A Hydrogel-Based Localized Release of Colistin for Antimicrobial Treatment of Burn Wound Infection

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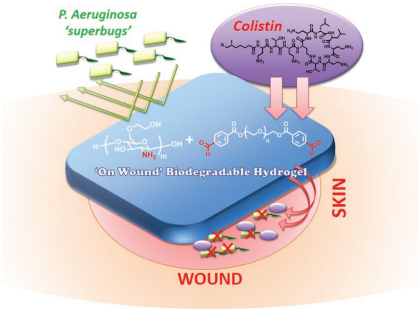
There is an urgent unmet medical need for new treatments for wound and burn infections caused by multidrug-resistant Gram-negative "superbugs," especially the problematic *Pseudomonas aeruginosa*. In this work, the incorporation of colistin, a potent lipopeptide into a self-healable hydrogel (via dynamic imine bond formation) following the chemical reaction between the amine groups present in glycol chitosan and an aldehyde-modified poly(ethylene glycol), is reported. The storage module (*G'*) of the colistin-loaded hydrogel ranges from 1.3 to 5.3 kPa by varying the amount of the cross-linker and colistin loading providing different options for topical wound healing. The majority of the colistin is released from the hydrogel within 24 h and remains active as demonstrated by both antibacterial in vitro disk diffusion and time-kill assays. Moreover and pleasingly, the colistin-loaded hydrogel performs almost equally well as native colistin against both the colistin-sensitive and also colistin-resistant *P. aeruginosa* strain in the in vivo animal "burn" infection model despite exhibiting a slower killing profile in vitro. Based on this antibiotic performance along with the biodegradability of the product, it is believed the colistin-loaded hydrogel to be a potential localized wound-healing formulation to treat burn wounds against microbial infection.



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1. Introduction

In view of the staggering annual costs of wound care in Australia ($2.6 billion AUD), the United Kingdom (£2.3–3.1 billion), and the United States ($50 billion USD),[1] the development of an antibiotic wound care patch for the treatment of wound and burn infections caused by multidrug-resistant (MDR) "superbugs" will undoubtedly result in improved health care on a global scale. Chronic wound infections remain a major source of morbidity and severe pain in burn patients.[2] The emergence of MDR Gram-negative "superbugs" threatens to undermine the efficacy of treatments in both the developed and developing world.[3] *P. aeruginosa*, for example, is a particularly problematic pathogen that is of concern worldwide.[2b,3d,4] These problematic bacteria can easily spread within confined environments such as hospitals to immunodeficient

patients,[2b,4b] making the treatment of these patients more complex and problematic. Polymyxins remain one of the effective treatment options for MDR Gram-negative infections.[5] The two clinically used polymyxins, polymyxin B and colistin (polymyxin E) specifically bind to the lipopolysaccharide (LPS) of the outer membrane of the Gram-negative bacteria and bacterial cell death through permeabilization of the bacterial outer membrane.[6] Systemic administration of polymyxins remains limited due to their high incidence of nephrotoxicity.[7] To date, several approaches have been developed to reduce this toxicity.[8] It has been demonstrated that the localized application of colistin to the infection site can reduce toxicity. For example, the clinical treatment of cystic fibrosis lung infection patients by local delivery of colistin using nebulized and dry powder aerosolized formulations has proved encouraging.[9]

The incorporation of therapeutics in hydrogel formulations has been widely employed to facilitate and manage many wound-healing processes, particularly burn wounds.[10] Unlike other treatments, the application of hydrogels can prevent the loss of the body fluid, keep the wound surface hydrated, promote the healing process, and also act as a barrier against bacterial infections.[11] Properties such as degradability,[12] environmental responsiveness,[13] and self-healing[14] can be easily fine-tuned to support the curing process of specific wound types. Among these, chitosan-based hydrogels have attracted significant attention due to their unique properties, such as biocompatibility, biodegradability, and the ability to accelerate the healing of wounds in humans.[15]

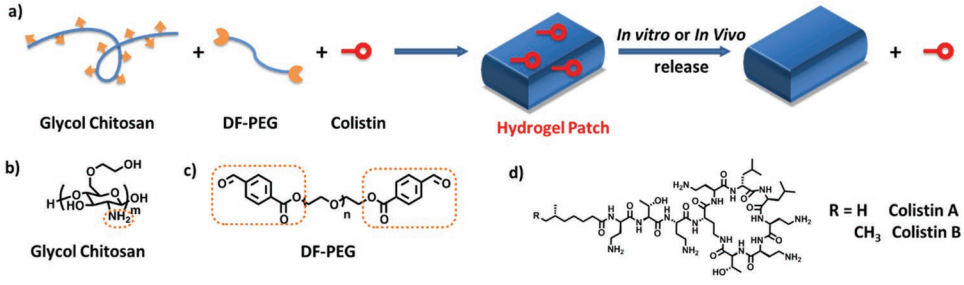
As colistin is a small lipopeptide antibiotic that is sensitive to many thermal, enzymatic, and chemical conditions, a hydrogel-based delivery platform with facile preparation, easy operation, mild gel formation environment, and high drug loading capacity could be a suitable candidate to deliver colistin in a local and effective manner to wounds. Recently, Wei and co-workers developed an injectable chitosan-based hydrogel through the utilization of dynamic imine bond chemistry, which exhibits high biocompatibility and allows for the controlled storage and release of proteins, and even cells.[16] An inexpensive hydrogel can be formed using commercially available glycol chitosan and an easily prepared low-toxic aldehyde-modified poly(ethylene glycol) derivative (DF-PEG),[16b] is a transparent material with self-healing properties, which can provide better monitoring of the wound through direct observation, and improved adhesion to the wound. The quick and efficient chemistry used allows this hydrogel to be formed either at room or body temperatures, which is convenient for topical delivery. More importantly, the starting materials of this hydrogel, as well as colistin, are highly water soluble, suggesting that colistin could be loaded and distributed into the system evenly during the gel formation.

In this present study, we have prepared dynamic glycol chitosan/DF-PEG hydrogels loaded with colistin. The effects of DF-PEG and colistin concentration on the gel material properties were investigated. The release profile of colistin was determined both in phosphate-buffered saline (PBS) and bacterial growth media by high-performance liquid chromatography (HPLC) analysis. Furthermore, the antibacterial activity of the released colistin was evaluated in vitro via disk diffusion and time-kill experiments. Proof-of-concept in vivo experiments were performed using a mouse "burn" infection model.

2. Results and Discussion

2.1. Gel Formation and Characterization

The synthesis of a colistin-loaded hydrogel was achieved by integrating colistin into a modification of an established hydrogel formulation (Scheme 1).[16b] Instead of the original protocol, PBS buffer was used as the solvent in an attempt to more closely mimic the wound environment. A standard hydrogel was prepared from mixing a 3% w/w glycol chitosan solution with either 5% or 10% (w/w) DF-PEG solution at a volume ratio of 2.8:1 to achieve the targeted strains. Colistin was added either to the glycol chitosan solution or to the DF-PEG solution, and after mixing the two solutions a transparent hydrogel was formed instantly (typically < 1.6 min).



Scheme 1. a) Illustration of the synthesis of colistin-containing hydrogel. The chemical structure of b) glycol chitosan, c) DF-PEG, and d) colistin A and B.

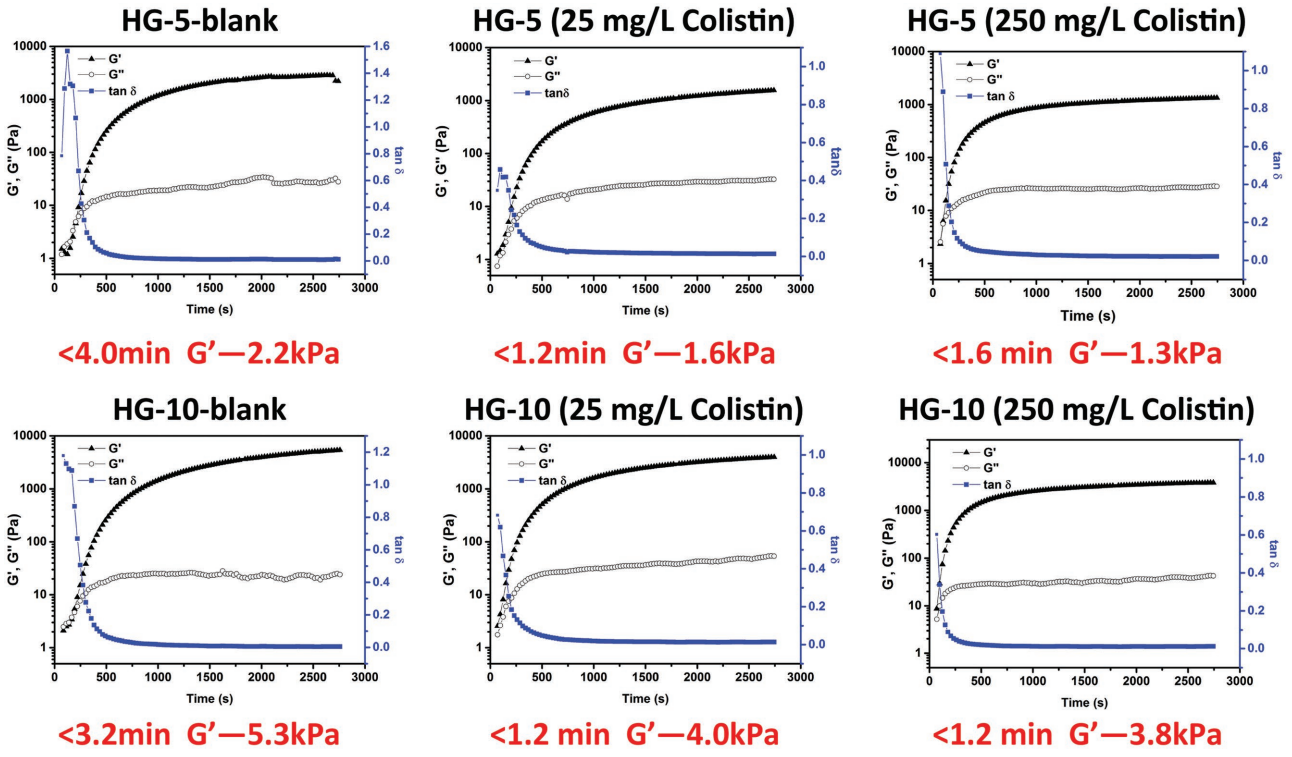


Figure 1. Storage modulus *G'* and loss modulus *G"* analyses during gelation process for different hydrogels. (Black: storage modulus; white: loss modulus; blue: phase angle; 37 °C; frequency: 1.0 Hz; strain: 5.0%.)

Even though colistin itself contains five primary amines capable of imine formation, an initial attempt to prepare a hydrogel directly from mixing colistin and DF-PEG in PBS at the same (20 mg colistin with 500 µL 10% (w/w) DF-PEG PBS solution) or a more concentrated condition (20 mg colistin with 500 µL 20% (w/w) DF-PEG PBS solution) was unsuccessful (Figure S1, Supporting Information). This may be attributed to the dynamic reversibility of the imine bonds under these conditions. In order to act as a stable cross-linking point, at least three amines on each colistin need to form imine bonds with DF-PEG at all times, which is not necessarily easy as the individual imine bond is weak and hydrolytically reversible. However, it is more likely to happen with a multi-functional amine-rich material such as glycol chitosan. Also, a more stable imine bond can be formed from the glucosamine unit on glycol chitosan. Thus, glycol chitosan is necessary to this system to obtain a stable hydrogel network.

Since the hydrogel network is mainly dominated by glycol chitosan and DF-PEG, the mechanical properties of the colistin-loaded hydrogel can be tuned by adjusting the amount of the DF-PEG cross-linker. Hydrogels with two different amounts of cross-linker were prepared and investigated. The hydrogel formation process was not affected by the addition of colistin, and more interestingly, the presence of colistin in the formulation accelerated the gelation process, which we attribute to the increased number of amines in the system (Figure 1). The hydrogels prepared using 10% (w/w) DF-PEG solution (HG-10) have a larger strain compared to the ones prepared from 5% (w/w) DF-PEG solution (HG-5). The strain range of the latter is suitable for application as a topical gel, while the higher strain of HG-10 makes it a suitable candidate for patch processing.

The self-healing property provided by the dynamic imine bond of the original hydrogel has proven to be helpful to tissue repair.[16c] To observe whether the self-healable gel would be affected by the addition of colistin, the rheology of the colistin-loaded hydrogels was examined (Figure S2, Supporting Information). As colistin does not interrupt the formation of the main dynamic cross-linked network of the hydrogels, both hydrogels demonstrated self-healing properties in the presence of colistin while the strain of the hydrogels only reduced slightly after three cycles, implying the colistin-loaded hydrogels can still provide similar protection on the wound after suffering some small damage.

2.2. Colistin Release from the Hydrogel

Since the integration of colistin into the hydrogels was successful, the potential for tuning the colistin release was investigated. The release rate of colistin was first tested in PBS using both HG-5 and HG-10 formulations

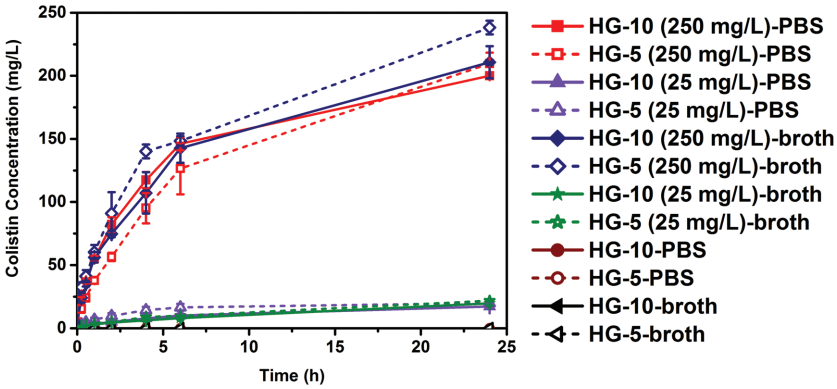


Figure 2. The release study in PBS and cation-adjusted Mueller–Hinton broth. The data of HG-5 are described in dashed lines and HG-10 in solid lines. (The release traces of the blank hydrogels overlap with the *x*-axis.)

for comparison. Due to the potency of colistin, the release experiment was carried out at relatively low concentrations. A high (5 mg colistin per hydrogel; final colistin concentration in solution, 250 mg L^{-1}) and a low dose (0.5 mg colistin per hydrogel; final colistin concentration in solution, 25 mg L^{-1}) colistin were loaded into both hydrogels for the test. In order to confirm colistin is incorporated within the hydrogel as opposed to being located at the surface, a washing-up step was conducted prior to the release study. The recovered wash solutions were systematically analyzed by HPLC and revealed no detectable signals, suggesting near quantitative encapsulation of colistin. Interestingly, although adjusting the amount of the DF-PEG cross-linker is used to tune the physical properties of the hydrogels, it has little impact upon the colistin release rate under the formulation conditions used herein (Figure 2). The colistin concentration difference at each sampling time is sufficiently insignificant not to have any biological implication for both hydrogels loaded with the same amount of colistin. We attribute this observation to the dynamic properties of the hydrogel cross-linked network and the weak interactions between colistin and the hydrogel scaffold. The low colistin loading may also be one of the reasons for the little difference of the release rate between the HG-5 and HG-10 formulations.

Similarly, the release of colistin was tested in cation-adjusted Mueller–Hinton (CAMHB) bacterial growth media to evaluate the effect of a complex biological media on the colistin release rate. Even though the CAMHB contains amino acids, proteins, and various ions, which is very different to PBS, the release profiles of both colistin-loaded hydrogels were found to be very similar (Figure 2). Again, under these conditions the amount of the cross-linker in the hydrogel did not have a great influence on the release of colistin due to similar reasons described above. All of these results suggest that the antibiotic activity between these two colistin-loaded hydrogels should be similar.

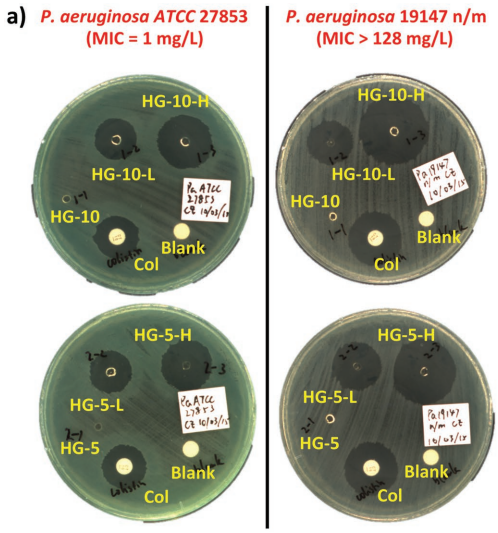
2.3. In Vitro Bio-Activity Evaluation of Colistin-Loaded Hydrogels

The activity of the colistin-loaded gels was investigated in vitro against two *P. aeruginosa* strains, a colistin-sensitive strain (ATCC 27853, minimum inhibitory concentration (MIC) = 1 mg L^{-1}) and a colistin-resistant strain cystic fibrosis isolate (19147 n/m, MIC > 128 mg L^{-1}).

First, a disk diffusion assay was used to evaluate the release of colistin from the hydrogel through the interface (Figure 3). Briefly, the colistin-loaded hydrogels were placed on an agar plate surface that was swabbed with a bacterial isolate solution and the plate was then incubated for 24 h. A clear bacteria-free zone, also known as zone of inhibition (ZoI), would be found from the samples that have antibiotic activity and the larger size of ZoI indicates a higher potency of the sample.

Although the hydrogel per se did not show any inhibition against the bacteria, clear ZoIs were found from the colistin-sensitive *P. aeruginosa* strain when colistin-loaded hydrogels were applied (Figure 3a, left lane), suggesting that colistin is effectively released from the colistin-loaded hydrogels to the bacterial interface and more importantly, that the released colistin remains active.

ZoIs of similar size were found between both colistin-loaded hydrogels (HG-5 and HG-10) against the *P. aeruginosa* strain, in accordance with the similar colistin release after 24 h from the release profile (Figure 2), proposing the two colistin-loaded hydrogels have similar antibacterial properties despite the difference of their physical properties. More importantly, the colistin-loaded hydrogels showed similar activity to commercial colistin disks containing the same amount of colistin (10 μg), indicating the released colistin has almost the same activity against the *P. aeruginosa* bacteria as the native one and the hydrogel does not severely hinder or alter the activity of the colistin. Similar results were also found from the colistin-resistant *P. aeruginosa* strain (Figure 3a, right lane). Owing to the high loading capacity of the hydrogel, it was possible to increase the loading of colistin into the hydrogel of the same size. With the higher loading of the colistin (100 μg colistin per hydrogel), the hydrogels showed the expected enhanced antibiotic performance against the bacteria when compared to the commercial disks. In order to obtain a better understanding of the killing kinetics of the colistin-loaded hydrogels, time-dependent bacterial inhibition experiments (time-kill experiments) were conducted (Figure 4). Briefly, the colistin-loaded hydrogels were introduced into the broth containing bacteria at the logarithmic phase of growth and the broth samples were taken periodically for viable



b)

	ATCC 27853		19147 n/m	
	D/cm	ZoI/cm	D/cm	ZoI/cm
Blank	0.60	0	0.60	0
Col (10 μg)	0.60	1.60	0.60	1.93
HG-10 (0 μg)	0.30	0	0.28	0
HG-10-L (10 μg)	0.36	1.52	0.30	1.65
HG-10-H (100 μg)	0.35	1.97	0.31	2.49
Blank	0.60	0	0.60	0
Col (10 μg)	0.60	1.58	0.60	1.94
HG-5(0 μg)	0.27	0	0.34	0
HG-5-L (10 μg)	0.36	1.53	0.30	1.78
HG-5-H (100 μg)	0.31	1.86	0.33	2.36

D: Disk/Hydrogel Diameter
ZoI: Zone of Inhibition

Figure 3. a) Disk diffusion assay for the colistin-loaded hydrogels against the colistin-sensitive (left) and colistin-resistant (right) *P. aeruginosa* strains. b) Zone of inhibition (ZoI) results of the colistin-loaded hydrogels. Blank: commercial blank disc; Col: commercial colistin disk (contains 10 μg colistin); HG-10/5: blank hydrogels; HG-10/5-L: hydrogels loaded with 10 μg colistin; HG-10/5-H: hydrogels loaded with 100 μg colistin.

counts. As the growth of the colistin-sensitive *P. aeruginosa* strain can be easily inhibited at a very low colistin concentration, only the colistin-resistant strain was investigated in these time-kill studies. Although the MIC of the colistin-resistant strain is greater than 128 mg L^{-1}, colistin solution at a final concentration of 25 mg L^{-1} inhibited the growth of the bacteria from 0.5 to 6 h. On the contrary, the lower colistin dose (0.5 mg colistin per hydrogel; final colistin concentration in solution, 25 mg L^{-1}) hydrogels, showed a lower level of activity. This is mainly due to the difference diffusion profiles obtained from the two solutions and the hydrogel–solution interface. The slower colistin release provided by colistin-loaded hydrogel in the first few hours results in a lower local concentration of released colistin, compared with the colistin solution, thus inhibition of the growth of bacteria at that dose is not observed. To increase the initial local colistin concentration, a higher concentration of colistin was loaded into the hydrogels (5 mg colistin per hydrogel; final colistin concentration in solution, 250 mg L^{-1}). At this loading, colistin-loaded hydrogels showed a much better performance against the resistant *P. aeruginosa* strain. Fitted with the previous colistin release curve (Figure 2), the difference of the killing profiles between HG-5 and HG-10 was again very subtle, indicating similar concentrations of colistin with similar antibiotic ability were released from both colistin-loaded hydrogels. Although a slightly slower killing profile was still observed compared to solution containing the same amount of colistin, due to the reduced initial local concentration provided from the hydrogels, the survival bacteria number was significantly decreased (Figure 4).

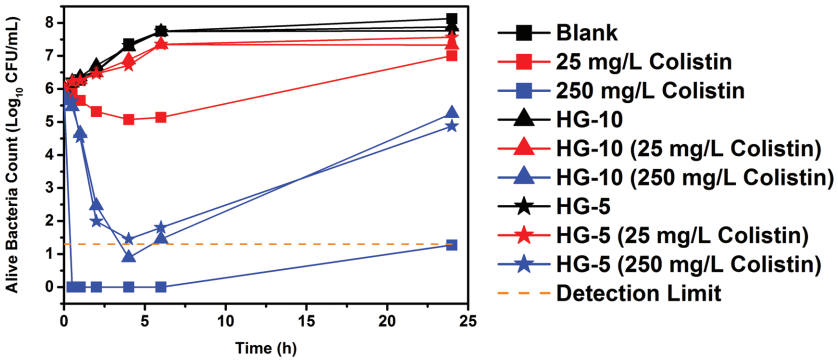


Figure 4. Time-kill test of the colistin-loaded hydrogels. Black lines: blank control and blank hydrogels; red lines: samples loaded with 0.5 mg colistin (final concentration: 25 mg L^{-1}); blue lines: samples loaded 5 mg colistin (final concentration: 250 mg L^{-1}). The detection limit is shown in dashed line.

2.4. In Vivo Animal "Burn" Infection Model Test of Colistin Hydrogels

An animal "burn" infection model was also developed to evaluate the gel performance in vivo (see the Supporting Information for the details). Animal experiments were approved by the institutional animal ethics committee and animals were maintained in accordance with the criteria of the Australian code of practice for the care and use of animals for scientific purposes. Both the colistin-sensitive strain and the resistant strain were tested. Due to the biodegradability of the hydrogels,

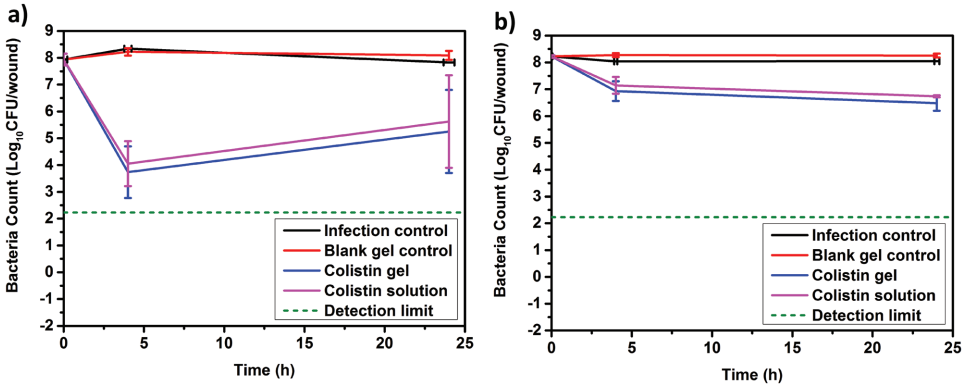


Figure 5. The "burn" infection model test of the colistin-loaded hydrogel against a) colistin-sensitive and b) colistin-resistant strain. Black line: blank infection control; red line: blank HG-10 hydrogel; blue line: HG-10 with colistin (0.3 mg/wound for sensitive strain and 1.5 mg/wound for resistant one); pink line: colistin solution (0.3 mg/wound for sensitive strain and 1.5 mg/wound for resistant one). The detection limit is shown in dashed line.

a visible weight loss was found in both colistin-loaded hydrogels during the test (Figure S4, Supporting Information). Since the HG-5 hydrogel required a longer gelation time and it exhibited a faster degradation rate in vivo, only the HG-10 formulation was examined in vivo. 100 μL 10^9 CFU mL^{-1} of bacteria at the early logarithmic growth phase was inoculated onto the wound (1 cm^2) of each mouse, 2 h in advance of the test. Then patches made from colistin solution, colistin-loaded hydrogel, or blank hydrogels were applied onto the wound at time = 0 h. Colistin (0.3 mg) was loaded into the hydrogel to kill the colistin-sensitive strain. Surprisingly, in spite of the slower colistin release in vitro, the colistin-loaded hydrogel performed as comparably to colistin solution in vivo (Figure 5). In the case of the resistant strain, the colistin concentration was not sufficient to kill the bacteria independently if it was applied in the hydrogel or as a solution (Figure S5, Supporting Information). Therefore, a higher dose (1.5 mg colistin) was used instead to kill the resistant strain. Although the killing profile is slower due to the nature of the resistant strain, the colistin-loaded hydrogel again showed a similar activity as the colistin solution, leading to ≈2 logarithm reduction of the bacteria population (i.e., ≈99% bacteria population was killed) (Figure 5). This implies that the hydrogel formulation did not inhibit the activity of the colistin in vivo even though it has a slower release rate than that observed in the time-kill study. This may partially be attributed to the similar diffusion profile obtained from the wound interface but also through the enhanced degradation of the hydrogel in the biologically complex nature of the local wound environment. Moreover, since the colistin-loaded hydrogels were applied directly onto the burn wound, it appears that the local concentration on the wound was high enough to kill the bacteria while all the tested mice survived suggesting the overall systemic concentration and toxicity remained low.

3. Conclusion

In summary, we have successfully prepared self-healing colistin-loaded hydrogels by incorporating colistin into an inexpensive, biocompatible, and biodegradable chitosan hydrogel. The hydrogel structure was not affected by the addition of the colistin lipopeptide and the colistin-loaded hydrogels inherited all of the properties from the original hydrogel without disrupting their desired physical properties. Interestingly, the hydrogel formation process could be accelerated in the presence of the colistin. Colistin is released from the hydrogel at a similar rate at body temperature in different buffers. The disk diffusion assay showed that the released colistin remained active and had comparable antibacterial activity as the colistin solution. Although the colistin-loaded hydrogels showed a slower killing profile in vitro compared with the colistin solution, it performed as well as the colistin solution in vivo thanks to the biodegradable and dynamic nature of this glycol chitosan hydrogel. Furthermore, a wide range of colistin loading was easily achievable, thus allowing the tuning of the release of colistin for different applications. Indeed, we demonstrated that even the colistin-resistant *P. aeruginosa* strains can easily be killed in vivo in a mouse burn infection model. Finally, we have demonstrated this hydrogel can achieve the localized release of active colistin without causing any toxicity to the mice. This colistin-loaded hydrogel holds significant potential for the treatment of chronic wound infections caused by problematic Gram-negative "superbugs."

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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[1] a) Anitibiotic resistance threats in the United States **2013**, www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf (accessed: June 2016); b) Tackling a global health crisis: Initial steps, https://amr-review.org/sites/default/files/Report-52.15.pdf (accessed: June 2016); c) Responding to the threat of antimicrobial resistance, https://www.health.gov.au/internet/main/publishing.nsf/Content/1803C433C71415CACA257C8400121B1F/$File/amr-strategy-2015-2019.pdf (accessed: June 2016).

[2] a) D. Church, S. Elsayed, O. Reid, B. Winston, R. Lindsay, *Clin. Microbiol. Rev.* **2006**, *19*, 403; b) E. A. Azzopardi, E. Azzopardi, L. Camilleri, J. Villapalos, D. E. Boyce, P. Dziewulski, W. A. Dickson, I. S. Whitaker, *PloS One* **2014**, *9*, e95042.

[3] a) G. W. Waterer, R. G. Wunderink, *Crit. Care Med.* **2001**, *29*, N75; b) H. W. Boucher, G. H. Talbot, J. S. Bradley, J. E. Edwards, D. Gilbert, L. B. Rice, M. Scheld, B. Spellberg, J. Bartlett, *Clin. Infect. Dis.* **2009**, *48*, 1; c) A. P. Zavascki, C. G. Carvalhaes, R. C. Picão, A. C. Gales, *Expert Rev. Anti-Infect. Ther.* **2010**, *8*, 71; d) A. P. Magiorakos, A. Srinivasan, R. Carey, Y. Carmeli, M. Falagas, C. Giske, S. Harbarth, J. Hindler, G. Kahlmeter, B. Olsson-Liljequist, *Clin. Microbiol. Infect.* **2012**, *18*, 268.

[4] a) J. B. Lyczak, C. L. Cannon, G. B. Pier, *Microbes Infect.* **2000**, *2*, 1051; b) E. E. Tredget, H. A. Shankowsky, R. Rennie, R. E. Burrell, S. Logsetty, *Burns* **2004**, *30*, 3; c) G. H. Talbot, J. Bradley, J. E. Edwards, D. Gilbert, M. Scheld, J. G. Bartlett, *Clin. Infect. Dis.* **2006**, *42*, 657.

[5] a) H. W. Boucher, G. H. Talbot, D. K. Benjamin, J. Bradley, R. J. Guidos, R. N. Jones, B. E. Murray, R. A. Bonomo, D. Gilbert, The Infectious Diseases Society of America, *Clin. Infect. Dis.* **2013**, *56*, 1685; b) T. Velkov, K. D. Roberts, R. L. Nation, P. E. Thompson, J. Li, *Future Microbiol.* **2013**, *8*, 711.

[6] a) T. Velkov, P. E. Thompson, R. L. Nation, J. Li, *J. Med. Chem.* **2010**, *53*, 1898; b) S. Biswas, J.-M. Brunel, J.-C. Dubus, M. Reynaud-Gaubert, J.-M. Rolain, *Expert Rev. Anti-Infect. Ther.* **2012**, *10*, 917.

[7] a) M. E. Falagas, P. I. Rafailidis, *Clin. Infect. Dis.* **2009**, *48*, 1729; b) H. Spapen, R. Jacobs, V. Van Gorp, J. Troubleyn, P. M. Honoré, *Ann. Intensive Care* **2011**, *1*, 14; c) J. A. Ordooei, S. Shokouhi, Z. Sahraei, *Eur. J. Clin. Pharmacol.* **2015**, *71*, 801.

[8] a) P. M. Honoré, R. Jacobs, O. Joannes-Boyau, S. Lochy, W. Boer, E. De Waele, V. Van Gorp, J. De Regt, V. Collin, H. D. Spapen, *Blood Purif.* **2014**, *37*, 291; b) K. S. Akers, M. P. Rowan, K. L. Niece, I. J. Stewart, K. Mende, J. M. Cota, C. K. Murray, K. K. Chung, *Antimicrob. Agents Chemother.* **2015**, *59*, 46; c) R. L. Nation, J. Li, O. Cars, W. Couet, M. N. Dudley, K. S. Kaye, J. W. Mouton, D. L. Paterson, V. H. Tam, U. Theuretzbacher, *Lancet Infect. Dis.* **2015**, *15*, 225.

[9] a) P. Beringer, *Curr. Opin. Pulm. Med.* **2001**, *7*, 434; b) A. Michalopoulos, S. K. Kasiakou, Z. Mastora, K. Rellos, A. M. Kapaskelis, M. E. Falagas, *Crit. Care* **2005**, *9*, R53.

[10] a) J. S. Boateng, K. H. Matthews, H. N. Stevens, G. M. Eccleston, *J. Pharm. Sci.* **2008**, *97*, 2892; b) T. Vermonden, R. Censi, W. E. Hennink, *Chem. Rev.* **2012**, *112*, 2853; c) S. V. Murphy, A. Skardal, A. Atala, *J. Biomed. Mater. Res., Part A* **2013**, *101*, 272; d) M. Madaghiele, C. Demitri, A. Sannino, L. Ambrosio, *Burns Trauma* **2014**, *2*, 153; e) V. W. Ng, J. M. Chan, H. Sardon, R. J. Ono, J. M. García, Y. Y. Yang, J. L. Hedrick, *Adv. Drug Delivery Rev.* **2014**, *78*, 46; f) N. S. Goodwin, A. Spinks, J. Wasiak, *Int. Wound J.* **2015**, *13*, 519.

[11] a) T. Dai, M. Tanaka, Y.-Y. Huang, M. R. Hamblin, *Expert Rev. Anti-Infect. Ther.* **2011**, *9*, 857; b) G. Sun, X. Zhang, Y.-I. Shen, R. Sebastian, L. E. Dickinson, K. Fox-Talbot, M. Reinblatt, C. Steenbergen, J. W. Harmon, S. Gerecht, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 20976.

[12] a) D. Seliktar, *Science* **2012**, *336*, 1124; b) P. M. Kharkar, K. L. Kiick, A. M. Kloxin, *Chem. Soc. Rev.* **2013**, *42*, 7335.

[13] a) P. Gupta, K. Vermani, S. Garg, *Drug Discovery Today* **2002**, *7*, 569; b) Y. Qiu, K. Park, *Adv. Drug Delivery Rev.* **2012**, *64*, 49.

[14] a) A. Phadke, C. Zhang, B. Arman, C.-C. Hsu, R. A. Mashelkar, A. K. Lele, M. J. Tauber, G. Arya, S. Varghese, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 4383; b) Y. Zhang, B. Yang, L. Xu, X. Zhang, L. Tao, Y. Wei, *Acta Chim. Sin.* **2013**, *71*, 485; c) Z. Wei, J. H. Yang, J. Zhou, F. Xu, M. Zrínyi, P. H. Dussault, Y. Osada, Y. M. Chen, *Chem. Soc. Rev.* **2014**, *43*, 8114.

[15] a) H. Ueno, H. Yamada, I. Tanaka, N. Kaba, M. Matsuura, M. Okumura, T. Kadosawa, T. Fujinaga, *Biomaterials* **1999**, *20*, 1407; b) D. K. Singh, A. R. Ray, *J. Macromol. Sci., Polym. Rev.* **2000**, *40*, 69; c) N. Bhattarai, J. Gunn, M. Zhang, *Adv. Drug Delivery Rev.* **2010**, *62*, 83.

[16] a) Y. Zhang, L. Tao, S. Li, Y. Wei, *Biomacromolecules* **2011**, *12*, 2894; b) B. Yang, Y. Zhang, X. Zhang, L. Tao, S. Li, Y. Wei, *Polym. Chem.* **2012**, *3*, 3235; c) T.-C. Tseng, L. Tao, F.-Y. Hsieh, Y. Wei, I.-M. Chiu, S.-H. Hsu, *Adv. Mater.* **2015**, *27*, 3518.