Published in final edited form as:

Adv Mater. 2015 June 10; 27(22): 3437–3443. doi:10.1002/adma.201501071.

Hydrogel Retaining Toxin-Absorbing Nanosponges for Local Treatment of Methicillin-Resistant *Staphylococcus aureus* Infection

Fei Wang,

Department of NanoEngineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

Department of Pharmaceutics, School of Pharmacy, Fudan University, and Key Laboratory of Smart Drug Delivery (Fudan University), Ministry of Education, Shanghai 201203, P. R. China

Weiwei Gao,

Department of NanoEngineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

Soracha Thamphiwatana,

Department of NanoEngineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

Brian T. Luk,

Department of NanoEngineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

Pavimol Angsantikul,

Department of NanoEngineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

Qiangzhe Zhang,

Department of NanoEngineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

Che-Ming J. Hu,

Department of NanoEngineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

Ronnie H. Fang,

Department of NanoEngineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

Jonathan A. Copp,

Department of NanoEngineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

Dissaya Pornpattananangkul,

*; Email: zhang@ucsd.edu, Tel: +1-858-246-0999

Department of Chemistry, Faculty of Science, Chulalongkorn University, Pathumwan, Bangkok, Thailand

Weiyue Lu, and

Department of Pharmaceutics, School of Pharmacy, Fudan University, and Key Laboratory of Smart Drug Delivery (Fudan University), Ministry of Education, Shanghai 201203, P. R. China

Liangfang Zhang*

Department of NanoEngineering and Moores Cancer Center, University of California, San Diego, La Jolla, CA 92093, USA

Keywords

hydrogel; nanosponge; detoxification; pore-forming toxin; MRSA infection

Targeting virulence factors such as bacterial toxins represents an attractive antimicrobial approach with potential advantages of expanding the repertoire of bacterial targets, preserving the host endogenous microbiome, and lowering selective pressure for resistance development.^[1, 2] Among various toxins, pore-forming toxins (PFTs) are the most common class of bacterial protein toxins and constitute important bacterial virulence factors.^[3] These toxins disrupt cells by forming pores on cellular membranes and altering their permeability for bioactivity.^[4] However, the majority of current toxin targeting strategies, such as antisera,^[5] monoclonal antibodies,^[6, 7] small-molecule inhibitors,^[8, 9] and molecularly imprinted polymers,^[10] relies primarily on structure-specific epitopic binding and custom synthesis is required to match specific toxins. As a result, the enormous diversity of PFTs presents a serious challenge to devise an effective detoxification platform against bacterial infections. To address this challenge, a unique red blood cell (RBC) membrane-coated nanoparticle system has been recently developed by wrapping intact RBC membrane onto polymeric nanoparticles (denoted ‘nanosponges’) for broad detoxification applications.^[11, 12] The term ‘nanosponges’ is used to describe the unique capability of the RBC membrane-coated nanoparticles for non-specifically ‘soaking up’ a broad spectrum of PFTs. Different from existing detoxification strategies, the nanosponges target the membrane-disrupting mechanism common to PFTs; thereby offering an all-purpose toxin decoy strategy to absorb various types of PFTs regardless of their molecular structures.^[12]

Meanwhile, on the front of nanotechnology development, therapeutic nanoparticles are increasingly combined with other biomaterials to form hybrid nanostructures for improved therapeutic index. Particularly, loading nanoparticles into hydrogels have received much attention.^[13] Hydrogels are hydrophilic 3D polymer networks with extensive uses in tissue engineering and drug delivery. With appropriate compositions, hydrogels can not only preserve the structural integrity and the functionalities of the contained nanoparticles but also offer additional engineering flexibility to improve the therapeutic efficacy. For example, biopolymers were linked together by selective adsorption to nanoparticles, forming self-assembled hydrogels with shear-thinning and self-healing properties.^[14] Modifying hydrogel composition has also allowed for controllable nanoparticle release and tunable viscoelasticity suitable for topical applications.^[15, 16] Hydrogels made from responsive

polymer matrices have also been attempted to load nanoparticles for controlled releases triggered by environmental cues such as pH, ionic strength, and temperature.^[17, 18] Recently, polymeric nanoparticles were also loaded into liver lobule-mimicking hydrogel for toxin entrapment.^[19] Inspired by the numerous advantages of hydrogels in retaining nanoparticles and enhancing their properties, herein, we report on an advanced hybrid nanoformulation that integrates toxin nanosponge with hydrogel for local treatment of bacterial infection. Such nanosponge-hydrogel formulation judiciously integrates two distinct materials into one robust hybrid system with unique physicochemical and biological properties that either one of the two building blocks cannot achieve independently.

Among various bacterial pathogens, *Staphylococcus aureus* (*S. aureus*) is a prominent Gram-positive bacterium and the leading cause for a vast range of human skin and wound infections.^[20, 21] Over the past a few decades, *S. aureus* has experienced several waves of antibiotic resistance and now displays broad resistance to the entire beta-lactam class of antibiotics, including penicillins, cephalosporins, and carbapenems.^[22] To keep up the pace of antibiotic resistance, new antibiotics including vancomycin, linezolid, tedezolid, daptomycin, ceftaroline, and tigecycline have been developed and introduced in recent years. However, emergence of resistant *S. aureus* strains, especially toward vancomycin, has significantly increased.^[23] Currently, virulent strains of methicillin-resistant *S. aureus* (MRSA) have become increasingly prevalent, imposing a paramount clinical challenge that threatens public health.^[24-26] Collectively, these facts underscore the undisputed and urgent need to develop new and effective therapeutic approaches for MRSA treatment.

Notably, MRSA infections are commonly localized to skin and soft tissues.^[27] In these infections, a critical element of virulence results from a diverse arsenal of PFTs secreted by the bacteria, which attack the host cells.^[28] These distinctive features of MRSA infections make the nanosponge-hydrogel hybrid formulation an attractive treatment strategy against such infections (Figure 1A). The hydrogel composition can be optimized to effectively retain nanosponges within its matrix without compromising toxin transport for neutralization. In the study, we confirm the *in vitro* and *in vivo* toxin neutralization capabilities of the nanosponge-hydrogel formulation. When injected *in situ*, the hydrogel can effectively retain nanosponges at the injection sites, a property favoring toxin absorption. In a subcutaneous MRSA mouse model, the hybrid formulation shows significant anti-virulence therapeutic efficacy, evidenced by the markedly reduced MRSA skin lesion development. Overall, we demonstrate the potential of the nanosponge-loaded hydrogel as a new and effective detoxification strategy for the treatment of localized MRSA infection.

The preparation of nanosponge-loaded hydrogel (denoted “NS-gel”) was divided into two steps. In the first step, we prepared nanosponges by mechanically extruding purified mouse RBC membrane with 100 nm of poly(lactic-co-glycolic acid) (PLGA) polymeric cores. Following membrane fusion, the diameter of the nanoparticles increased from 72.7 ± 0.2 nm to 88.4 ± 0.3 nm, corresponding to the addition of a bilayered RBC membrane onto the polymeric cores. Meanwhile, the surface zeta potential changed from -27.7 ± 1.1 mV to -13.9 ± 0.9 mV, which was likely due to charge screening by RBC membranes (Figure 2B). The samples were then stained with uranyl acetate and visualized by transmission electron microscopy (TEM). Figure 2C shows that all nanosponges had a spherical core-shell

structure, in which the PLGA core was wrapped by a thin shell. Collectively, these results confirmed the successful coating of RBC membranes onto the PLGA cores.^[11, 29] In the second step, we mixed pre-formed nanosponges with acrylamide as the monomer and poly(ethylene glycol) dimethacrylate (PEGDMA) as the cross-linker. Hydrogelation was initiated by adding ammonium persulfate and tetramethylethylenediamine (TEMED), and allowed to proceed for 12 h at room temperature.^[16]

The hydrogel composition was optimized for effective nanosponge retention while maintaining a low viscosity suitable for injection. To this end, we first labeled the nanosponges with 1,1'-dioctadecyl-3,3,3',3' tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt (DiD) (excitation/emission=644 nm/655 nm), a hydrophobic fluorophore with negligible leakage from PLGA polymer matrix.^[11, 30] Then we fixed the concentrations of nanosponges, acrylamide, ammonium persulfate, and TEMED as 2 mg/mL (PLGA content), 40 mg/mL, 1 mg/mL and 1 μ L/mL, respectively, but varied PEGDMA concentrations and accordingly examined the nanosponge release from the corresponding hydrogels. As shown in Figure 1D, the accumulated release of nanosponge over 24 h decreased abruptly from approximately 53% at 0.5 (w/v)% crosslinker concentration to no more than 5% at 0.6 (w/v)%, suggesting that the latter PEGDMA concentration was adequate in forming a hydrogel for effectively retaining nanosponges. This crosslinker concentration was used to prepare NS-gel for the following studies.

The NS-gel was further characterized with dynamic rheological measurements of the storage modulus (G') and the loss modulus (G'') as a function of frequency (Figure 1E). In the study, G' exceeded G'' over the entire frequency range, a clear viscoelastic behavior indicating the formation of a hydrogel network.^[16] We observed slightly higher values of G' and G'' at high frequencies for the NS-gel formulation as compared to those of the empty hydrogel (without nanosponges), which is likely due to adhesive interactions between the nanosponges and the acrylamide polymer matrix.^[15, 31] Nevertheless, G' and G'' values measured from NS-gel were generally close to those measured from the empty hydrogel, suggesting a negligible effect of the loaded nanosponges on the gel's rheological characteristics. We then freeze-dried the NS-gel and observed its structure under scanning electron microscope (SEM). The sample showed characteristic porous sponge-like structures with some irregular lamellar features (Figure 1F). At a higher magnification, nanosponge particles with a diameter of approximately 100 nm embedded within the hydrogel were also observed (Figure 1F, insert).

We then compared the ability of the NS-gel in absorbing α -toxin to that of the empty gel. When α -toxin solution was added to the empty gel of an equal volume, a gradual decrease of toxin concentration was observed within the first 1 h of incubation until it reached approximately 50% of the original concentration (Figure 1G). No further decrease of the toxin concentration was observed with prolonged incubation time, suggesting a slow diffusion process of α -toxin into the hydrogel network until equilibrium was reached. In contrast, when α -toxin solution was incubated with the NS-gel, a sharp decrease of toxin concentration was observed within the first 30 min of incubation. After 1 h, no more than 5% of the initial toxin was left in the solution. Such significantly increased ability of the NS-gel in absorbing α -toxin suggests its potential for detoxification applications.

To test the ability of the NS-gel to neutralize PFTs, we used a hemolytic assay where α -toxin was mixed with the NS-gel and then added to purified mouse RBCs.^[12] PBS and the empty gel were tested in parallel as controls. As shown in Figure 2A, NS-gel sample showed a clear supernatant, suggesting the RBCs were undamaged. In contrast, noticeable differences were observed with other samples, which showed significant RBC lysis. The degree of hemolysis was quantified by measuring the absorbance of the released hemoglobin in the supernatant at 540 nm (Figure 2B). The capability of the NS-gel to absorb toxins secreted by MRSA bacteria was further examined by mixing the NS-gel with MRSA culture medium known to contain multiple virulent PFTs.^[32, 33] As shown in Figure 2C and D, a similar reduction of RBC hemolysis by the NS-gel was observed as compared to the control groups, demonstrating the NS-gel's applicability in neutralizing multiple types of RBC membrane-targeted PFTs secreted by the bacteria.

Next, we evaluated the retention of the nanosponges by the hydrogel upon *in vivo* administration. In the study, we formulated the NS-gel with DiD-labeled nanosponges and injected the NS-gel subcutaneously on the left flank of the mice. As a control, the same amount of nanosponges suspended in PBS was injected to the right flank of the same mice. For both groups, the whole body imaging revealed the confinement of fluorescence at the injection sites within 48 h (Figure 3A). However, a more rapid decay of fluorescence intensity was observed at the site injected with nanosponges suspended in PBS, indicating a faster loss of nanoparticles through diffusion to surrounding tissues. Quantification of the fluorescence intensity showed that nearly 80% of the free nanosponges diffused away from the injection site within 2 h. In contrast, the NS-gel had negligible loss of the nanosponge payloads within the initial 2 h and only lost approximately 20% of the total nanosponge during the 48 h testing period (Figure 3B). This study, together with the previous *in vitro* nanosponge release results (Figure 1B), clearly demonstrated the prolonged retention of the nanosponges conferred by the hydrogel formulation. These results further indicate that the NS-gel could be a competent formulation for the treatment of local bacterial infection, in which the pathogens reside on a localized area of a tissue.

The ability of the NS-gel to neutralize α -toxin was further examined *in vivo* by subcutaneous injection of α -toxin (50 μ L at a concentration of 40 μ g/mL in PBS) immediately followed by injecting empty gel or NS-gel (100 μ L), respectively, beneath the right flank skin of mice. For the mice treated with empty gel, 72 h after the injection obvious skin lesions were induced with demonstrable oedema and inflammation (Figure 4A). Closer examination of the skin tissue showed typical indications of toxin-induced damages, including necrosis, apoptosis and inflammatory infiltrate of neutrophils with dermal oedema (Figure 4B).^[12, 34] Moreover, the toxin damaged the underlying muscle tissue, as indicated by interfibril oedema, tears on muscles fibres and a significant number of extravasating neutrophils from the surrounding vasculature (Figure 4C).^[12] However, mice treated with NS-gel showed no observable damage on the skin (Figure 4D). The tissue samples showed normal epithelial structures in skin histology (Figure 4E) and intact fibrous structures with no visible infiltrate in muscle histology (Figure 4F).

Finally, we proceeded to examine the *in vivo* anti-MRSA efficacy of the NS-gel. To establish a subcutaneous MRSA infection model, we injected 1×10^9 CFU MRSA bacteria under the

flank skin of each ICR mouse.^[34] The infected mice were randomly divided into two groups ($n = 9$) and then injected at the infection sites with the NS-gel and empty gel, respectively. Herein, to evaluate therapeutic efficacy, we chose to compare the size of MRSA-induced skin lesion observable on the superficial layer of the epidermis, a common index reflecting the severity of the infection.^[34–36] After 24 h, mice in both groups developed visible skin lesions but the size difference was non-significant (Figure 5). At 48 h, the lesion sizes increased in both groups; however, the mice treated with the NS-gel showed a significantly smaller skin lesion when compared to those treated with the empty gel. At 72 h and 96 h, the lesion sizes continued to increase in both groups as the infection continued to progress. The lesion size of the NS-gel treated group remained significantly smaller than that of the group treated with the empty-gel. As toxin neutralization by the nanosponges is dependent on both the dosage and dosing frequency, which need to be further optimized in order to completely eradicate MRSA-induced skin lesion. Nevertheless, a superior efficacy observed with the NS-gel demonstrates its potential as an effective local treatment regimen against MRSA infection.

Previously, we reported that RBC-membrane coated nanoparticles (*i.e.*, nanosponges) were effective in neutralizing PFTs including α -toxin, streptolysin-O, and melittin.^[12] The current study took this platform one important step further by directly demonstrating its effectiveness in treating MRSA infection in a mouse model. This advancement is enabled by the combined advantages of the nanosponges and the hydrogel: while the nanosponges absorbing and diverting PFTs away from the cellular targets, the hydrogel retained nanosponges at the infection sites, promoting localized toxin neutralization for better therapeutic efficacy. The observed anti-virulence efficacy reflects the synergy of such combination. With improved versatility and functionality, such advanced hybrid materials are expected to offer a practical and reliable formulation that facilitates downstream preclinical and clinical tests.

The rapid technological developments in nanotechnology and biomaterials provide a promising potential for engineering advanced materials through hybridization.^[37, 38] On one hand, nanoparticle platforms are gaining an increasing attention for broad-spectrum toxin neutralization.^[39, 40] For example, we have recently extended the nanosponges to neutralize other chemical and biological molecules that target RBCs such as pathological antibodies driving prevalent autoimmune diseases.^[30] Meanwhile, cell membrane coating technology has also been increasingly explored to functionalize various types of nanoparticles, including those made from gold,^[41, 42] silica,^[43] and gelatin,^[44] opening unprecedented capability to harness natural functionalities for innovative therapeutics. On the other hand, the technological advance has also led to the development of hydrogels featuring highly interactive, integrative, and biocompatible characteristics for increasingly advanced drug delivery and tissue engineering applications.^[37, 45–47] Integrating hydrogel with nanosponges, or cell membrane coated nanoparticles in general, is anticipated to result in advanced materials that harness the advantages of each building material. We believe that this design strategy will open unique opportunities for a wide range of therapeutic applications.

In summary, we developed an advanced hybrid material that integrates a unique toxin-absorbing nanosponge with hydrogel for anti-virulence treatment of local bacterial infection. The nanosponges were made by wrapping intact RBC membrane onto polymeric nanoparticles made from PLGA, followed by loading into hydrogels. The hydrogel composition was optimized to effectively retain nanosponges within its matrix without compromising toxin transport for neutralization. Following subcutaneous injection to mice, nanosponges were effectively retained at the injection sites. In a MRSA subcutaneous mouse model, we demonstrated for the first time that mice treated with the nanosponge-hydrogel hybrid showed markedly reduced MRSA skin lesion development. These results collectively indicate that the nanosponge-hydrogel hybrid formulation represents a new and effective detoxification strategy for the treatment of localized bacterial infection. Given the critical roles played by pore-forming toxins in pathogenesis of a wide range of bacteria, we expect the nanosponge-hydrogel formulation to be capable for treatment of infectious diseases caused by various types of bacteria. More importantly, no antibiotics are involved in this new treatment, which is therefore unlikely to be affected by the existing bacterial antibiotic-resistance mechanisms and will not exert selective pressure to bacteria for developing new resistance.

Experimental Section

Preparation of nanosponges

The nanosponge was prepared following previously described methods.^[11, 12] Briefly, poly(DL-lactide-co-glycolide) (PLGA) polymeric nanoparticles with a diameter of about 100 nm were prepared with 0.67 dL/g of carboxy-terminated 50:50 PLGA polymer (LACTEL Absorbable Polymers) through a nanoprecipitation process. Specifically, the PLGA polymer was first dissolved in acetone at a concentration of 10 mg/mL. Then 1 mL of the solution was added to 3 mL of deionized water. The mixture was then stirred in open air for 1 h and placed in vacuum for another 3 h. The resulting nanoparticle solution was filtered using an Amicon Ultra-4 centrifugal filter with a molecular weight cutoff of 10 kDa (Millipore). To prepare fluorescently labeled nanosponges, 0.1 wt% 1,1'-diiododecyl-3,3',3'-tetramethylindodicarbocyanine perchlorate (DiD; excitation/emission = 644/665 nm; Life Technologies) was added to the polymers prior to PLGA nanoparticle synthesis. RBC membrane coating was then completed by fusing RBC membrane-derived vesicles with PLGA nanoparticles via sonication using an FS30D bath sonicator at a frequency of 42 kHz and a power of 100 W for 2 min. Dynamic light scattering (DLS) measurements on the resulting nanosponges (Malvern ZEN 3600 Zetasizer) showed an average hydrodynamic diameter of about 100 nm and 115 nm before and after the membrane coating process, respectively. The core-shell structure of the nanosponge was confirmed by using transmission electron microscope (TEM, FEI 200 kV Sphera microscope).

Preparation of nanosponge-loaded hydrogel (NS-gel)

To prepare hydrogel, acrylamide (used as the monomer), poly(ethylene glycol) dimethacrylate (PEGDMA, used as the cross-linker), tetramethylethylene diamine (TEMED), and ammonium persulfate (both used as initiators) were purchased from Sigma-Aldrich. For the empty hydrogel, the final concentrations of acrylamide, ammonium

persulfate and TEMED were kept constant at 40 mg/mL, 1 mg/mL, and 1 μ L/mL, respectively, while PEGDMA concentration was tested in the range of 0 – 1% w/w. The liquid mixture was vortexed for 1 min and then placed in a vacuum chamber at room temperature for 2 h to allow complete gelation to occur. For NS-gel, nanosponges with a final concentration of 2 mg/mL were mixed the aforementioned chemicals followed by gelation.

To measure nanosponge release rate from the hydrogel, DiD dye-labeled nanosponges were used to prepare NS-gels at different PEGDMA concentrations. Then 0.5 mL NS-gel was submerged into 50 mL PBS. The mixture was incubated at 37°C for 24 h and the DiD signal from the supernatant was measured. The rheological analysis was carried out at $37 \pm 0.1^\circ\text{C}$ on a strain-controlled AR-G2 rheometer with 22 mm diameter parallel-plate geometry (TA Instruments Inc., New Castle, DE). Oscillatory rheological measurements were performed in the linear viscoelastic regime. The strain was kept at 0.1% and a dynamic frequency sweep from 0.1 to 10 rad/s was conducted to measure the storage modulus G' and loss modulus G'' . To study hydrogel morphology, NS-gel was lyophilized and the flakes of the gel were placed on a silicon wafer. The samples were coated with iridium and then examined with scanning electron microscope (SEM). Absorption of α -toxin by NS-gel was examined by incubating 2 mL α -toxin (2 μ g/mL) with 0.5 mL NS-gel at 37 °C for 24 h. Empty hydrogel was used as a control group. At 4, 8, and 24 h time points, α -toxin concentration in the supernatants was quantified with ELISA (R&D Systems, Inc., Minneapolis, MN).

In vitro α -toxin neutralization study

To evaluate α -toxin neutralization by NS-gel *in vitro*, a hemolytic assay was conducted. Briefly, 3 μ L of α -toxin solution (1 mg/mL) was mixed with 0.5 mL NS-gel, empty hydrogel, and PBS, respectively. Then 1 mL of 5% purified mouse RBCs was added to the top of each sample, followed by incubation at 37°C for 1 h. Then the samples were carefully centrifuged. The extent of RBC lysis was quantified by measuring the absorption of the supernatants at 540 nm. To neutralize toxins in bacterial supernatant, MRSA culture (from an overnight culture of 1×10^9 CFU/mL of MRSA 252) was used to dissolve the chemicals for the hydrogel preparation. All experiments were performed in triplicate.

Live whole-body imaging of mice to study nanosponge retention

Prior to the study, the back of the mice (6 week-old male ICR mice from Charles River Laboratories, n=3) was carefully shaved. Then 50 μ L DiD labeled NS-gel was injected subcutaneously to the left flanks of the mice. As a control, nanosponges suspended in PBS (2 mg/mL, 50 μ L) were injected subcutaneously to the right flanks the same mice. At the designated time points (0, 6, 24, and 48 h), mice were anesthetized with isoflurane and imaged with a Xenogen IVIS 200 system. Fluorescence intensities were quantified and normalized across the time points. Heat maps were overlaid on bright field images.

In vivo α -toxin neutralization study

Neutralization of α -toxin by the NS-gel was conducted by subcutaneously injecting 50 μ L of 40 μ g/mL of α -toxin solution into the flank region of 6 week-old male ICR mice (Charles River Laboratories, n=3), immediately followed by injecting 100 μ L empty gel or NS-gel

into the same region. After 72 h the mice were imaged. Then the mice were sacrificed and the skin and muscle samples were removed. The tissues were frozen, cut, and then stained with hematoxylin and eosin (H&E) for histological analysis.

In vivo detoxification efficacy against localized MRSA infection

To evaluate *in vivo* detoxification efficacy of the NS-gel against MRSA infection, a MRSA subcutaneous infection mouse model was used. Briefly, prior to the study, the back of 18 ICR mice (6 week-old male, Charles River Laboratories) was carefully shaved. Then 1×10^9 CFU of MRSA 252 suspended in 50 μ L PBS was injected subcutaneously into the flank region. Then the mice were randomly divided into two groups (n=9). For the treatment group, 0.2 mL NS-gel was injected into the infection region. For the control group, the empty gel instead of NS-gel was injected. The lesion of each mouse was carefully monitored. The lesion was photographed and the lesion size was measured from the photography by using Image J.

Acknowledgements

This work is supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health under Award Number R01DK095168.

References

1. Clatworthy AE, Pierson E, Hung DT. Nat. Chem. Biol. 2007; 3:541. [PubMed: 17710100]
2. Rasko DA, Sperandio V. Nat. Rev. Drug Discov. 2010; 9:117. [PubMed: 20081869]
3. Los FCO, Randis TM, Aroian RV, Ratner AJ. Microbiol. Mol. Biol. Rev. 2013; 77:173. [PubMed: 23699254]
4. Gilbert RJC. Cell. Mol. Life Sci. 2002; 59:832. [PubMed: 12088283]
5. Beghini DG, Hernandez-Oliveira S, Rodrigues-Simioni L, Novello JC, Hyslop S, Marangoni S. Toxicon. 2004; 44:141. [PubMed: 15246761]
6. Cheng LW, Henderson TD II, Patfield S, Stanker LH, He X. Toxins. 2013; 5:1845. [PubMed: 24152988]
7. Oganessian V, Peng L, Damschroder MM, Cheng L, Sadowska A, Tkaczyk C, Sellman BR, Wu H, Dall'Acqua WF. J. Biol. Chem. 2014; 289:29874. [PubMed: 25210036]
8. Hung DT, Shakhnovich EA, Pierson E, Mekalanos JJ. Science. 2005; 310:670. [PubMed: 16223984]
9. McCormick CC, Caballero AR, Balzli CL, Tang A, O'Callaghan RJ. Invest. Ophthalmol. Vis. Sci. 2009; 50:2848. [PubMed: 19136695]
10. Hoshino Y, Koide H, Furuya K, Haberaecker WW III, Lee S-H, Kodama T, Kanazawa H, Oku N, Shea KJ. Proc. Natl. Acad. Sci. USA. 2012; 109:33. [PubMed: 22198772]
11. Hu C-MJ, Zhang L, Aryal S, Cheung C, Fang RH, Zhang L. Proc. Natl. Acad. Sci. USA. 2011; 108:10980. [PubMed: 21690347]
12. Hu C-MJ, Fang RH, Copp J, Luk BT, Zhang L. Nat. Nanotechnol. 2013; 8:336. [PubMed: 23584215]
13. Schexnailder P, Schmidt G. Colloid. Polym. Sci. 2009; 287:1.
14. Appel EA, Tibbitt MW, Webber MJ, Mattix BA, Veiseh O, Langer R. Nat. Commun. 2015; 6 article number 6295.
15. Sekine Y, Moritani Y, Ikeda-Fukazawa T, Sasaki Y, Akiyoshi K. Adv. Healthc. Mater. 2012; 1:722. [PubMed: 23184823]
16. Gao W, Vecchio D, Li J, Zhu J, Zhang Q, Fu V, Li J, Thamphiwatana S, Lu D, Zhang L. ACS Nano. 2014; 8:2900. [PubMed: 24483239]

17. Ramanan RMK, Chellamuthu P, Tang LP, Nguyen KT. *Biotechnol. Prog.* 2006; 22:118. [PubMed: 16454501]
18. Xiang Y, Chen D. *Eur. Polym. J.* 2007; 43:4178.
19. Gou M, Qu X, Zhu W, Xiang M, Yang J, Zhang K, Wei Y, Chen S. *Nat. Commun.* 2014; 5 article number 3774.
20. Kuehnert MJ, Kruszon-Moran D, Hill HA, McQuillan G, McAllister SK, Fosheim G, McDougal LK, Chaitram J, Jensen B, Fridkin SK, Killgore G, Tenover FC. *J. Infect. Dis.* 2006; 193:172. [PubMed: 16362880]
21. Singer AJ, Talan DA. *New Engl. J. Med.* 2014; 370:1039. [PubMed: 24620867]
22. Chambers HF, DeLeo FR. *Nat. Rev. Microbiol.* 2009; 7:629. [PubMed: 19680247]
23. Weigel LM, Clewell DB, Gill SR, Clark NC, McDougal LK, Flannagan SE, Kolonay JF, Shetty J, Killgore GE, Tenover FC. *Science.* 2003; 302:1569. [PubMed: 14645850]
24. Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, Fukuchi Y, Kobayashi I. *Lancet.* 1997; 350:1670. [PubMed: 9400512]
25. Klevens RM, Edwards JR, Tenover FC, McDonald LC, Horan T, Gaynes R. S. *Natl Nosocomial Infections. Clin. Infect. Dis.* 2006; 42:389. [PubMed: 16392087]
26. McKenna M. *Nature.* 2012; 482:23. [PubMed: 22297951]
27. Gordon RJ, Lowy FD. *Clin. Infect. Dis.* 2008; 46:S350. [PubMed: 18462090]
28. Gorwitz RJ. *J. Infect. Dis.* 2008; 197:179. [PubMed: 18173362]
29. Hu C-MJ, Zhang L. *Nano Today.* 2014; 9:401. [PubMed: 25285152]
30. Copp JA, Fang RH, Luk BT, Hu C-MJ, Gao W, Zhang K, Zhang L. *Proc. Natl. Acad. Sci. USA.* 2014; 111:13481. [PubMed: 25197051]
31. Thevenot C, Khoukh A, Reynaud S, Desbrieres J, Grassl B. *Soft Matter.* 2007; 3:437.
32. Wang R, Braughton KR, Kretschmer D, Bach T-HL, Queck SY, Li M, Kennedy AD, Dorward DW, Klebanoff SJ, Peschel A, DeLeo FR, Otto M. *Nat. Med.* 2007; 13:1510. [PubMed: 17994102]
33. Li M, Cheung GYC, Hu J, Wang D, Joo H-S, DeLeo FR, Otto M. *J. Infect. Dis.* 2010; 202:1866. [PubMed: 21050125]
34. Yeaman MR, Filler SG, Chaili S, Barr K, Wang H, Kupferwasser D, Hennessey JP, Fu Y, Schmidt CS, Edwards JE Jr, Xiong YQ, Ibrahim AS. *Proc. Natl. Acad. Sci. USA.* 2014; 111:E5555. [PubMed: 25489065]
35. Guo Y, Ramos RI, Cho JS, Donegan NP, Cheung AL, Miller LS. *Antimicrob. Agents Chemother.* 2013; 57:855. [PubMed: 23208713]
36. Das D, Tulkens PM, Mehra P, Fang E, Prokocimer P. *Clin. Infect. Dis.* 2014; 58:S51. [PubMed: 24343833]
37. Kopecek J, Yang J. *Angew. Chem. Int. Ed. Engl.* 2012; 51:7396. [PubMed: 22806947]
38. Kanie K, Kurimoto R, Ebara M, Idota N, Narita Y, Honda H, Kato R. *J. Tissue Eng. Regen. Med.* 2014; 8:220.
39. Forster V, Signorell RD, Roveri M, Leroux J-C. *Sci. Transl. Med.* 2014; 6 article number 258ra141.
40. Weisman A, Chen YA, Hoshino Y, Zhang H, Shea K. *Biomacromolecules.* 2014; 15:3290. [PubMed: 25093686]
41. Gao W, Hu C-MJ, Fang RH, Luk BT, Su J, Zhang L. *Adv. Mater.* 2013; 25:3549. [PubMed: 23712782]
42. Piao J-G, Wang L, Gao F, You Y-Z, Xiong Y, Yang L. *ACS Nano.* 2014; 8:10414. [PubMed: 25286086]
43. Parodi A, Quattrocchi N, van de Ven AL, Chiappini C, Evangelopoulos M, Martinez JO, Brown BS, Khaled SZ, Yazdi IK, Vittoria Enzo M, Isenhardt L, Ferrari M, Tasciotti E. *Nat. Nanotechnol.* 2013; 8:61. [PubMed: 23241654]
44. Li L-L, Xu J-H, Qi G-B, Zhao X, Yu F, Wang H. *ACS Nano.* 2014; 8:4975. [PubMed: 24716550]
45. Burdick JA, Murphy WL. *Nat. Commun.* 2012; 3 article number 1269.
46. Li Y, Rodrigues J, Tomas H. *Chem. Soc. Rev.* 2012; 41:2193. [PubMed: 22116474]
47. Hoffman AS. *Adv. Drug Del. Rev.* 2012; 64:18.

Figure 1. Formulation and characterization of nanosponge-loaded hydrogel (NS-gel)

(A) Schematic illustration of a hydrogel retaining toxin-absorbing nanospheres for local treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infection. The toxin nanosponge was constructed with a polymeric core wrapped in natural red blood cell (RBC) bilayer membrane and was subsequently embedded into an acrylamide-based hydrogel. (B) Hydrodynamic size (diameter, nm) and surface zeta potential (ζ , mV) of PLGA polymeric cores before and after RBC membrane coating measured by dynamic light scattering (DLS). (C) A representative transmission electron microscopy (TEM) image showing the spherical core-shell structure of the nanospheres under negative staining with uranyl acetate. The scale bar represents 50 nm. (D) Release of the toxin nanosponge from hydrogels made with different crosslinker concentrations. Error bars represent the standard deviations ($n=3$). (E) Rheological characterization of the hydrogel (0.6 wt% crosslinker) either without nanospheres (open markers) or loaded with 2 mg/mL nanospheres (solid markers). The storage modulus G' and loss modulus G'' were plotted logarithmically against frequency (0.1–10 Hz at 37°C). (F) A representative scanning electron microscopy (SEM) image of the NS-gel. The scale bar represents 1 μ m. (G) Absorption of α -toxin was studied by incubating 1 mL α -toxin solution (2 μ g/mL in PBS) with 1 mL NS-gel or empty gel. The concentrations of α -toxin in the supernatant at different incubation times were quantified using ELISA. Error bars represent standard deviations ($n = 3$).

Figure 2. *In vitro* toxin neutralization

(A) Centrifuged RBCs after incubation with α -toxin mixed in PBS, empty gel, and NS-gel, respectively. (B) Hemolysis quantification of the samples in (A). (C) Centrifuged RBCs after incubation with MRSA-culturing medium mixed with PBS, empty gel, and NS-gel, respectively. (D) Hemolysis quantification of the samples in (D). Error bars represent standard deviations ($n = 3$).

Figure 3. *In vivo* nanosponge retention by hydrogel

Nanosponges labeled with DiD fluorescent dye was used to formulate NS-gel, which was then injected subcutaneously under the loose skin over the left flank of the mice. Free suspended nanosponges (without hydrogel) were injected as a control group at the right flank of the same mice. (A) Fluorescence images taken at different time points show the retention of the nanosponges under mouse skin. (B) Quantification of the fluorescence intensity as observed in (A). All images are representative of 3 mice per group and the error bars represent the standard deviation ($n = 3$).

Figure 4. *In vivo* toxin neutralization

A–C, Mice injected with α -toxin followed by empty gel. The dashed lines depict the approximate tissue-hydrogel boundary. **(A)** Skin lesions occurred 72 h following toxin injection. **(B)** Hematoxylin and eosin (H&E) stained histological sections revealed inflammatory infiltrate, apoptosis, necrosis and oedema in the epidermis. **(C)** Tears on muscle fibres, interfibril oedema and extravasation of neutrophils from surrounding vasculature indicate muscular damage. **D–F**, Mice injected with α -toxin followed by NS-gel. **(D)** No skin lesion occurred. **(E)** No abnormality was observed in the epidermis. **(F)** Normal muscle structure was observed. Scale bar represents 50 μ m, $n = 6$ for each group.

Figure 5. *In vivo* treatment of MRSA infection

1×10^9 CFU of MRSA 252 was mixed with 0.2 mL of 2 mg/mL NS-gel or empty gels, followed by subcutaneous injection under the loose skin on the back of the mice ($n = 9$ per group). Skin lesions were monitored and photographed on day 1 to 4 after the injections and the lesion sizes were measured. Bars represent median values. * $P < 0.05$, n.s.: not significant.