injury. Findings suggest that the soluble form of ACE2 decreases viral spread through competitively binding with 2019-nCoV and neutralizes the virus similar to a decoy receptor. In this way, it protects the lung from injury through saving cellular ACE2 activity and negatively regulates the RAS. Non-covalent attachments of NPs to the surface of RBCs (RBCs-NPs complex) increases their level in the blood without detectable changes in RBC circulation. This complex improves the blood pharmacokinetics and provides transient accumulation in the lungs while concurrently avoids their uptake by liver and spleen [18, 19, 24]. After several hours, NPs detach from the surface of RBCs, presumably due to shear force and interactions with vascular cells and ultimately are taken up by the liver and the spleen [24]. Recently, it has been reported that when RBCs are squeezed through the lung narrow capillaries, the NPs detach from the RBCs and transfer to the pulmonary capillary endothelial cells. Therefore, NPs, but not RBCs, accumulate transiently in the pulmonary vasculature [18, 19]. It can be a potential therapeutic approach for coronavirus infection. To use ACE2 as a treatment to

2019-nCoV-infected patients, we propose to fuse soluble form of recombinant ACE2 (rACE2) into the exposed surface of NPs that were attached to RBCs (therapeutic triple complex) and thereby we expect to achieve several results including:

- Increase the transient accumulation of rACE2 in highly vascularized organs like lungs, heart and kidneys.
- Extending the lifespan of the circulating rACE2.
- The therapeutic triple complex acts as a bioreactor in the blood circulation and decreases serum level of Ang II with cleavage it and inhibits severe acute lung injury/ARDS.

For this purpose, at first NPs adsorb onto the RBCs *ex vivo*. Spherical (PSNP) NPs can readily adhere to RBCs membrane by incubation at particle/RBC ratios up to 100:1. Particle adhesion to RBCs membrane is likely due to electrostatic and hydrophobic interactions between PSNP and the RBC. After adsorption of NPs to RBCs, rACE2 passively will be adsorbed onto the exposed carboxylated surface of NPs. After injection via an intravascular catheter, the therapeutic triple complex augments the accumulation of rACE2 in highly vascularized organs, first of all, lungs. NPs attached to the rACE2, through the lung narrow capillaries, transfer from the RBCs to the pulmonary capillary endothelium under the shear force and interactions with vascular cells [18, 19] (Fig. 1).

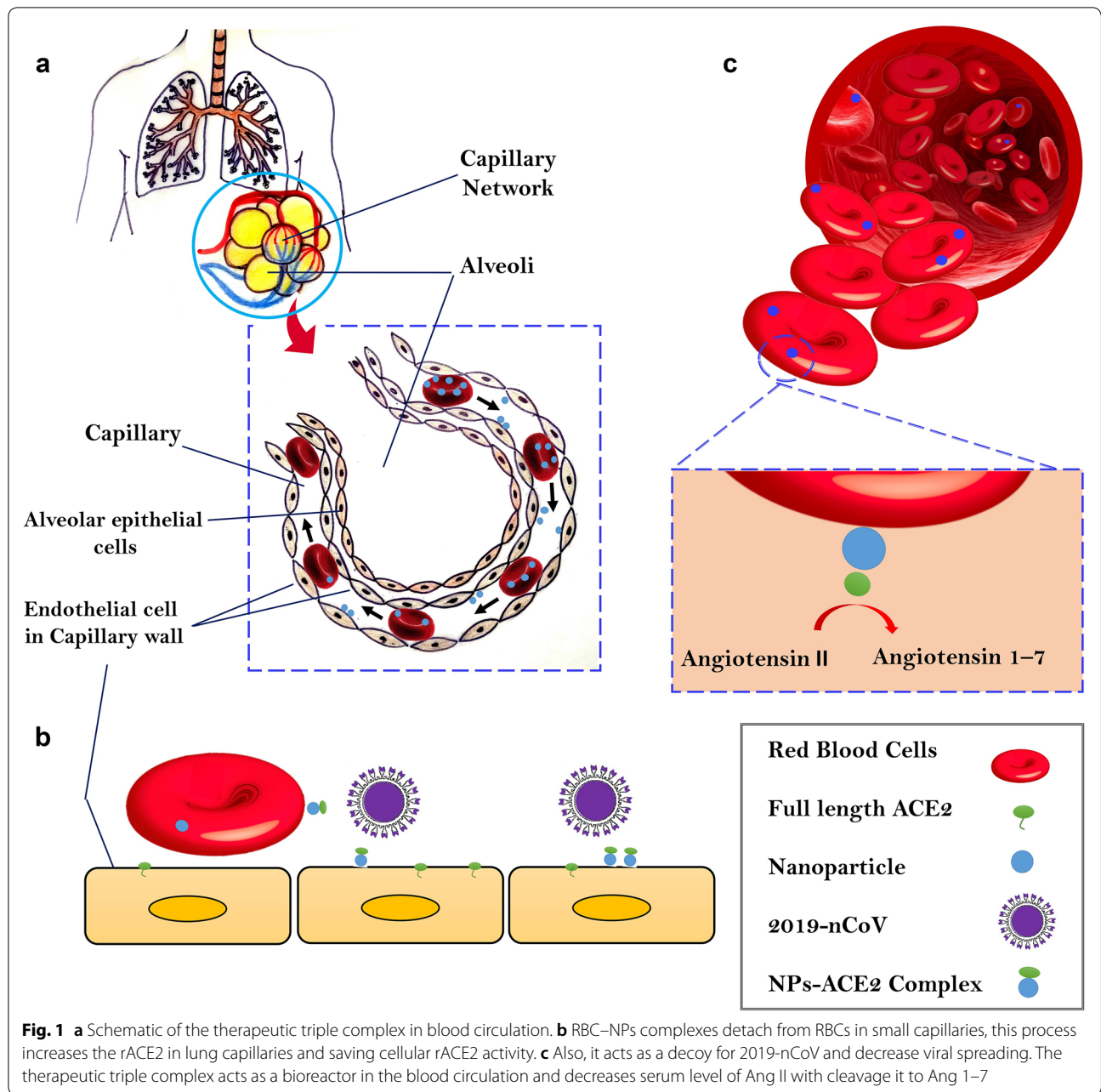
### **Effect of nanoparticles on biocompatibility of carrier RBCs**

RBCs are naturally able to transport various cargoes throughout the circulatory system. RBC drug delivery systems represent viable (and, in some cases, preferable) alternatives to synthetic carriers; however, impairment of biocompatibility of the carrier RBCs and other adverse and unintended effects may cause other problems. Dosing, timing, and regimens of administration of carrier RBCs or targeted agents need to be assessed separately and adjusted for these new parameters of their behavior and influences in the body [25]. Attachment of NPs to the surface of carrier RBCs can lead to changes in circulation time, immunogenicity, biodistribution elimination pathways, and pharmacodynamics [26]. These changes in the pharmacokinetics of NPs imposed by carrier

RBCs are beneficial for many drug delivery targets. To be clinically useful, NPs must not begin severe adverse effects on RBCs at loadings necessary for therapeutic applications [27]. In several studies, using two types of distinct NPs [PSNP and Lysozyme-dextran nanogels (LDNGs)], potential adverse and sensitizing effects of surface adsorption of NPs on the mouse and human RBCs were assessed. The seminal studies reported that RBCs may serve as “super-carriers” for NPs, as non-covalent attachment of PSNP to murine RBCs markedly alters the biodistribution of the NP in a manner advantageous to treatment of many diseases. PSNPs were used as a representative NP to evaluate RBCs as cellular carriers for NP delivery [27, 28]. According to these reports, the attachment of PSNP particle does not alter RBCs morphology [18, 19, 21, 24]. However, different studies have reported that the adsorption of PSNP beads onto RBCs causes their agglutination and sensitizes RBCs to damage by osmotic, mechanical and oxidative stress [27], whereas the adsorption of NPs made from soft biodegradable materials, LDNGs, does not induce negative effects on RBCs. Probable key physicochemical differences between LDNGs and PSNPs are likely responsible for the observed differences in their RBC sensitization and compatibility. LDNGs are less rigid, biodegradable and biocompatible compared to PSNPs. LDNGs do not induce NP-mediated damage to RBCs at loadings which PSNPs induce adverse effects [27, 28]. Therefore, the selection of the appropriate NPs types based on the targets of the experiments is an important factor and should be carefully studied.

### **Conclusion**

Recombinant ACE2 protein could not only be a treatment to block the spreading of 2019-nCoV, but modulation of the RAS could also be used to protect individuals with SARS. Fusing rACE2 into the exposed surface of NPs that were attached to RBCs, cause to extending the lifespan of the circulating rACE2 and increasing lung highly vascularized organs targeting and also act as a bioreactor in the blood circulation to decrease serum level of Ang II. Possibly this approach could also be used to protect individuals infected with other viruses such as SARS-CoV and avian influenza A strains, from developing acute severe lung failure and acute respiratory distress syndrome.



**Abbreviations**

ACE: Angiotensin-converting enzyme; ACE2: Angiotensin-converting enzyme II; Ang: Angiotensin; ARDS: Acute respiratory distress syndrome; LDNGs: Lysozyme-dextran nanogels; 2019-nCoV: 2019 Novel Coronavirus; NPs: Nanoparticles; PSNP: Polystyrene; rACE2: Recombinant ACE2; RAS: Renin-angiotensin system; RES: Reticuloendothelial system; RBCs: Red blood cells.

**Acknowledgements**

Not applicable.

**Authors' contributions**

AZS and MSA: participated in the data analyses and intellectual discussions of the data and manuscript writing. BDM: coordinated and supervised the study and critically revised the final manuscript. All authors read and approved the final manuscript.

**Funding**

Not applicable.

**Availability of data and materials**

Not applicable.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

The authors guarantee the contribution to the work and agree to publish the manuscript.

**Competing interests**

The authors declare that they have no conflicts of interest.

**Author details**

<sup>1</sup> Department of Molecular Medicine, School of Advanced Technologies, Shahrekord University of Medical Sciences, Shahrekord, Iran. <sup>2</sup> Department of Medical Biotechnology, School of Advanced Technologies, Shahrekord University of Medical Sciences, Shahrekord, Iran. <sup>3</sup> Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran.

Received: 26 May 2020 Accepted: 28 November 2020

Published online: 07 December 2020

**References**

- Tetro JA. Is COVID-19 receiving ADE from other coronaviruses? *Microbes Infect.* 2020;22:72–3.
- Zumla A, Hui DS, Azhar EI, Memish ZA, Maeurer M. Reducing mortality from 2019-nCoV: host-directed therapies should be an option. *Lancet.* 2020;395:e35–6.
- Lei C, Qian K, Li T, Zhang S, Fu W, Ding M, et al. Neutralization of SARS-CoV-2 spike pseudotyped virus by recombinant ACE2-Ig. *Nat Commun.* 2020;11:2070.
- Zou X, Chen K, Zou J, Han P, Hao J, Han Z. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. *Front Med.* 2020;14:185–92.
- Zhao Y, Zhao Z, Wang Y, Zhou Y, Ma Y, Zuo W. Single-cell RNA expression profiling of ACE2, the receptor of SARS-CoV-2. *Am J Respir Crit Care Med.* 2020;202:756–9.
- Guan W-J, Ni Z-Y, Hu Y, Liang W-H, Ou C-Q, He J-X, et al. Clinical characteristics of coronavirus disease, in China. *N Engl J Med.* 2019;2020(382):1708–20.
- Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395:497–506.
- Monteil V, Kwon H, Prado P, Hagelkrüys A, Wimmer RA, Stahl M, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell.* 2020;181:905–13.
- Yang X-H, Deng W, Tong Z, Liu Y-X, Zhang L-F, Zhu H, et al. Mice transgenic for human angiotensin-converting enzyme 2 provide a model for SARS coronavirus infection. *Comp Med.* 2007;57:450–9.
- Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, et al. Tumor necrosis factor- $\alpha$  convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). *J Biol Chem.* 2005;280:30113–9.
- Ferrario CM, Trask AJ, Jessup JA. Advances in biochemical and functional roles of angiotensin-converting enzyme 2 and angiotensin-(1–7) in regulation of cardiovascular function. *Am J Physiol Heart Circ Physiol.* 2005;289:H2281–90.
- Tikellis C, Thomas MC. Angiotensin-converting enzyme 2 (ACE2) is a key modulator of the renin angiotensin system in health and disease. *Int J Pept.* 2012;2012:256294.
- Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature.* 2005;436:112–6.
- Sanchis-Gomar F, Lavie CJ, Perez-Quilis C, Henry BM, Lippi G. Angiotensin-Converting Enzyme 2 and antihypertensives (angiotensin receptor blockers and angiotensin-converting enzyme inhibitors) in Coronavirus Disease 2019. *Mayo Clin Proc.* 2020;95:1222–30.
- Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med.* 2005;11:875–9.
- Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature.* 2003;426:450–4.
- Zou Z, Yan Y, Shu Y, Gao R, Sun Y, Li X, et al. Angiotensin-converting enzyme 2 protects from lethal avian influenza A H5N1 infections. *Nat Commun.* 2014;5:3594.
- Anselmo AC, Gupta V, Zern BJ, Pan D, Zakrewsky M, Muzykantov V, et al. Delivering nanoparticles to lungs while avoiding liver and spleen through adsorption on red blood cells. *ACS Nano.* 2013;7:11129–37.
- Brenner JS, Pan DC, Myerson JW, Marcos-Contreras OA, Villa CH, Patel P, et al. Red blood cell-hitchhiking boosts delivery of nanocarriers to chosen organs by orders of magnitude. *Nat Commun.* 2018;9:2684.
- Xia Q, Zhang Y, Li Z, Hou X, Feng N. Red blood cell membrane-camouflaged nanoparticles: a novel drug delivery system for antitumor application. *Acta Pharm Sin B.* 2019;9:675–89.
- Villa CH, Pan DC, Zaitsev S, Cines DB, Siegel DL, Muzykantov VR. Delivery of drugs bound to erythrocytes: new avenues for an old intravascular carrier. *Ther Deliv.* 2015;6:795–826.
- Liu Y, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci.* 2020;63:364–74.
- Guo J, Huang Z, Lin L, Lv J. Coronavirus disease 2019 (COVID-19) and cardiovascular disease: a viewpoint on the potential influence of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers on onset and severity of severe acute respiratory syndrome coronavirus 2 infection. *J Am Heart Assoc.* 2020;9:e016219.
- Chambers E, Mitragotri S. Prolonged circulation of large polymeric nanoparticles by non-covalent adsorption on erythrocytes. *J Control Release.* 2004;100:111–9.
- Villa CH, Seghatchian J, Muzykantov V. Drug delivery by erythrocytes: “Primum non nocere.” *Transfus Apher Sci.* 2016;55:275–80.
- Glassman PM, Villa CH, Ukidve A, Zhao Z, Smith P, Mitragotri S, et al. Vascular drug delivery using carrier red blood cells: focus on RBC surface loading and pharmacokinetics. *Pharmaceutics.* 2020;12:440.
- Pan DC, Myerson JW, Brenner JS, Patel PN, Anselmo AC, Mitragotri S, et al. Nanoparticle properties modulate their attachment and effect on carrier red blood cells. *Sci Rep.* 2018;8:1615.
- Pan D, Vargas-Morales O, Zern B, Anselmo AC, Gupta V, Zakrewsky M, et al. The effect of polymeric nanoparticles on biocompatibility of carrier red blood cells. *PLoS ONE.* 2016;11:e0152074.

**Publisher's Note**

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.