Enhanced antibacterial function of a supramolecular artificial receptor-modified macrophage (SAR-Macrophage)
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Bacterial infection has become a global concern owing to the significant morbidity and mortality. Although the phagocytosis of bacteria by immune cells acts as the front line to protect human body from invading pathogens, the relatively slow encounter and insufficient capture of bacteria by immune cells often lead to an inefficient clearance of pathogens. Herein, a supramolecular artificial receptor-modified macrophage (SAR-Macrophage) was developed to enhance the recognition and latch of bacteria in the systemic circulation, mediated via strong and multipoint host–guest interactions between the artificial receptors (cucurbit[7]uril) on the macrophage and the guest ligands ( adamantane) selectively anchored on Escherichia coli (E. coli). As a result, the SAR-Macrophage could significantly accelerate the recognition of E. coli, catch and internalize more pathogens, which subsequently induced the M1 polarization of macrophages to generate ROS and effectively kill the intracellular bacteria. Therefore, the SAR-Macrophage represents a simple, yet powerful anti-bacterial approach.

Bacterial infection has attracted increasing attention due to the acquired resistance of bacteria to various antibiotics, which poses a serious threat to public health. To avoid antibiotic resistance, increasing studies are turning to the natural immune system to combat bacterial infection. Macrophages play a key role in immune protection from bacterial pathogens by recognizing, engulfing and digesting bacteria through a phagocytosis process. Although the macrophage endocytic process is a natural response to pathogenic bacterial infection, it is often difficult for a macrophage to efficiently encounter, latch and catch pathogens, resulting in low sensitivity and elimination efficiency of macrophages towards bacteria, thus inevitably leading to a high infection rate. Various strategies and mechanisms have been explored to improve the antibacterial activity of macrophages. For instance, Casanova et al. reported that the overexpression of BAII (brain angiogenesis inhibitor 1) enhanced the macrophage’s encounter, engulfment and clearance of Gram-negative bacteria through the recognition of their surface lipopolysaccharides. Similarly, Chimenti-Gbaguidi et al. found that the pretreatment of macrophages with Liver X Receptor agonists resulted in an enhanced recognition of lipopolysaccharides and an increased reactive oxygen species (ROS) generation to exert antibacterial activities. In another study, Biswal et al. discovered that the activation of Nrf2 (nuclear erythroid-related factor 2) upregulated its downstream MARCO (the scavenger receptor) of defective macrophage, which was suppressed in chronic obstructive
pulmonary disease, leading to restored bacterial recognition and phagocytosis in alveolar macrophages. In spite of these successes, all of these approaches relied on the upregulation of natural surface receptors of macrophages, which may imbalance their innate immunity and inevitably lead to undesirable side effects. On the other hand, numerous nanomaterials have been extensively studied as antibiotic carriers or to generate ROS to directly combat with bacteria. In spite of the promising antibacterial activity, these nanomaterials still face a series of obstacles, including nonspecific toxicity and increased risk of drug resistance. Herein, through simply engineering the natural macrophage with artificial receptors that have specific, bioorthogonal recognition of bacteria may mitigate various risks, such as drug-resistance and undesirable side effects.

Indeed, artificial host–guest interactions offer a new paradigm for biomedical applications, displaying advantages in enabling bio-orthogonal, specific recognition, targeting and latching in vitro and in vivo. For instance, Kim et al. leveraged an ultrastable synthetic host–guest pair between cucurbit[7]uril (CB[7]) and adamantyl-(ADA) guests for the specific and bio-orthogonal protein imaging and bioimaging in vivo. Wang et al. demonstrated that CB[7]-grafted hyaluronic acid could recognize, latch and aggregate ADA-modified mitochondria to further induce mitochondrial aggregation and fusion intracellularly. On the basis of such a bio-orthogonal, high specificity and binding affinity between CB[7] and ADA in the complex biological environment, and inspired by the clinical success of CAR-T (Chimeric Antigen Receptor T-cell) and CAR-Macrophage, here, we designed a facile, yet powerful SAR-Macrophage (supramolecular artificial receptor macrophage), reminiscent of CAR-T and CAR-Macrophage, by anchoring CB[7] as an artificial receptor on the surface of macrophage, thus enhancing the recognition, latching and internalization of E. coli, which was specifically decorated with ADA as an artificial ligand, mediated via strong and multipoint host–guest interactions for significantly improving the antibacterial activity of the macrophage against E. coli (Scheme 1).

First, as d-mannose can be recognized and adsorbed onto E. coli owing to its strong and specific interactions with FimH (Type 1 fimbrin d-mannose specific adhesion protein) on the pathogens, a mannose-ADA conjugate was designed and synthesized via a thiol–ene click reaction between allyl-d-mannopyranoside and 1-adamantanethiol to decorate the surface of E. coli with ADA via mannose-FimH interactions. The successful synthesis was confirmed by 1H and 13C NMR spectroscopy (Fig. S2–S4, ESI†). As shown in Fig. S5 (ESI†), mannose-ADA (up to 500 µg mL⁻¹) exhibited negligible cytotoxicity against E. coli after incubation for 24 h via MTT assays. Cy5-conjugated CB[7] (CB[7]–Cy5) was subsequently applied to investigate the successful decoration of E. coli with ADA. As shown in Fig. S6 (ESI†), after incubation with CB[7]–Cy5 for 10 min, E. coli pre-incubated with mannose-ADA (100 µg mL⁻¹, 20 min) exhibited strong red fluorescence of Cy5 on the surface, attributed to the strong host–guest interactions between CB[7] of CB[7]–Cy5 and ADA from the E. coli surface. In contrast, no

Scheme 1  Supramolecular artificial receptor (SAR)-Macrophages rapidly and highly specifically recognize E. coli through strong and multipoint host–guest interactions, thus improving the latching and internalization of E. coli, and inducing M1 polarization of macrophages to generate ROS, and effectively kill the intracellular bacteria.
fluorescence was observed on the *E. coli* without pre-incubation with mannose-ADA, indicating that the successful recognition of CB[7]–Cy5 by *E. coli* was achieved via the CB[7]–ADA host–guest interaction.

**SAR-Macrophage** was constructed by decorating macrophage (Raw 264.7 cells) with CB[7] on the surface via simply inserting DSPE-PEG-CB[7] (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol)-CB[7]) into the surface membrane of the cells.27,28 The amount of CB[7] on the cell surface was quantitatively determined to be approximately 0.43 × 10^{-12} mol per cell, by incubation of the SAR-Macrophage with ferrocene (as a strong guest molecule of CB[7]) that was quantified via ICP-MS analysis. In addition, DSPE-PEG-CB[7] exhibited negligible cytotoxicity on RAW 264.7 cells after incubation for 24 h at a concentration up to 50 μM (Fig. S7, ESI†). Fluorescein isothiocyanate (FITC)-modified ADA (ADA-FITC) was utilized to evaluate the availability and stability of the surface CB[7] decorated on the macrophage. As shown in Fig. S8 (ESI†), RAW 264.7 cells incubated with DSPE-PEG-CB[7] for 1, 2, 4, 12 and 24 h, respectively, were subsequently treated with ADA-FITC for 5 min, and the green fluorescence of FITC maintained on the cell membrane for up to 12 h, indicating the good stability of DSPE-PEG-CB[7] in the cell membrane. The rapid recognition and stable host–guest interactions between CB[7] and ADA may ensure efficient recognition and latch of ADA-decorated *E. coli* by the SAR-Macrophage.

We subsequently investigated the recognition and internalization of ADA-modified *E. coli* by the SAR-Macrophage. In this study, the following treatment groups were prepared: macrophages were incubated with *E. coli* (CB[7](−), ADA(−)); SAR-Macrophages were incubated with *E. coli* (CB[7](+), ADA(−)); macrophages were incubated with ADA-decorated *E. coli* (CB[7](−), ADA(+)) and SAR-Macrophages were incubated with ADA-decorated *E. coli* (CB[7](+), ADA(+)) at different multiplicity of infection (MOI) ratios ranging from 1 : 1 to 10 : 1. When the macrophages were incubated with *E. coli* at MOI = 1, strong green fluorescence from GFP-expressing *E. coli* was observed in the cytoplasm of RAW 264.7 cells in the (CB[7](+), ADA(+)) group, in contrast to the rather weak green fluorescence observed in the other three groups (Fig. 1A and Fig. S9A, ESI†), suggesting that the efficient recognition and internalization of ADA-decorated *E. coli* was realized by the SAR-Macrophage via strong host–guest interactions between CB[7] from the surface of the macrophage and ADA of *E. coli*. As shown in Fig. 1B, the

![Fig. 1](image-url)
SAR-Macrophages exhibited a significantly improved recognition of *E. coli*, ca. 14-fold of that recognized by regular macrophages (quantitated according to the fluorescence intensity).

When the infected *E. coli* was increased to MOI = 10, the recognition of *E. coli* by the macrophages was significantly accelerated. As shown in Fig. 1C and Fig. S9B (ESI†), after incubation with various formulations for 20, 40 and 100 min, the GFP fluorescence (of *E. coli*) increased more rapidly in the (CB[7](+), ADA(+)) group of macrophages, resulting in a 32-fold increase when compared with regular macrophages in 100 min, indicating that host–guest interactions indeed promoted the recognition and uptake of *E. coli* by the SAR-Macrophages (Fig. 1D). In both MOI = 1 and MOI = 10 CLSM (confocal laser scanning microscopy) images, the SAR-Macrophages were found to be morphologically deformed along with the formation of pseudopodia, indicating the polarization of macrophages to M1 would be beneficial to elimination of the intracellular bacteria. In contrast, the macrophages of other groups maintained the regular spherical morphology. The bacterial recognition and morphology of macrophages incubated with different formulations under MOI = 10 were further examined under scanning electron microscopy (SEM). As shown in Fig. 2A, abundant ADA-decorated *E. coli* were observed on the surface of the SAR-Macrophage, accompanied by the appearance of pseudopodia. In comparison, the rest three groups of macrophages showed relatively low efficiency in catching *E. coli* and exhibited a smooth cell membrane surface and a spherical shape, consistent with the observations under CLSM.

The M1 polarization of macrophages was evaluated via flow cytometry assays after the internalization of *E. coli* (MOI = 10). As shown in Fig. S10 (ESI†), in the (CB[7](+), ADA(+)) group, the M1 polarization ratio (CD11c+ cells) increased to 40.88% in comparison with 1.71%, 4.07%, 8.43% and 10.27% observed in free macrophage, (CB[7](−), ADA(−)) group, (CB[7](+), ADA(−)) group and (CB[7](−), ADA(+)) group, respectively, indicating that the M1 polarization of the SAR-Macrophages was induced by the promoted recognition and arrest of *E. coli*. In addition, no M2 polarization (CD206+) was observed in all of these groups.

To further confirm the antibacterial efficacy of the M1-polarized macrophages, we infected different formulations of macrophages with *E. coli* at MOI = 10, followed by the evaluation of the intracellular ROS by staining with 2’,7’-dichlorofluorescein diacetate (DCFHDA).29 According to the CLSM results (Fig. 2B), the (CB[7](+), ADA(+)) group of macrophages showed obvious intracellular green fluorescence due to the host–guest mediated recognition and arrest of *E. coli*, which promoted the intracellular ROS production. Conversely, the macrophages of other groups showed rather weak intracellular fluorescence, indicating the insufficient internalization of *E. coli* into the macrophages. The intracellular ROS generation was further quantitatively analyzed via flow cytometry upon DCFHDA staining. As shown in Fig. S11 (ESI†), the fluorescence intensity of (CB[7](+), ADA(+)) was 2.1-fold higher than that of the control group, while the (CB[7](−), ADA(−)) group, (CB[7](+), ADA(−)) group and (CB[7](−), ADA(+)) group showed modestly elevated fluorescence (1.3–1.5-fold that of the control group), consistent with the results observed under CLSM. As intracellular ROS play a key role in killing the internalized *E. coli*, the viability of the intracellular bacteria was evaluated by using the bacterial live/dead staining assays at 18 h after infection. Intense red signals were found inside the macrophages of all groups, suggesting that most of the intracellular *E. coli* were dead. Of a significant note, more red fluorescence, indicative of dead bacteria, was observed in the SAR-Macrophage (Fig. S12, ESI†). These results indicated that the SAR-Macrophages exhibited an enhanced recognition and
arrest of extracellular bacteria and effectively killed bacteria via M1 polarization.

Plate counting assay was subsequently adopted to confirm the antibacterial activities of the SAR-Macrophage. It was shown that after treatment with \( \text{[CB}[7]\text{](+), ADA(+)} \), plate counting results confirmed the remarkable antibacterial activity of the SAR-Macrophage towards \textit{E. coli}, which was comparable to, but not as good as, the first-line antibiotics, such as ampicillin and ofloxacin, which was not unexpected because here \textit{E. coli} is a regular strain sensitive to antibiotics (Fig. S13, ESI†). To assess the effective therapeutic dose range, different quantities of SAR-Macrophages were added to \textit{E. coli}, as shown in Fig. S14 (ESI†), and the SAR-Macrophage (CB\[7\](+),ADA(+)\) exhibited decent antibacterial effects even at a concentration of \(10^3 \text{ mL}^{-1}\). In contrast, in the presence of the same quantity of regular macrophages, there were much more bacteria that survived, confirming that the strong and specific host–guest interaction was the main reason for the enhanced antibacterial properties.

Furthermore, in order to show the unique advantages of the SAR-Macrophage against drug-resistant bacteria, Uropathogenic \textit{Escherichia coli} Y9 (UPEC), which is a type of clinically isolated drug-resistant bacterium,\(^{30}\) was employed in our antibacterial studies and compared against a couple of classic antibiotics. As shown in Fig. S15 (ESI†), the first-line antibiotics, such as ampicillin and ofloxacin, exhibited little antibacterial activities against UPEC due to the well-developed antibiotic resistance of UPEC. In contrast, after treatment with the SAR-Macrophage (CB\[7\](+),ADA(+)\), the survival of UPEC decreased significantly, indicating the effective antibacterial capability of the SAR-Macrophage to the drug-resistant strains, which was otherwise not achievable by regular macrophages.

The recognition and arrest of \textit{E. coli} by the SAR-Macrophages were further investigated in zebrafish in vivo, as immune-capture of bacteria usually takes place in the circulatory system, and this transparent animal modul allows a direct visualization of the arrest process in the fish body at a single cell level.\(^{31}\) After sequentially injecting DiD-labeled RAW 264.7 cells into the common cardinal vein (CCV) of zebrafish larvae (48 hpf (hours post fertilization)) and GFP-expressed \textit{E. coli} into the dorsal vein for free circulation, the red-fluorescence macrophages could not catch the green-fluorescence \textit{E. coli} efficiently in the fast-circulating blood system (Fig. 3). In dramatic contrast, the SAR-Macrophages caught ADA-decorated \textit{E. coli} in a rather efficient manner, as was evidenced by the overlapped DiD and GFP fluorescence in vivo. These data showed that the DiD-labeled SAR-Macrophages efficiently recognized and captured pathogens in living zebrafish.

To further evaluate the applicability of the SAR-Macrophage in the fight against pathogen infection in vivo, a mouse wound

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Fig. 3  (A) Scheme showing a two-step injection of the macrophage and \textit{E. coli} into zebrafish. (B) Representative CLSM images and corresponding quantification of GFP- (green) and DiD- (red) co-localization in zebrafish, showing the binding between DiD-loaded RAW 264.7 and GFP-expressed \textit{E. coli}, with different formulations, including \( \text{[CB}[7]\text{](−), ADA(−)} \), \( \text{[CB}[7]\text{](+), ADA(−)} \), \( \text{[CB}[7]\text{](−), ADA(+)\) and \( \text{[CB}[7]\text{](+), ADA(+)\), respectively. For the macrophage, approximately 100–200 cells with DiD fluorescent labeling were injected into the common cardinal vein (CCV) of the fish larvae (48 hpf). For bacteria, approximately 1000–2000 CFU were injected into the vein to allow circulation.
Infection model was established, where full-thickness wounds were created with a 6 mm biopsy punch on the back of mice and were subsequently infected with \(E.\ coli\) (10 \(\mu L\), 1 \(\times\) \(10^9\) CFU per mL). After the mice underwent infection for 4 h, the wound area was treated with mannose-ADA and the SAR-Macrophage (10^5 cells) sequentially, with a 1 h gap between the two injections for the (CB[7](+), ADA(+)) group (Fig. 4A). As shown in Fig. 4B and C, the (CB[7](+), ADA(+)) group of mice exhibited a rapid wound healing process, with the scabbed wound of only 22% that of the original area on Day 5, attributed to the fast recognition and elimination of mannose-ADA labeled \(E.\ coli\) by the SAR-Macrophage. In contrast, in the other treatment groups, the wounds changed from a clear raw appearance to yellowish dense mucus, and recovered slowly. The wound tissues from the infected areas were removed on Day 5 for the quantitative analysis of viable bacteria. As shown in Fig. 4D and Fig. S16 (ESI†), (CB[7](+), ADA(+)) exhibited superior antibacterial effects and significantly lowered the...
A SAR-Macrophage was developed and applied for the first time to enhance the recognition and latching of bacteria, mediated \textit{via} strong and multipoint host–guest interactions between the artificial receptors (CB[7]) on the macrophage and the guest ligands (ADA) on \textit{E. coli}. The SAR-Macrophage was shown to rapidly recognize, efficiently internalize and exterminate guest-anchored SAR (file no.: 0065/2021/A2), the National Natural Science Foundation of China (21871301 and 22071275), and Dr. Stanley Ho Medical Development Foundation (SHMDF-OIRFS/2021/002) are gratefully acknowledged for providing financial support to this work.

**Notes and references**