Intracellular Carbon Monoxide Release Unveils Selective Antibacterial Effects

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Carbon monoxide (CO) has gained recognition as a significant gasotransmitter. Thus far, CO has demonstrated diverse biological functions, including anti-tumor, anti-inflammatory, and antibacterial effects when administered through CO gas or CO-releasing molecules (CORMs). It is noteworthy that CO gas exhibits no apparent bactericidal effect, while conventional CORMs like metal carbonyls display broad-spectrum antibacterial activities. However, it is crucial to acknowledge that the antibacterial activity in metal carbonyls may be attributed to the presence of transition metal ions. Berreau and coworkers rationally designed membrane-permeable and membrane-impermeable CO donors.[1] These donors produced similar anti-inflammatory activities in mammalian cells, with extracellular CO release found to be less toxic. This preliminary result suggests that the spatial location of CO release may indeed influence the biological functions of CO.

In this study, we have constructed a small library of nonmetallic CO-releasing micelles containing 3-HF moieties within the cores as potential CO donors and investigated whether the location of CO release affects its antibacterial activity (Figure 1a). We found that small-sized BP1 micelles exhibit excellent antibacterial properties, while large-sized BP1 micelles are mainly located outside *S. aureus*, resulting in weaker antibacterial properties (Figure 1b, c). We also found that the monomer sequences within the copolymer chains can significantly influence the self-assembly behaviors and subsequent antimicrobial activity of CO-releasing micelles (Fig. 1d). In addition, we found that bacteria uptake micelles with different shells differently, thus affecting their antibacterial activities (Figure 1e).

The generation of hydroxyl radicals (•OH) after intracellular CO release may contribute to the bactericidal effect (Figure 1f). Furthermore, CO-releasing micelles concurrently eliminate bacterial pathogens and inhibit the inflammatory response in a skin abscess murine model (Figure 1g). This study, for the first time, unveils that the spatial location of CO release can significantly affect its physiological functions, providing valuable insights for the development of novel CO-based antibacterial agents.

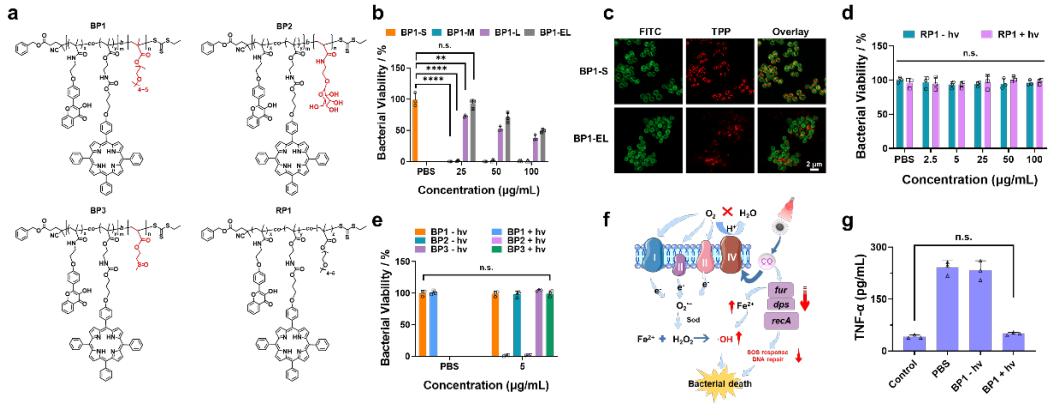

Figure 1 (a) Chemical structures of CO-releasing copolymers of BP1, BP2, BP3, and RP1. (b) Antibacterial activities of micelles of different particle sizes. (c) Colocalization of micelles with different particle sizes and bacteria. Antibacterial activities of micelles with (d) different chain sequences and (e) different shells. (f) Antibacterial mechanism of intracellular CO release. (g) Quantification of inflammatory cytokine levels of TNF-α.

[1]T. Soboleva, C. R. Simons, A. Arcidiacono, A. D. Benninghoff, L. M. Berreau, *J. Med. Chem.* 2019, *62*, 9990-9995.