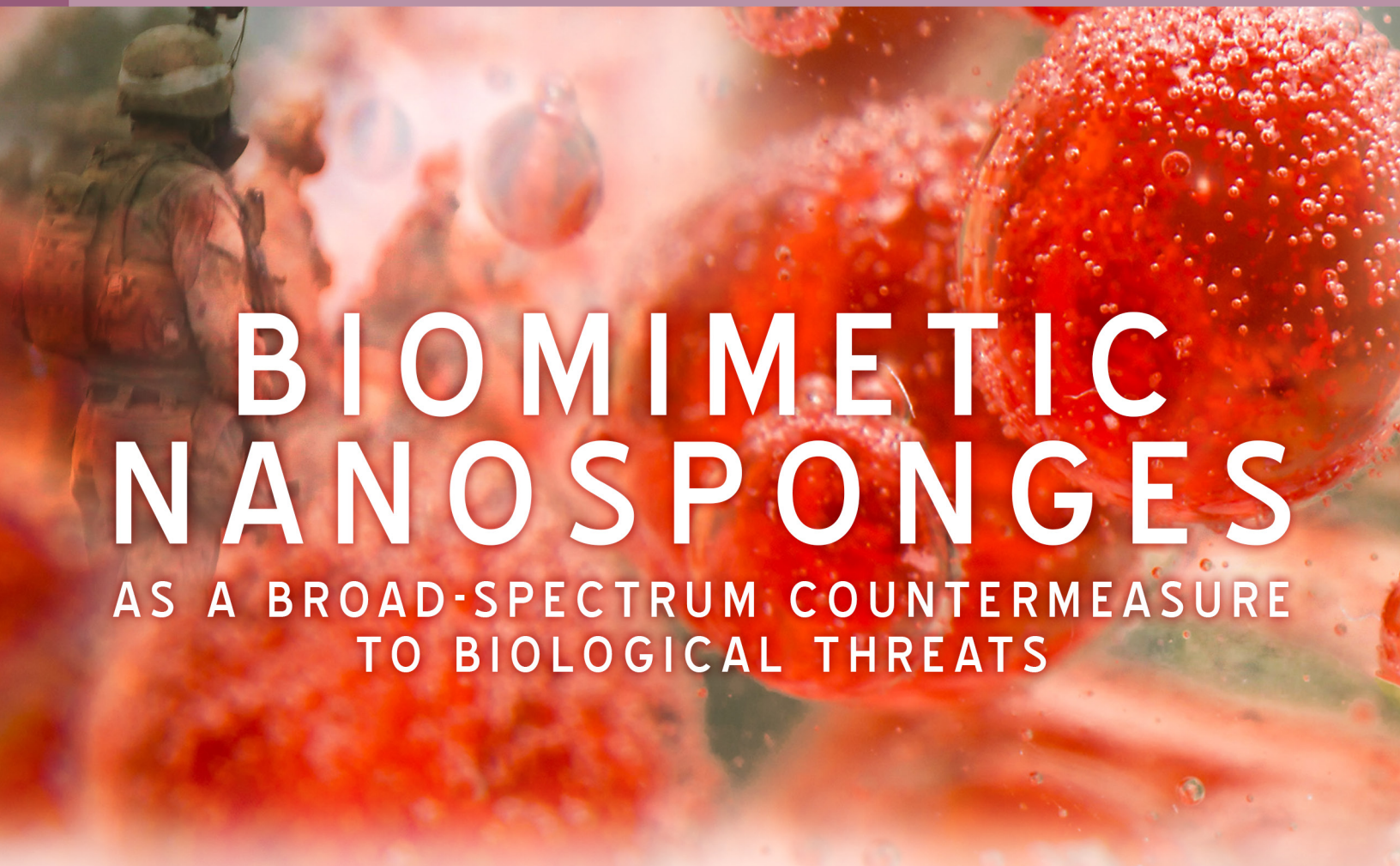
HDIAC JOURNAL
The Journal of the Homeland Defense & Security Information Analysis Center

Volume 6
Issue 3
Fall 2019

BIOMIMETIC NANOSPONGES

AS A BROAD-SPECTRUM COUNTERMEASURE TO BIOLOGICAL THREATS



BIOMIMETIC NANOSPONGES

AS A BROAD-SPECTRUM COUNTERMEASURE TO BIOLOGICAL THREATS

Ronnie H. Fang & Liangfang Zhang

A number of organisms secrete biological toxins that can cause significant harm to the human body. For example, pathogenic bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), utilize a number of different virulence factors to attack their host and enhance colonization [1]. Poisonous animals use their venom, which can contain a mixture of cytolytic and neurotoxic agents, to immobilize prey [2]. Military personnel are potentially at risk of catching a difficult-to-treat infection, being subject to envenomation, or coming under attack from a chemical nerve agent (e.g., sarin, tabun) while deployed—which could significantly compromise health and mission readiness. As such, research and development efforts focused on developing effective and efficient countermeasure therapeutics are of considerable interest to the Department of Defense (DoD).

Most traditional means of toxin neutralization work by a "lock and key" mechanism, whereby a single therapeutic is used to counteract the biological effect of a single target toxin [3]. These can be highly effective in certain scenarios, particularly when the exact nature of the threat is known. However, there are many situations—combat and forward-deployed environments in particular—in which this type of approach is not practical. This includes cases where the threat has not yet been identified, or when there is insufficient time to develop a treatment against an identified threat. And as many organisms secrete multiple toxins at once, some of which microbiologists are still characterizing, the development and fabrication of antidotes and countermeasures can be complicated.

Developing a New Class of Biomimetic Countermeasure Therapeutics

A common feature of most biological toxins, regardless of how they exert their activity, is that they must interact with cells via their plasma membrane. In the case of hemolysins, these toxins dock onto the membrane and physically disrupt integrity or form pores on the surface [4]. Other toxins engage surface receptors and are transported into the cell, whereupon they can affect vital intracellular processes [5]. Taking advantage of these toxin–cell interactions, our research group at the Department of NanoEngineering at the University of California San Diego developed a new class of biomimetic countermeasure therapeutics [6]. "Nanosponges" are generated by coating a layer of cell membrane onto a nanoparticulate substrate. The resulting nanoparticles can then act as nanoscale decoys that are capable of attracting and neutralizing a wide range of toxins, preventing them from destroying healthy cells.

Nanosponges differ from traditional platforms for toxin neutralization by employing a function-driven approach that bypasses the need to identify and characterize individual toxins [7]. Instead of focusing on toxin structure, a greater emphasis is placed on their natural interactions with cells. By faithfully presenting cell membrane material on their surfaces, nanosponges are inherently multi-specific, and a single nanoparticle can protect against a wide range of toxins. This greatly streamlines the development process and makes the platform applicable to a broad array of DoD missions and requirements, where one formulation can be indicated against a number of different CBRN agents, infections, or even venomous injuries. Research into biomimetic toxin-absorbing nanosponges meets

Image Credit | *Photo illustration created by HDIAC and adapted from a U.S. Marine Corps photo (available for viewing at: https://media.defense.gov/2016/Aug/22/2001612174/-1/-1/0/160818-M-LV138-299.JPG) and Adobe Stock.*

the mission of DoD's Chemical and Biological Defense Program (CBDP), which seeks to examine "factors which influence the behavior of chemicals, toxins, and pathogens in relation to the host or target [8]." Future research may also advance biomimetic nanosponge applications to include pre-emptive anti-toxin vaccines for warfighters, to inoculate them in advance of deployment into hostile environments.

Cell Membrane-Coated Nanoparticles

Nanosponges are based on the cell membrane-coated nanoparticle concept (see Figure 1), which is part of a new wave of biomimetic nanotechnologies that have become increasingly popular over the past decade [9, 10]. By taking design inspirations from nature and leveraging natural components, highly functional nanoparticle platforms can be fabricated without having to start from ground zero. Cell membrane-coated nanoparticles are made using a top-down approach that first involves deriving the plasma membrane from cells with a combination of mechanical disruption and differential centrifugation. The purified membrane material is then fused onto the surface of nanoparticulate cores, resulting in a characteristic core–shell structure. The coating of the membrane onto a solid substrate helps with stabilization, preventing the lipid material from fusing with other membranes. At the same time, the core can be used to deliver functional payloads, such as drugs and imaging agents.

Importantly, the coating process has shown to successfully transfer membrane surface markers from the original cells onto the final nanoparticle formulations. For example, CD47 is a marker highly present on red blood cells (RBCs) that acts as a "don't eat me" signal to prevent phagocytic uptake [11]. When RBC membrane is used as a coating

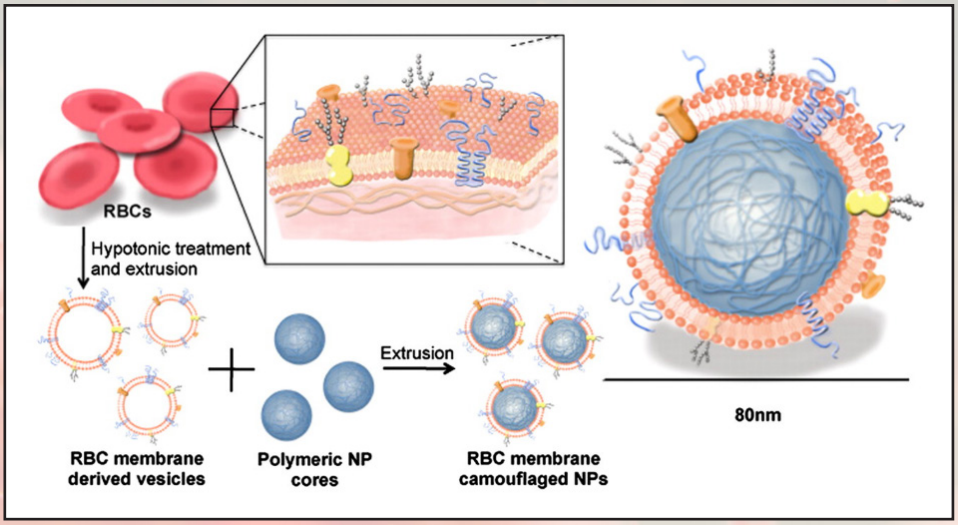


Figure 1. The cell membrane-coated nanoparticle. Cells are employed as a source of membrane material, which contains a wide range of functional ligands. The membrane is then fused onto nanoparticulate cores, resulting in a core–shell, cell-mimicking nanostructure that can be used for a variety of biomedical applications [10]. (Reprinted with Permission)

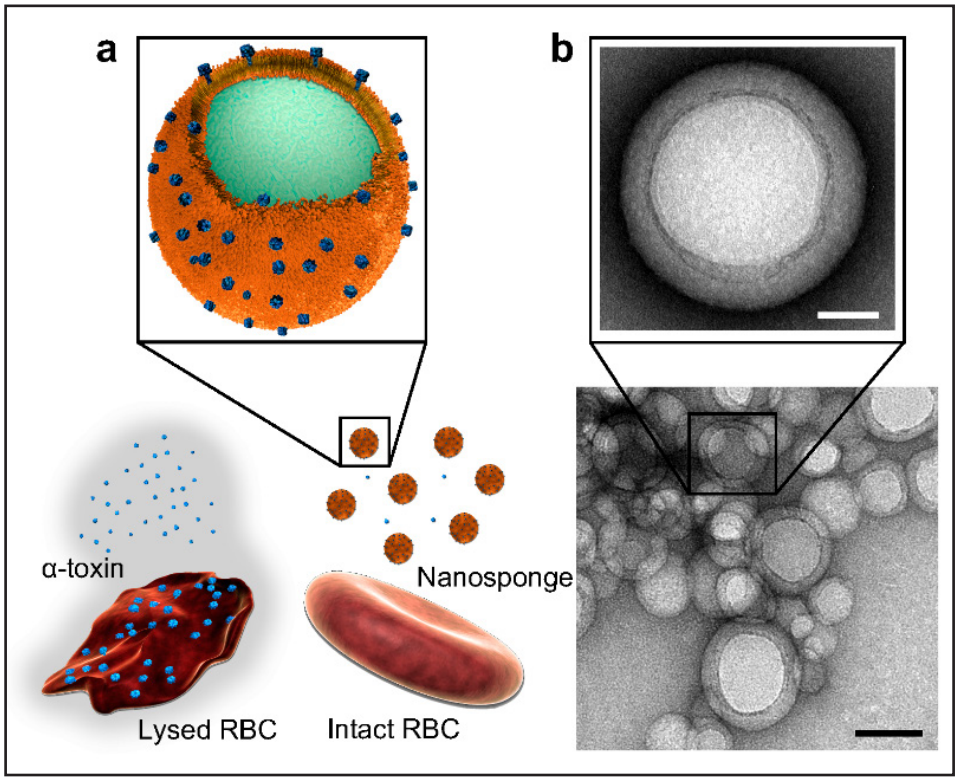


Figure 2. Biomimetic nanosponges for pore-forming toxin neutralization. (a) Under normal circumstances, hemolytic toxins such as staphylococcal α-toxin can attack RBCs and cause lysis. When nanosponges are introduced, these nanodecoys neutralize the toxins and leave the RBCs intact. (b) Transmission electron micrographs reveal the core–shell structure of the nanosponges (top scale bar = 20nm; bottom scale bar = 80nm) [6]. (Reprinted with Permission)

material, the resulting RBC membrane-coated nanoparticles display CD47 in a right-side-out orientation and at the same density as the source cells [12]. Taking advantage of this protein's functionality, the nanoparticles have exhibited reduced uptake by macrophages compared with uncoated particles. Similarly, comprehensive protein analysis of nanoparticles coated with platelet membrane has demonstrated successful functionalization with a panel of platelet surface markers, including those that can be leveraged for immune evasion and targeted delivery [13]. In essence, membrane-coated nanoparticles present themselves as miniaturized versions of the cells from which they are sourced and exhibit many of the same functions as their live counterparts.

As a result of their cell-mimicking properties, membrane-coated nanoparticles have been successfully used for a number of different biomedical applications [9]. This includes the nanodelivery of therapeutic and diagnostic agents, which benefit greatly from the long circulation and enhanced disease site localization afforded by the membrane functionalization. Depending on the source of the membrane material used, formulations capable of targeting cancer, atherosclerotic plaque, and bacteria have all been reported. When loaded with an immunostimulatory agent, it is possible to generate an immune response against the antigenic material on the nanoparticle surface, and this has been leveraged for the design of potent anticancer vaccines [14]. Coating with biological membrane is not limited to nanoparticle substrates however, as nanofibers and planar sensors have also been successfully functionalized [15, 16].

Toxin Neutralization

One unique application of cell membrane-coated nanoparticles is their ability to serve as biomimetic nanosponges for toxin neutralization (see Figure 2) [6]. The nanosponges work by taking advantage of the natural interactions that exist between biological toxins and cellular membrane. This interaction can be nonspecific, as is the case with some small peptide toxins like honeybee melittin, which inserts itself into lipid bilayers and causes membrane disruption [17]. Very often the interaction of toxins with cell membrane is mediated by specific surface receptors [18]. This is the case with *S. aureus* α-hemolysin, which has greater affinity to membranes with a high expression of ADAM10. Streptolysin-O, one of the characteristic toxins for *Streptococcus pyogenes*, uses cholesterol as its receptor; the toxin belongs to a family of cholesterol-dependent cytolysins that all work by similar mechanisms.

Due to differences in surface marker expression, biological toxins are likely to exhibit differential affinities for different cell types. For example, while hemolysins may prefer to attack and lyse RBCs [19], bacterial endotoxins exert their activity through engagement of white blood cells (WBCs) [20]. With the nanosponge technology, this can be easily addressed by fabricating the nanoparticles using membrane derived from cells with the highest affinity for the target of interest. Using next-generation proteomic analysis, it has been demonstrated that nanosponges fabricated using different membrane sources preferentially enrich varying protein subsets from crude bacterial secretions [21].

Not only were the nanosponges able to bind known toxins, but they also demonstrated affinity towards proteins with functions that have yet to be elucidated. A biomimetic "virulomics" approach employing nanosponge enrichment could ultimately be used to identify novel virulence factors, and this may have major implications for the future development of new antibacterial therapies. Research and development into new therapies may provide the warfighter with both pre-deployment antidotes and battlefield countermeasures, increasing force survivability and resilience.

The function-driven approach for toxin neutralization employed by nanosponge technology represents one of its biggest advantages. As suggested by the aforementioned proteomic study, it is not necessary to know the specific identity of a toxin before designing a nanosponge to neutralize it [21]. The only knowledge required is the type of cell most affected by exposure to the toxin. Because this approach does not follow a one-to-one neutralization scheme, it is possible to leverage the numerous receptors found on natural cell membranes to achieve one-to-many multivalency. For example, RBC-based nanosponges have been shown to concurrently bind and neutralize the activity of α-hemolysin, Panton–Valentine leukocidin, and γ-hemolysin from *S. aureus*—all at the same time [22]. With their broad neutralization capabilities, nanosponges can be

applied to many types of diseases and conditions, even in some cases where previously no good solution existed.

Detoxification Applications

With the rising incidence of antibiotic resistance, significant efforts have been dedicated to finding novel means of managing bacterial infections. One approach that has shown promise is antivirulence therapy, which seeks to disarm bacteria by neutralizing the toxins used to promote survival and host colonization [23]. Given their ability to neutralize biological toxins broadly, nanosponges have been explored for a number of antibacterial applications. It was demonstrated that, in a murine model of lethal toxin challenge using staphylococcal α-hemolysin, RBC nanosponges can significantly improve survival in both protective and therapeutic scenarios [6]. The benefit of these nanosponges has also been demonstrated using lethal challenge with multi-toxin bacterial secretions [24], as well as during live infection with group A streptococcus (see Figure 3) [25].

In addition to their ability to neutralize bacterial toxins, nanosponges are also adept at binding endogenous proteins with pathological properties. For example, both RBC and platelet-based nanosponges have been used to neutralize autoimmune antibodies in models of autoimmune hemolytic anemia and thrombocytopenia, respectively [26, 27]. WBC nanosponges can be used to bind proinflammatory cytokines and have shown exceptional utility for treating chronic inflammatory conditions such as arthritis [28].

Additionally, with their bacterial toxin-binding capacity, WBC nanosponges can blunt the effect of bacteria-induced sepsis, which is associated with poor outcomes in the clinic and is oftentimes lethal. WBC nanosponges could serve the dual purpose of binding gram-negative endotoxin as well as cytokines, thereby reducing the immune activation that is characteristic of sepsis and enhancing survival in a murine model of the condition [29].

Nanosponges have potential use against pathogens other than bacteria, including parasites and viruses. In one work, it was demonstrated that WBC nanosponges could be used to bind virulence factors from protein secretions derived from the eggs of *Schistosoma mansoni*, one of the most common parasites in humans [21]. More recently,

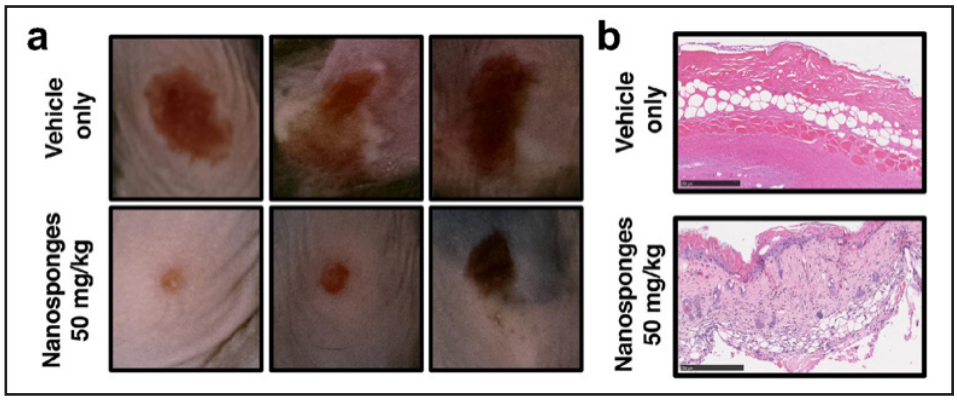


Figure 3. Nanosponges protect against group A streptococcus skin infection. (a) At three days post-infection, mice treated with nanosponges exhibited significantly reduced lesion sizes. (b) Histopathologic analysis of lesion biopsies showed that nanosponge-treated mice had less necrotic tissue injury compared with vehicle-treated mice [25]. (Reprinted with Permission)

WBC nanosponges derived from CD4-expressing T cells were used to bind and neutralize human immunodeficiency virus (HIV) [30]. It is well-known that HIV evolves in a manner that ultimately outpaces the immune system's capacity to address it, and broadly neutralizing antibodies against the virus are highly sought after. The use of $CD4^+$ nanosponges represents a completely different approach that relies on the natural receptors the virus employs to gain cellular entry, which may ultimately enable the nanoparticles to retain their HIV-binding capabilities over time.

Similarly, nanosponges fabricated using mosquito cell membranes have recently been used against Zika virus, greatly improving survival in animal models of infection [31]. This application would be of particular benefit to DoD, since significant numbers of personnel serve in Areas of Responsibility where Zika, Dengue virus, and other arboviruses are common [32].

Although the majority of work using nanosponges has centered on toxins of biological origin, there are also examples of the platform's application to chemical agents. In one example, RBC nanosponges were shown to bind and neutralize dichlorvos, a neurotoxic organophosphate compound that covalently binds and deactivates acetylcholinesterase [33]. Mice treated with the nanosponges were protected from the lethal effects of the compound. Membrane-coated magnetic particles have also been used as tools for affinity-based drug screening and identification [34, 35]. Future research may delineate the efficacy of biomimetic nanosponges against weaponized chemical agent exposure or chemical intoxication.

While the nanosponge platform has mostly been employed for biodetoxification to protect against toxin exposure in therapeutic and prophylactic scenarios, it has also been used in the design of more effective anti-toxin vaccine formulations [36]. Since toxins that are complexed with nanosponges are completely neutralized, they can be delivered back into the body in order to train the immune system against subsequent exposure. This method of using nanoparticle-based toxin detainment for formulating vaccines overcomes many of the challenges associated with traditional toxoid vaccines, which require harsh denaturation techniques that can affect both the immunogenicity and antigenicity of the final formulation. This approach has been used to successfully protect against live MRSA infection, significantly reducing bacterial load in vaccinated mice [37]. The generation of multiantigenic formulations is facile and simply requires coincubation of the nanosponges with bacterial secretions [22]. More recently, active propulsion using micromotor technology has shown to enhance intestinal delivery for eliciting anti-toxin titers in the mucosa [38]. The provision of anti-toxin vaccines to warfighters prior to active-duty service or deployment will inoculate them against toxin exposure, without encumbering them with another medical countermeasure device or medication that must be carried with them into the field.

Conclusion and Outlook

Cell membrane-coated nanosponges are a new class of biomimetic nanoparticle that can be employed in a number of different biodetoxification applications—many of which are of considerable interest to the DoD. Military forces may be supplied with anti-toxin

and anti-agent vaccines or injections prior to deployment, or supplied with a broad-spectrum single-point countermeasure available in the event of a suspected CBRN exposure. Future developments in biomimetic nanosponge research may also lead to their use in defending the public and DoD from novel microbiological weapons, a threat posed by rapid advances in bioengineering and synthetic biology [39]. The platform functions by targeting the working mechanism of biological toxins, most of which must interact with cell membrane in some fashion. With this function-driven approach, a single nanosponge can neutralize many toxins at once, including those whose precise function has not yet been elucidated. The technology has major implications for the treatment and prevention of biological threats, particularly some pathogens that have traditionally been hard to manage.

Looking forward, more work can be done along the lines of applying nanosponges towards envenomation or chemical intoxication. Successful translation of the platform will also require efforts to significantly scale-up production to clinically relevant levels. While RBC-based nanosponges have been the most commonly used up to this point, other types of nanosponges, including those based on platelets and WBCs, should excel depending on the specific condition to be treated. Cocktail treatments, or the use of fusion membranes [40], could be considered to further increase the applicability of nanosponges. Overall, future prospects for this biomimetic platform are encouraging, and continued development will enable the realization of its full potential.

References

1. Powers, M. E., & Bubeck Wardenburg, J. (2014). Igniting the fire: *Staphylococcus aureus* virulence factors in the pathogenesis of sepsis. *PLoS Pathogens, 10*(2), e1003871. doi:10.1371/journal.ppat.1003871
2. Andreeva-Kovalevskaya Zh, I., Solonin, A. S., Sineva, E. V., & Ternovsky, V. I. (2008). Pore-forming proteins and adaptation of living organisms to environmental conditions. *Biochemistry (Moscow), 73*(13), 1473–1492. doi:10.1134/S0006297908130087
3. Hua, L., Hilliard, J. J., Shi, Y., Tkaczyk, C., Cheng, L. I., Yu, X., . . . Sellman, B. R. (2014). Assessment of an anti-alpha-toxin monoclonal antibody for prevention and treatment of Staphylococcus aureus-induced pneumonia. *Antimicrobial Agents and Chemotherapy, 58*(2), 1108–1117. doi:10.1128/AAC.02190-13
4. Geny, B., & Popoff, M. R. (2006). Bacterial protein toxins and lipids: Pore formation or toxin entry into cells. *Biology of the Cell, 98*(11), 667–678. doi:10.1042/Bc20050082
5. Lord, J. M., Smith, D. C., & Roberts, L. M. (1999). Toxin entry: How bacterial proteins get into mammalian cells. *Cellular Microbiology, 1*(2), 85–91. doi:10.1046/j.1462-5822.1999.00015.x
6. Hu, C. M., Fang, R. H., Copp, J., Luk, B. T., & Zhang, L. (2013). A biomimetic nanosponge that absorbs pore-forming toxins. *Nature Nanotechnology, 8*(5), 336–340. doi:10.1038/nnano.2013.54
7. Fang, R. H., Luk, B. T., Hu, C. M., & Zhang, L. (2015). Engineered nanoparticles mimicking cell membranes for toxin neutralization. *Advanced Drug Delivery Reviews, 90*, 69–80. doi:10.1016/j.addr.2015.04.001
8. Department of Defense. (2019, March). *Department of Defense Fiscal Year (FY) 2020 Budget Estimates, Chemical and Biological Defense Program, Defense-Wide Justification Book Volume 4 of 5*. Retrieved from https://comptroller.defense.gov/Portals/45/Documents/defbudget/fy2020/budget_justification/pdfs/03_RDT_and_E/RDTE_Vol4_CBDP_RDTE_PB20_Justification_Book.pdf
9. Fang, R. H., Kroll, A. V., Gao, W., & Zhang, L. (2018). Cell membrane coating nanotechnology. *Advanced Materials, 30*(23), 1706759. doi:10.1002/adma.201706759
10. Hu, C. M., Zhang, L., Aryal, S., Cheung, C., Fang, R. H., & Zhang, L. (2011). Erythrocyte membrane-camouflaged polymeric nanoparticles as a biomimetic delivery platform. *Proceedings of the National Academy of Sciences of the United States of America, 108*(27), 10980–10985. doi:10.1073/pnas.1106634108
11. Oldenborg, P. A., Zheleznyak, A., Fang, Y. F., Lagenaur, C. F., Gresham, H. D., & Lindberg, F. P. (2000). Role of CD47 as a marker of self on red blood cells. *Science, 288*(5473), 2051–2054. doi:10.1126/science.288.5473.2051
12. Hu, C. M., Fang, R. H., Luk, B. T., Chen, K. N., Carpenter, C., Gao, W., . . . Zhang, L. (2013). 'Marker-of-self' functionalization of nanoscale particles through a top-down cellular membrane coating approach. *Nanoscale, 5*(7), 2664–2668. doi:10.1039/c3nr00015j
13. Hu, C. M., Fang, R. H., Wang, K. C., Luk, B. T., Thamphiwatana, S., Dehaini, D., . . . Zhang, L. (2015). Nanoparticle biointerfacing by platelet membrane cloaking. *Nature, 526*(7571), 118–121. doi:10.1038/nature15373
14. Kroll, A. V., Jiang, Y., Zhou, J., Holay, M., Fang, R. H., & Zhang, L. (2019). Biomimetic nanoparticle vaccines for cancer therapy. *Advanced Biosystems, 3*(1), 1800219. doi:10.1002/adbi.201800219
15. Chen, W., Zhang, Q., Luk, B. T., Fang, R. H., Liu, Y., Gao, W., & Zhang, L. (2016). Coating nanofiber scaffolds with beta cell membrane to promote cell proliferation and function. *Nanoscale, 8*(19), 10364–10370. doi:10.1039/c6nr00535g
16. Gong, H., Chen, F., Huang, Z., Gu, Y., Zhang, Q., Chen, Y., . . . Zhang, L. (2019). Biomembrane-modified field effect transistors for sensitive and quantitative detection of biological toxins and pathogens. *ACS Nano, 13*(3), 3714–3722. doi:10.1021/acsnano.9b00911
17. van den Bogaart, G., Guzman, J. V., Mika, J. T., & Poolman, B. (2008). On the mechanism of pore formation by melittin. *Journal of Biological Chemistry, 283*(49), 33854–33857. doi:10.1074/jbc.M805171200
18. Los, F. C., Randis, T. M., Aroian, R. V., & Ratner, A. J. (2013). Role of pore-forming toxins in bacterial infectious diseases. *Microbiology and Molecular Biology Reviews, 77*(2), 173–207. doi:10.1128/MMBR.00052-12
19. McCullough, J. (2014). RBCs as targets of infection. *Hematology-American Society of Hematology Education Program, 2014*(1), 404–409. doi:10.1182/asheducation-2014.1.404
20. Galanos, C., & Freudenberg, M. A. (1993). Bacterial endotoxins: Biological properties and mechanisms of action. *Mediators of Inflammation, 2*(7), S11–16. doi:10.1155/S0962935193000687
21. Lapek, J. D., Jr., Fang, R. H., Wei, X., Li, P., Wang, B., Zhang, L., & Gonzalez, D. J. (2017). Biomimetic virulomics for capture and identification of cell-type specific effector proteins. *ACS Nano, 11*(12), 11831–11838. doi:10.1021/acsnano.7b02650
22. Wei, X., Gao, J., Wang, F., Ying, M., Angsantikul, P., Kroll, A. V., . . . Zhang, L. (2017). *In situ* capture of bacterial toxins for antivirulence vaccination. *Advanced Materials, 29*(33), 1701644. doi:10.1002/adma.201701644
23. Dickey, S. W., Cheung, G. Y. C., & Otto, M. (2017). Different drugs for bad bugs: Antivirulence strategies in the age of antibiotic resistance. *Nature Reviews Drug Discovery, 16*(7), 457–471. doi:10.1038/nrd.2017.23
24. Chen, Y., Zhang, Y., Chen, M., Zhuang, J., Fang, R. H., Gao, W., & Zhang, L. (2019). Biomimetic nanosponges suppress *in vivo* lethality induced by the whole secreted proteins of pathogenic bacteria. *Small, 15*(6), 1804994. doi:10.1002/smll.201804994
25. Escajadillo, T., Olson, J., Luk, B. T., Zhang, L., & Nizet, V. (2017). A red blood cell membrane-camouflaged nanoparticle counteracts streptolysin O-mediated virulence phenotypes of invasive group A streptococcus. *Frontiers in Pharmacology, 8*, 477. doi:10.3389/fphar.2017.00477
26. Wei, X., Gao, J., Fang, R. H., Luk, B. T., Kroll, A. V., Dehaini, D., . . . Zhang, L. (2016). Nanoparticles camouflaged in platelet membrane coating as an antibody decoy for the treatment of immune thrombocytopenia. *Biomaterials, 111*, 116–123. doi:10.1016/j.biomaterials.2016.10.003
27. Copp, J. A., Fang, R. H., Luk, B. T., Hu, C. M. J., Gao, W., Zhang, K., & Zhang, L. (2014). Clearance of pathological antibodies using biomimetic nanoparticles. *Proceedings of the National Academy of Sciences of the United States of America, 111*(37), 13481-13486. doi:10.1073/pnas.1412420111
28. Zhang, Q., Dehaini, D., Zhang, Y., Zhou, J., Chen, X., Zhang, L., . . . Zhang, L. (2018). Neutrophil membrane-coated nanoparticles inhibit synovial inflammation and alleviate joint damage in inflammatory arthritis. *Nature Nanotechnology, 13*(12), 1182–1190. doi:10.1038/s41565-018-0254-4
29. Thamphiwatana, S., Angsantikul, P., Escajadillo, T., Zhang, Q. Z., Olson, J., Luk, B. T., . . . Zhang, L. (2017). Macrophage-like nanoparticles concurrently

absorbing endotoxins and proinflammatory cytokines for sepsis management. *Proceedings of the National Academy of Sciences of the United States of America, 114*(43), 11488–11493. doi:10.1073/pnas.1714267114
30. Wei, X., Zhang, G., Ran, D., Krishnan, N., Fang, R. H., Gao, W., . . . Zhang, L. (2018). T-cell-mimicking nanoparticles can neutralize HIV infectivity. *Advanced Materials, 30*(45), 1802233. doi:10.1002/adma.201802233
31. Rao, L., Wang, W., Meng, Q. F., Tian, M., Cai, B., Wang, Y., . . . Wu, J. (2018). A biomimetic nanodecoy traps Zika virus to prevent viral infection and fetal microcephaly development. *Nano Letters, 19*(4), 2215–2222. doi:10.1021/acs.nanolett.8b03913
32. Garg, H., & Joshi, A. (2019). Multivalent, virus-like particle vaccine and reporter virus assay for arboviruses. *Journal of the Homeland Defense & Security Information Analysis Center, 6*(1), 32–37. Retrieved from https://www.hdiac.org/wp-content/uploads/Multivalent-Virus-Like-Particle-Vaccine-and-Reporter-Virus-Assay-for-Arboviruses_V6I1.pdf
33. Pang, Z. Q., Hu, C. M. J., Fang, R. H., Luk, B. T., Gao, W., Wang, F., . . . Zhang, L. (2015). Detoxification of organophosphate poisoning using nanoparticle bioscavengers. *ACS Nano, 9*(6), 6450–6458. doi:10.1021/acsnano.5b02132
34. Bu, Y. S., Hu, Q., Ke, R. F., Sui, Y., Xie, X. Y., & Wang, S. C. (2018). Cell membrane camouflaged magnetic nanoparticles as a biomimetic drug discovery platform. *Chemical Communications, 54*(95), 13427–13430. doi:10.1039/c8cc08530g
35. Sherwood, J., Sowell, J., Beyer, N., Irvin, J., Stephen, C., Antone, A. J., . . . Ciesla, L. M. (2019). Cell-membrane coated iron oxide nanoparticles for isolation and specific identification of drug leads from complex matrices. *Nanoscale, 11*(13), 6352–6359. doi:10.1039/c9nr01292c
36. Angsantikul, P., Fang, R. H., & Zhang, L. (2018). Toxoid vaccination against bacterial infection using cell membrane-coated nanoparticles. *Bioconjugate Chemistry, 29*(3), 604–612. doi:10.1021/acs.bioconjchem.7b00692
37. Wang, F., Fang, R. H., Luk, B. T., Hu, C. J., Thamphiwatana, S., Dehaini, D., . . . Zhang, L. (2016). Nanoparticle-based antivirulence vaccine for the management of methicillin-resistant *Staphylococcus aureus* skin infection. *Advanced Functional Materials, 26*(10), 1628–1635. doi:10.1002/adfm.201505231
38. Wei, X., Beltran-Gastelum, M., Karshalev, E., Esteban-Fernandez de Avila, B., Zhou, J., Ran, D., . . . Zhang, L. (2019). Biomimetic micromotor enables active delivery of antigens for oral vaccination. *Nano Letters, 19*(3), 1914–1921. doi:10.1021/acs.nanolett.8b05051
39. Geisler, K. E., Elliott, T. C., & Saindon, J. M. (2019, January). Microbiological weapons: A potential consequence of CRISPR/Cas9 technologies. Homeland Defense & Security Information Analysis Center. Retrieved from https://www.hdiac.org/wp-content/uploads/HDIAC-Spotlight_Microbiological-Weapons-A-Potential-Consequence-of-CRISPR-Cas9-Technologies_January-2019-1.pdf
40. Dehaini, D., Wei, X., Fang, R. H., Masson, S., Angsantikul, P., Luk, B. T., . . . Zhang, L. (2017). Erythrocyte-platelet hybrid membrane coating for enhanced nanoparticle functionalization. *Advanced Materials, 29*(16), 1606209. doi:10.1002/adma.201606209

Ronnie H. Fang, Ph.D.
Assistant Project Scientist, University of California San Diego

Ronnie H. Fang is an assistant project scientist in the Department of NanoEngineering at the University of California San Diego. He received his Ph.D. in nanoengineering at the University of California San Diego. His research is focused on leveraging biomimetic nanoparticles for drug delivery and immunoengineering applications.

Liangfang Zhang, Ph.D.
Professor, University of California San Diego

Liangfang Zhang is a professor in the Department of NanoEngineering and Moores Cancer Center at the University of California San Diego. He received his Ph.D. in chemical and biomolecular engineering at the University of Illinois at Urbana-Champaign. His research aims to create cutting-edge biomimetic nanotechnologies and exploit them for various biomedical applications with a particular focus on drug delivery, biodetoxification, and vaccination.