Targeted Drug Delivery Systems for Eliminating Intracellular bacteria

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Abstract

The intracellular survival of pathogenic bacteria requires a range of survival strategies and virulence factors. These infections are a significant clinical challenge, wherein treatment frequently fails because of poor antibiotic penetration, stability, and retention in host cells. Drug delivery systems (DDSs) are promising tools to overcome these shortcomings and enhance the efficacy of antibiotic therapy. In this review, we elaborate on the classification and mechanisms of intracellular bacterial persistence. Furthermore, we describe, as well as the systematic design strategies applied to DDSs to eliminate intracellular bacteria, and highlight the strategies used for internalization, intracellular activation, bacterial targeting, and immune enhancement. This overview should provide guidance for constructing functionalized DDSs to effectively eliminate intracellular bacteria.

1. Introduction

Once pathogenic bacteria enter the body they are quickly recognized, and, under normal circumstances, phagocytosed and eliminated by cells of the host's immune system.[1] The majority of bacterial species are successfully killed in this manner, however, a small number of species have evolved mechanisms to survive within these phagocytic cells. The best described intracellular bacteria are *Mycobacterium tuberculosis* (*M. tuberculosis*),[2] *Salmonella enterica* (*S. enterica*),[3] and *Listeria monocytogenes* (*L. monocytogenes*).[4] Additionally, some species, classically considered

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extracellular bacteria, such as *Staphylococcus aureus* (*S. aureus*),[5] *Escherichia coli* (*E. coli*),[6] and *Pseudomonas aeruginosa* (*P. aeruginosa*)[7] can also invade and colonize a range of host cells including host defense cells. In all cases, these intracellular bacteria can potentially "hide" from host defense mechanisms either temporarily or over longer periods of time, with the potential for later escape and dissemination of the infection into deeper tissues. The escape of intracellular bacteria has been linked with a variety of serious complications, such as sepsis,[8] osteomyelitis,[9] meningitis,[10] gastroenteritis,[11] and pneumonia.[12]

The clinical treatment for intracellular bacterial infection consists of the long-term administration of high-dose antibiotics.[13] However, many antibiotics do not penetrate host cell membranes due to the low permeability of the membrane and mechanisms such as efflux pumps.[14] Furthermore, the strongly acidic phagolysosomes and high oxidoreductase activity within host cells can inactivate those antibiotics that do enter the cell.[15] Suboptimal antibiotic concentrations and prolonged use of antibiotics also contribute to the development of resistance within intracellular bacteria.[16] Consequently, many of the currently available antibiotic treatment strategies are incapable of completely eliminating intracellular bacteria, resulting in recurrent infection.[17]

Against this background, drug delivery systems (DDSs) offer great potential as a platform for breaking down drug transport barriers and improving drug efficacy in targeting intracellular bacterial infections.[18] In recent years, numerous DDSs have been developed and shown to efficiently enter host cells[19-21] and this review will summarize how they may be designed to pass through cell membranes, tolerate harsh intracellular microenvironment, arrive at the various locations where bacteria may reside intracellularly, e.g., phagolysosome, cytoplasm, and precisely release antibiotics to kill intracellular bacteria. The review provides insights and directions for the comprehensive design of functional DDSs to completely eradicate intracellular bacteria.

2. Host cells and intracellular bacteria

Professional phagocytes, which include macrophages and polymorphonuclear neutrophils (PMNs), are an important line of defense against invading bacterial pathogens.[22] Once these cells have phagocytosed bacteria, the phagosome in the cells tends to fuse with the lysosome, accompanied by maturation and acidification. This fusion further triggers the generation of antimicrobial substances such as reactive oxygen and nitrogen species, antimicrobial peptides, and cytokines.[23, 24] In addition, the acidification of the phagosome, metallization accumulation, and deprivation of essential nutrients (iron, fatty acids, or amino acids) further reinforce antibacterial processes within the cell.[22] However, certain bacterial species can secrete some virulence factors (e.g., α-hemolysin and

chemotactic inhibitory protein) to promote colonization, proliferation, and dissemination within host cells.[25] The fate of the infected cell often results in apoptosis and necroptosis due to nutrient depletion and intoxication from the bacteria.[26] Intracellular bacteria are then released and possibly reabsorbed by other phagocytes leading to a continuous cycle of lysis and uptake maintaining the population of intracellular bacteria. Alternatively, the release of such viable bacteria from phagocytes may also invade non-professional phagocytes, such as epithelial cells,[27] endothelial cells,[28] osteoblasts,[29] and fibroblasts.[30] In contrast to professional phagocytes, these non-professional phagocytes lack sufficient capacity to defend against bacteria.[31] The bacteria, therefore, often escape from the phagosomes and replicate into the cytoplasm, ultimately killing the host cell from the inside.[32]

Intracellular bacteria have been divided into two groups: obligate and facultative intracellular pathogens. The obligate intracellular bacteria include *Chlamydia spp.* or *Rickettsia spp*, who have evolved to exploit host cells as replication niches by manipulating host cell death and survival pathways.[33] The other group consists of facultative intracellular bacteria, such as *M. tuberculosis*, *S. enterica,* and *S. aureus*. They can not only replicate within cells but also in environmental niches (e.g., blood and air). These facultative intracellular bacteria are further sub-classified according to their intracellular life cycles as intravacuolar or cytosolic (**Figure 1**).[34] Intravacuolar bacteria reside and replicate within the cell endomembrane system by interrupting the fusion between phagosomes with the lysosome or surviving in the harsh phagolysosomes.[35] For example*, S. enterica*, and *M. tuberculosis* are all intravacuolar bacteria. This vacuolar environment provides an ideal hiding place and protects them from the innate immune defenses of the host cells. Cytosolic bacteria refer to the bacteria that can escape from the phagosome or phagolysosomes and enter the cytoplasm, such as *L. monocytogenes* and *S. aureus*.[36] They can replicate in the nutrient-rich cytoplasm and manipulate the immune response of host cells.

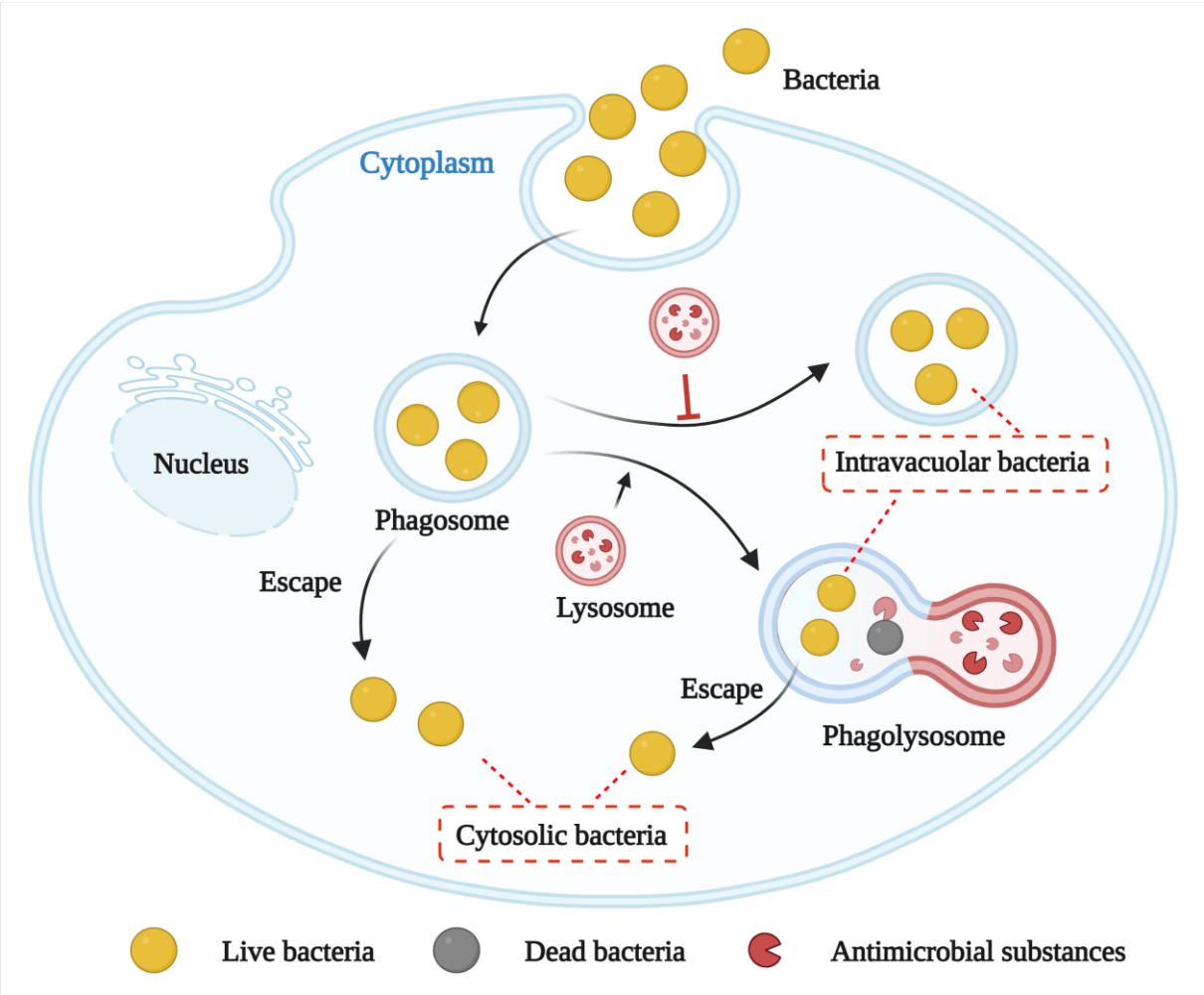

Figure 1. Formation and survival strategies of intracellular bacteria in macrophages. Intracellular bacteria are broadly categorized into intravacuolar and cytosolic bacteria according to their lifestyles. Created with BioRender.com

3. Antibiotics and DDSs

The most commonly used classes of antibiotics to treat intracellular bacterial infections include aminoglycosides, sulfonamides, quinolones, tetracyclines, and *β*-lactams.[37] These antibiotics have short-term intracellular retention and will accumulate in different cellular compartments at different concentrations.[14] Generally, weakly basic antibiotics, such as aminoglycosides and macrolides, tend to primarily accumulate in membrane-bound acidic compartments, like lysosomes. Weak acid antibiotics such as quinolones and *β*-lactams are mainly retained in the cytoplasm.[38] Antibiotics, however, are generally not efficient versus intracellular bacteria due to three major factors: i) membrane barriers of the host cells hinder the internalization of antibiotics, resulting in a low intracellular concentration that is below the minimum inhibitory concentration. ii) The harsh

intracellular macroenvironment (e.g., acidic pH, enzymes) attenuates the bactericidal activity of antibiotics. iii) Antibiotics distribute in different subcellular compartments but are not concentrated at the infection site (**Figure 2**).

To date, DDSs have been exploited to effectively overcome the limitations of antibiotics and have been widely used for treating intracellular bacterial infections *in vitro* (e.g., macrophages and epithelial cells) and *in vivo* (e.g., bacteria-induced pneumonia and peritonitis, two classical animal models). These DDSs can be classified into three groups based on the composition of DDSs, including organic, inorganic, and organic/organic hybrid nanoparticles (NPs, **Table 1**).

Table 1. The DDSs used for eradicating intracellular bacteria.

DDS type		Pathogenic bacteria	Host cells	Drug	Ref.
Organic	PEG	*M. tuberculosis*	THP-1	INH	[39]
		MRSA	J774A.1	Van	[40]
	PLGA	*K. pneumoniae*	MH-S macrophages	Gen	[12]
		C. trachomatis	McCoy cells	Rif	[41]
		S. aureus	Mouse osteoblast cells	Nafcillin	[42]
	PEA	*M. smegmatis*	NR8383 macrophages	Rif	[43]
	Chitosan	*L. monocytogenes*	RAW264.7	Gen	[44]
		S. aureus	THP-1	Tetracycline	[45]
	Alginate	*S. aureus* SCV	RAW264.7	Enrofloxacin	[46]
	Liposomes	*S. enterica*	Caco-2 cells	Colistin	[47]
		S. aureus	A549 cells	PenG	[48]
	SLN	*B. melitensis*	J774A.1	Doxycycline	[49]
		Salmonella CVCC541	RAW264.7	Enrofloxacin	[50]
	MSN	*S. aureus* SCV	RAW264.7	Rif	[51]
		Salmonella	RAW264.7	Cip	[52]
Inorganic	ZnO/Ag NPs	*M. tuberculosis*	THP-1	Rif	[53]
Organic-Inorganic	MOF	*S. aureus*	RAW264.7	Eetracycline	[54]
	Peptide/Au NPs	*S. Typhi*	THP-1	Antimicrobial	[55]

hybrid				peptide	
	Mannose/Se NPs	*M. tuberculosis*	THP-1	INH	[56]
	PLGA/Ag NPs	*S. enterica*	J774A.1	Pexiganan	[57]

Abbreviations: PEG, poly(ethylene glycol); PLGA, poly(lactide-*co*-glycolide) acid; PEA, poly(esteramide); SLN, solid lipid nanoparticles; MSN, mesoporous silica nanoparticles; MOF, metal-organic framework; Au NPs, gold nanoparticles; Se NPs, selenium nanoparticles; Ag NPs, silver nanoparticles; MRSA, methicillin-resistant *staphylococcus aureus*; *K. pneumoniae*, *Klebsiella pneumoniae*; *C. trachomatis*, *Chlamydia trachomatis*; *M. smegmatis*, *Mycobacterium smegmatis*; SCV, small colony variants; *B. melitensis*, *Brucella melitensis*; *S. Typhi*, *Salmonella Typhi*; INH, isoniazid; Van, vancomycin; Gen, gentamicin; Rif, rifampicin; PenG, penicillin G; Cip, ciprofloxacin; THP-1, THP-1 human acute monocytic leukemia macrophages; RAW264.7, RAW264.7 mouse mononuclear macrophages; J744A.1, J744A.1 mouse mononuclear macrophage.

In order to function efficiently in eliminating intracellular bacteria, DDSs must initially cross the cell membrane, reach the sites where bacteria are localized and release antibiotics. These steps involved include i) DDSs specifically target and enter host cells; ii) DDSs escape from phagolysosomes, which are acidic and highly enzymatically active; iii) DDSs target to eradicate intracellular bacteria through stimuli-responsive targeted or active targeted drug release and immune enhancement (**Figure 2**). Accordingly, the whole journey of the DDSs can be divided into extracellular processes and intracellular processes. The strategies used to internalize these DDSs and eliminate intracellular bacteria will be summarized in this review.

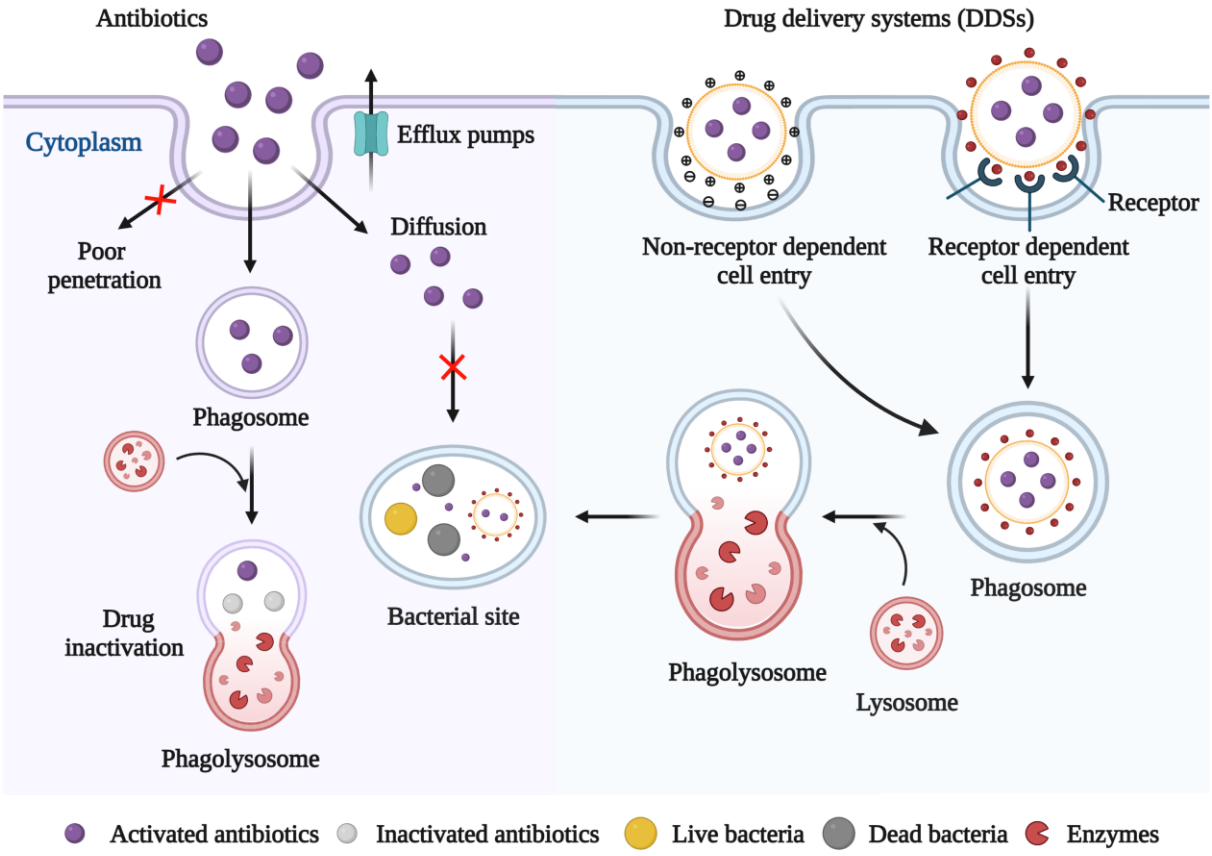

Figure 2. Factors involved in intracellular accumulation of antibiotics and DDSs. Left) The reasons for the failure of antibiotics to successfully treat the intracellular infection can be due to poor cellular membrane penetration, and diminished antibacterial activity in the harsh acidic and hydrolytic environment within phagolysosome. Right) Targeted DDSs can be used to deliver antibiotics to intracellular bacterial sites, protecting the antibiotic until arriving at the site of bacterial infection. Created with BioRender.com

4. DDSs for targeted internalization in infected phagocytes

In general, the properties of the DDSs are designed to exploit the internalization potential of phagocytes.[58] Macrophages, as major phagocytes, are extensively used to study intracellular pathogenic bacteria. Currently, the most common strategies for specific cellular internalization by macrophages include non-receptor-mediated internalization, receptor-mediated internalization, and biomimetic DDSs.

4.1. Non-receptor-mediated internalization

One of the most common approaches for selectively delivering drugs to phagocytes is by modulating the physical properties (size, surface charge, hydrophobicity, and morphology) of the DDSs. Phagocytic cells prefer the uptake of particles with larger size (500 nm−3 μm) and high surface charge over non-phagocytic cells.[59, 60] Using a non-receptor-mediated approach, it is possible to enter a wider population of cells, although one obvious limitation is that this does not allow for targeting specific cell types based on specific receptors.

(1) Size of DDSs

Yeo et al. designed multi-component polymer-constructed NPs (PpZEV NPs) composed of PLGA, PEG-PLGA conjugate, a chitosan derivative, and Eudragit E100 (E100, a copolymer consisting of dimethylaminoethyl methacrylate, butyl methacrylate, methyl methacrylate at a ratio of 2:1:1).[40] The PpZEV NPs with a size of 500−1000 nm could be selectively taken up by phagocytic cells, but not by non-phagocytic cells. The loaded Van, therefore, could be preferentially delivered into the MRSA-infected J774A.1 macrophage via the 500−1000 nm PpZEV NPs. Consistently, a truffles-resembled Np-pTA-Ag was designed by coating Ag NPs on PLGA NPs (**Figure 3A**).[57] The NP-pTA-Ag with a size of 600 nm was specifically taken up by phagocytic cells (J774A.1 macrophages) rather than by non-phagocytic cells (fibroblasts NIH3T3). Based on this, Ag NPs and the antibiotic pexiganan (Pex) were selectively delivered to J774A.1 macrophage with Pex@NP-pTA-Ag DDS by limiting its uptake by non-phagocytic cells. Besides, SLNs, as a new generation of submicron DDS, also were developed to explore the effect of size on endocytic efficiency in phagocytic cells.[61] The intracellular concentration of the antibiotic enrofloxacin was enhanced significantly as the size of docosanoic acid SLNs increased from 150 nm to 605 nm. Compared with free enrofloxacin, SLNs of 605 nm increased approximately 27.06−37.71 times the intracellular concentration of enrofloxacin and remarkably inhibited 99.97% of intracellular *Salmonella CVCC541* in RAW264.7 macrophages.

(2) Charge of DDSs

The macrophage cell membrane is negatively charged[62, 63] and so cationic NPs generally induce higher cellular internalization in macrophages compared to anionic NPs. Agarwal et al. synthesized PLGA microparticles of different sizes (500 nm, 1 μm, and 2 μm), with and without poly-L-lysine modification resulting in cationic and anionic PLGA microparticles, respectively.[64] Compared with anionic PLGA microparticles, cationic PLGA microparticles were easily phagocytosed by H37Rv *M. tuberculosis*-infected THP-1 macrophages and mouse bone marrow-derived macrophages (BMDM) regardless of their size (**Figure 3B**). This suggested that surface charge is more effective than size in regulating phagocytosis by macrophages when the size of microparticles was larger than 500 nm,

although the result did not rule out the specific influence of poly-L-lysine on phagocytosis. Accordingly, a series of hyperbranched polymers (HBP) were synthesized using three different polymer blocks (*i.e.* poly(ethylene glycol monomethyl ether methacrylate), poly(2-(dimethylamino)ethyl methacrylate), and poly(methacrylic acid)) by reversible addition-fragmentation chain transfer (RAFT) polymerization.[65] These three HBP with similar hydrodynamic radius (around 6–8 nm) but different surface charges (*i.e.* anionic, neutral, and cationic) were constructed by changing the ratio of these three blocks. Flow cytometry quantification revealed that the cationic HBPs tended to be more rapidly and efficiently taken up by RAW264.7 macrophages compared with neutral and anionic HBPs. These studies demonstrated that charge is an important factor to increase the specific internalization of DDSs in macrophages.

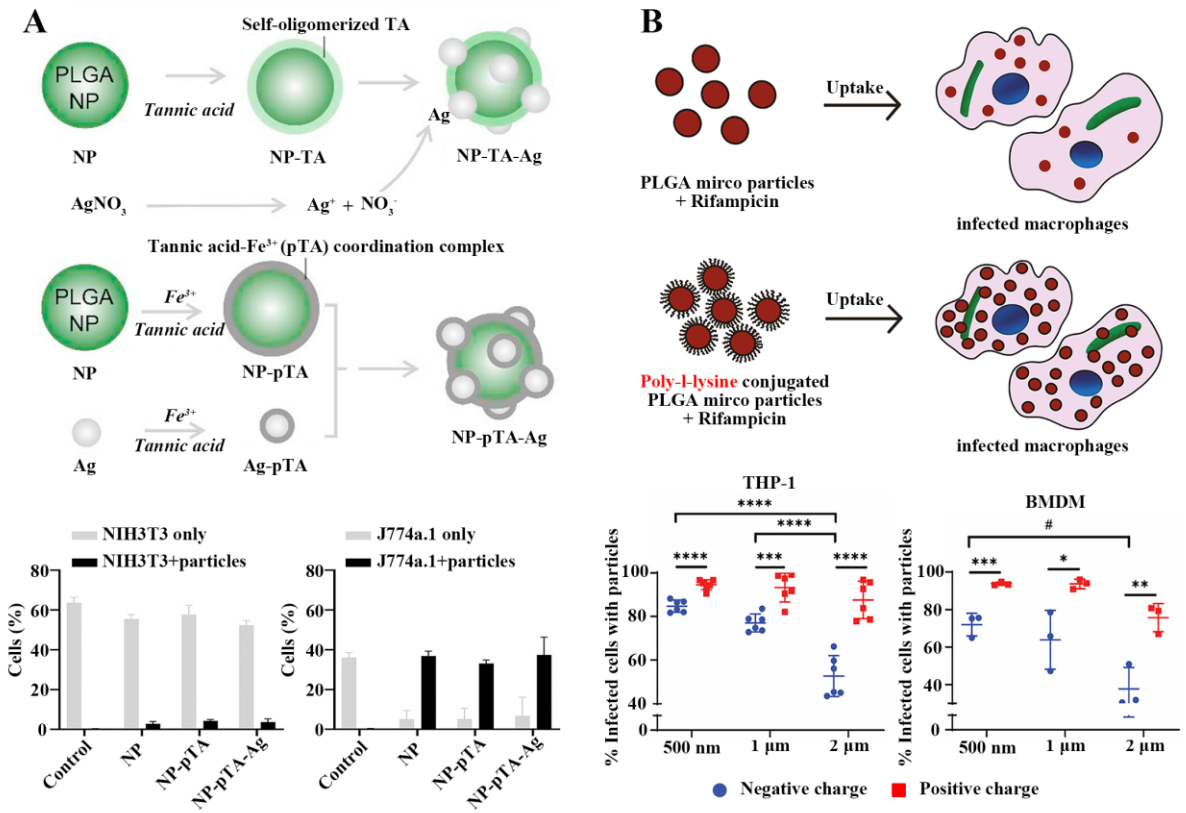

Figure 3. Non-receptor-mediated internalization strategies. A) Schematic diagram of NP-pTA-Ag NPs preparation and representative flow cytometry dot plots of NIH3T3 cells and J774A.1 macrophage co-culture incubated with different conditions. Reprinted with permission.[57] Copyright 2020, Elsevier. B) Schematic diagram of cationic inhalable microparticle for enhanced drug delivery to *M. tuberculosis*-infected macrophages and the effect of microparticle surface charge on uptake by *M. tuberculosis*-infected THP-1 macrophages and BMDMs. Reprinted with permission.[64] Copyright 2021, Elsevier.

4.2. Receptor-mediated internalization

Host cell-specific targeted internalization can be effectively regulated by non-receptor-mediated internalization, but it is difficult to further improve the level of internalization.[66] In contrast to non-receptor-mediated internalization mechanisms, receptor-mediated internalization strategies can more specifically increase the internalization efficiency of the drug by surface modification with specific surface moieties. When DDSs specifically bind to the unique or highly expressed receptors on the cell membrane of the target host cells, they trigger the assembly of lattice proteins under the plasma membrane, which is invaginated and thus taken up into the cell. It is clear that there are overexpressed receptors on the surface of macrophages, such as mannose, galactose, and hyaluronic acid receptors. This is an effective target, whereby conjugating DDSs with active targeting ligands, facilitates the increased intracellular accumulation of drugs in macrophages.

(1) Mannose receptor

Macrophage mannose receptor (MMR, cluster of differentiation 206, CD206), a C-type lectin, can recognize and lead to endocytosis a variety of microorganisms.[67] Therefore, they are mainly present on the surface of macrophages and immature dendritic cells.[68] Mannose is, therefore, commonly introduced into DDSs to improve their internalization in macrophages by specifically recognizing the highly expressed mannose receptors.

A mannosylated nanogel (MNG) with mannosyl ligands conjugated PEG arms and polyphosphoester crosslinked core was reported.[69] Van was loaded into the nanogel and formed MNG-V. MNG-V could preferentially enter into MRSA-infected RAW264.7 macrophages via the interaction of mannosyl ligands with mannose receptors. MNG-V reduced the survival rate of intracellular bacteria more than the free Van. Alveolar macrophages are important phagocytes of the pulmonary innate immune response. Daniel M. Ratner et al prepared a mannosylated ciprofloxacin polymeric prodrug poly(Man-*co*-Cipro) for delivery of drugs to pulmonary via targeting alveolar macrophages.[70] After being nebulized, which would allow it to reach the deep regions of the lung, poly(Man-*co*-Cipro) was internalized by alveolar macrophages via CD206-mediated uptake. Compared to free drugs, poly(Man-*co*-Cipro) delivered greater amounts of drug to the alveolar macrophages and showed efficient clearance of bacteria in mice model of *Francisella* pulmonary infection. Stolnik et al. further exploited the relationships between cellular internalization efficiency and the amount of mannose ligand on the surface of DDSs (**Figure 4A**).[71] They first synthesized a series length of mannose glycolipid ligands, including monovalent Chol-Man_1, oligo-valent Chol-$Man_{10,}$ and Chol-Man_{20} by a controlled radical polymerization. A set of liposome libraries were further fabricated based on different length (0, 1, 10, 20) and density surface distributions (0.5%, 1%,

5%, 10%) of mannose ligands, whose hydrodynamic diameters were between 110 and 170 nm. *In vitro* results showed that there was no significant difference in the internalization by RAW264.7 macrophages between those treated with non-mannosylated liposome L-Chol and monovalent mannosylated L-(Chol-Man_1)$_{10\%}$. However, compared with Chol-Man_1, Chol-Man_{10} and Chol-Man_{20} significantly improved the internalization by macrophages regardless of the surface density. The mechanism for this was proposed to be that the oligo-ligand mannose on the surface could instantaneously occupy multiple C-type lectin domains of mannose receptors. This study demonstrated a clear dependence of cellular internalization on the targeted ligand presented on the surface. Nevertheless, the internalization of mannose functionalized liposomes occurred across the entire population of cells, including uninfected and infected macrophages, and could not limit the exposure of drugs to only the infected macrophages.

(2) Galactose receptors

The macrophage galactose-binding lectin (MGL, cluster of differentiation 301, CD301) is a type II transmembrane glycoprotein containing a carbohydrate recognition domain, specifically for the monosaccharide galactose and *N*-acetylgalactosamine (GalNAc).[72] MGL can be an endocytic receptor for the recognition of glycosylated DDSs.[73] Zhang and co-workers compared the internalization efficacy of PLGA-PEG/lipid hybrid DDSs decorated with different carbohydrate moieties, including mannose, galactose, and dextran.[74] The results showed that the carbohydrate moiety-modified DDSs were all effective in enhancing internalization in RAW264.7 macrophages compared to those without carbohydrate modification. Comparatively, DDSs decorated with the mannose moiety outperformed those decorated with galactose in terms of endocytosis efficiency.

(3) Hyaluronic Acid receptor

The CD44 phagocytic receptor is a cell-surface glycoprotein and it is expressed in cancer cells, epidermal and dermal cells (keratin-forming cells, fibroblasts, and macrophages).[75-77] Compared to uninfected macrophages, the CD44 receptor was found to be overexpressed in infected macrophages.[78] The CD44 receptor can mediate cellular uptake of hyaluronic acid (HA), a linear polysaccharide composed of the alternating units of D-glucuronic acid and *N*-acetyl-D-glucosamine.[77, 79] HA and HA-based NPs were proven to primarily accumulate in intracellular lysosomes via CD44-mediated cellular uptake.[80] Therefore, HA was also used to improve the drug concentration in the lysosomes of infected macrophages. Duan and co-workers constructed an HA-streptomycin (Strep) conjugate via an acid hydrazone bond. The autophagy activator rapamycin (Rapa) was further loaded to form HAASD-Rapa.[81] The complex can enter infected RAW264.7 macrophages by CD44-mediated endocytosis and arrive at the acidic lysosomes, where the hydrazone bond was hydrolyzed and Strep and Rapa were rapidly released. They achieved a

synergistic killing capacity against intracellular *S. typhimurium* combined with bactericidal ability and Rapa-initiated activation of autophagy. Zeolitic imidazolate framework-8 (ZIF-8), a pH-sensitive DDS, was used to encapsulate antibiotic tetracycline (Tet@ZIF-8) to combat intracellular bacteria. Compared with Tet@ZIF-8, HA-modified Tet@ZIF-8 (Tet@ZIF-8@HA, abbreviated TZH) could be effectively internalized by *S. aureus*-infected RAW264.7 macrophages via the CD44-mediated pathway. They were mainly accumulated in the lysosomes, where bacteria loci, and achieved over 98% reduction of intracellular bacteria.[54]

4.3 Biomimetic DDSs

Biomimetic DDSs are novel type of DDSs developed by directly utilizing or mimicking complex biological structures and processes.[82] The most commonly used biomimetic DDSs include natural cell membranes or membranous structures released from the cell into the extracellular environment, such as extracellular vesicles (EVs),[83] exosomes,[84] apoptotic bodies,[85] microvesicles[86] and outer membrane vesicles (OMVs).[87] These DDSs can significantly improve the internalization efficiency of drugs and evade the action of the immune system due to their similarity to host cells.[88]

S. aureus-secreted Evs were collected and purified from culture supernatants of *S. aureus*, which were used to coat antibiotic-loaded PLGA NPs with the membrane-coating technique, forming NP-Antibiotic@EV.[83] These EVs constructs could actively target *S. aureus*-infected macrophages, which would present antigens after initial infection, and enable rapid internalization of NP-Antibiotic@EV. *In vivo* results indicated that NP-Antibiotic@EV improved efficacy in alleviating metastatic bacteremia infection. Mammalian cell-derived vesicles were also developed for intracellular antibiotic delivery. Ramasamy et al. used reconstituted apoptotic vesicles (ReApoBds) derived from cancer cells as a DDS to specifically deliver Van to *S. aureus*-infected macrophages.[85] Based on the natural immune recognition of macrophages, ReApoBds with abundant apoptosis effector proteins could be specifically phagocytosed by macrophages, demonstrating enhanced inhibition of intracellular *S. aureus*. As shown in **Figure 4B**, another approach involved the amphiphilic conjugation of triclosan and Cip, which was synthesized and self-assembled into antimicrobial NPs (ANPs).[89] Next, ANPs were encapsulated into isolated membranes from J774A.1 macrophages to obtain Me-ANPs. When bacteria were engulfed by macrophages, positively charged lysozyme was recruited and captured in the membrane to combat bacteria, resulting in less negatively charged cell membranes of infected macrophages compared to uninfected macrophages. Me-ANPs could be specifically phagocytosed by infected macrophages as a result of the retained high expression of Toll-like receptors that can recognize endogenous (host-derived) molecules and negative surface charge from membranes. Me-ANPs were found to be more effective than ANPs in eradicating acute

S. aureus peritonitis *in vivo*. In contrast to the receptor-targeted macrophage strategy, biomimetic DDSs can only be phagocytosed by infected macrophages, greatly improving DDSs specificity.

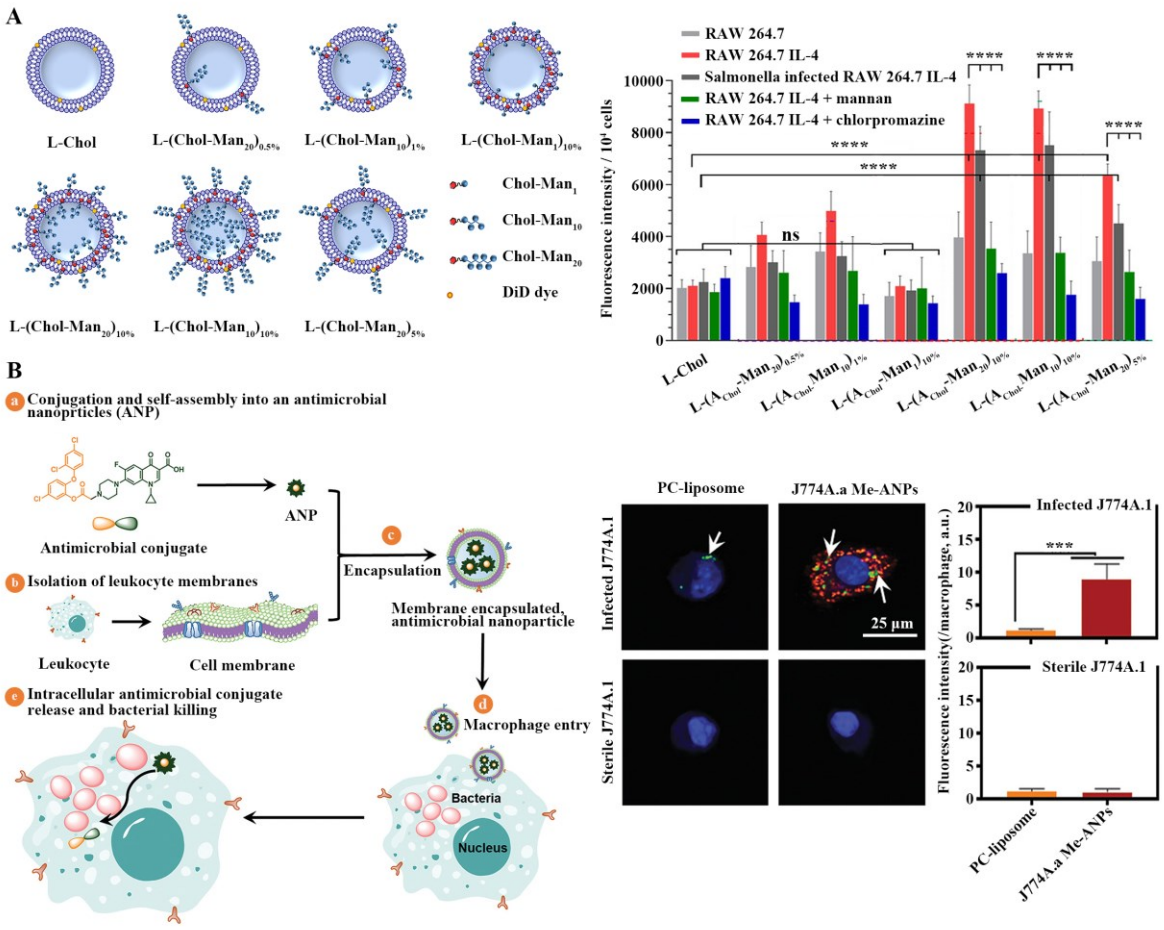

Figure 4. Receptor-mediated internalization strategies. A) Schematic representation of mannosylated liposomes; "L" denotes liposomes, "Chol-Man" denotes mannose ligands with a different number of mannose units: Man_1, Man_{10}, and Man_{20}, for 1, 10, and 20 units; $(Chol\text{-}Man)_{1\%}$, $(Chol\text{-}Man)_{5\%}$, and $(Chol\text{-}Man)_{10\%}$ indicate mol% of cholesterol-containing glycolipid anchor in the liposomal total lipid. And *in vitro* cellular internalization of liposomes by RAW264.7 macrophages under different conditions. Reprinted with permission.[71] Copyright 2021, Wiley-VCH. B) Schematic representation of macrophage-monocyte membrane-encapsulated, antimicrobial-conjugated nanoparticles (Me-ANPs) to kill intracellular bacterial pathogens, hiding inside leukocytes. And in vitro cellular internalization of PC-liposome and J774A.1 Me-ANPs by infected and sterile J774A.1 macrophage, respectively. Reprinted with permission.[89] Copyright 2020, Wiley-VCH.

5. DDSs for targeted eradication of intracellular bacteria

When antibiotics cross the barrier of the host cell and enter the intracellular compartment, their uneven distribution reduces the efficacy of killing bacteria.[14] Therefore, the ability of the DDSs to target the subcellular location occupied by the bacteria is the next major design goal in developing

more efficient DDSs for eradicating intracellular bacteria. As mentioned above, these subcellular locations mainly include intravacuolar, cytosolic, and phagolysosomes, which display a unique physiological environment such as pH, enzymes, and oxidizing/reducing substances. Accordingly, several methods have been used to trigger antibiotic release/DDSs targeting within subcellular locations, including stimuli (e.g., pH, enzyme, redox) responsive drug release and bacterial active targeting (**Figure 5**).

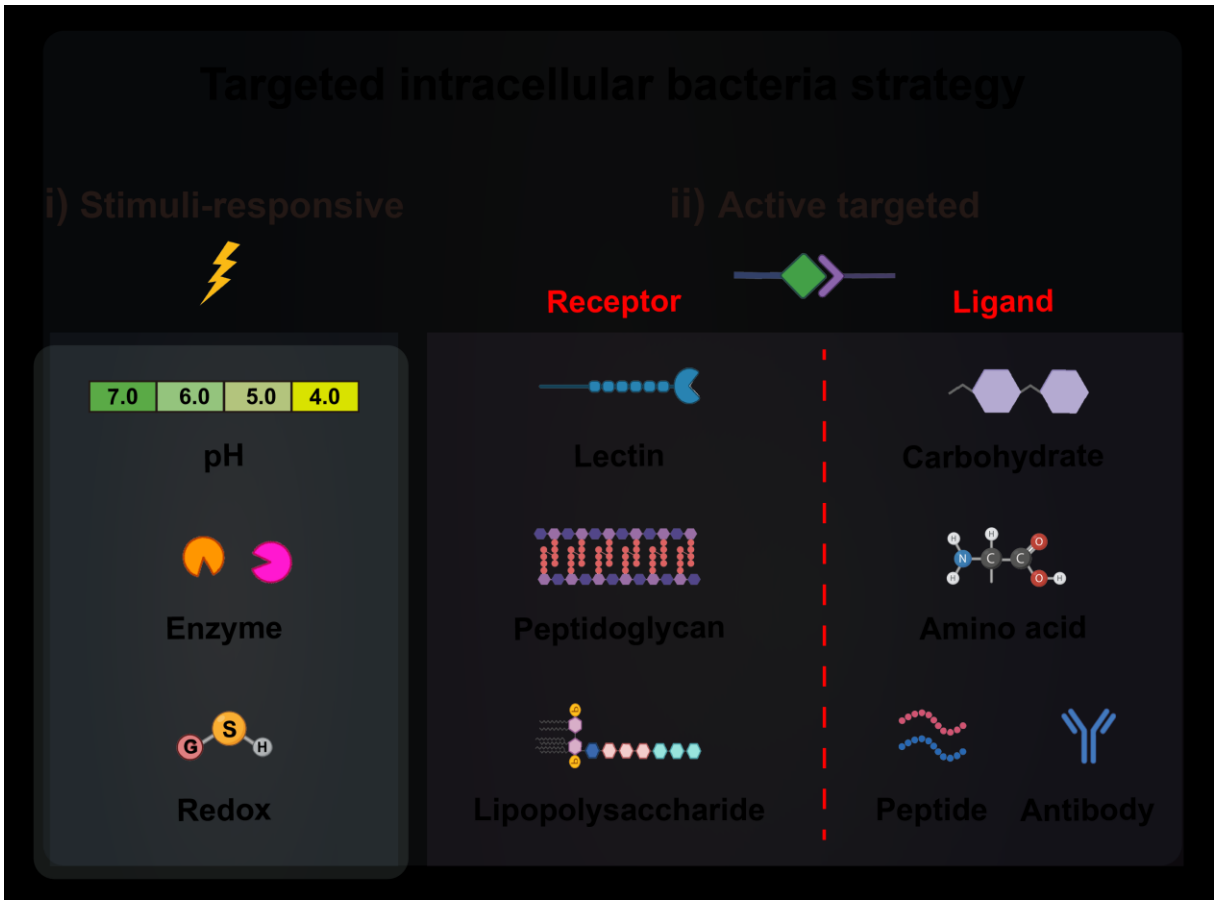

Figure 5. Schematic strategies for targeting intracellular bacteria, including stimuli-responsive and active targeted strategies.

5.1. Stimuli-responsive targeted strategy

DDSs can be designed to utilize the intracellular macroenvironment conditions, such as pH, enzymes, and redox, to enable stimulus-responsive targeted release of drugs on demand.[90]

(1) pH-responsive strategy

In general, when bacteria are internalized into the early phagosome (pH ~6) of the phagocyte, it undergoes a fusion with lysosomes to form phagolysosomes where V-ATPase is accumulated, accompanied by a pH drop to 4–5.[91, 92] Thereby, pH is an obvious potential trigger for stimuli-responsive drug release from DDSs.[93] Some polymers are designed for pH-responsive release drugs,

such as poly(diethylaminoethyl methacrylate) (PDEAEMA),[94] poly(methacrylic acid) (PMAA)[95] and poly(2-(diisopropylamino)ethyl methacrylate) (PDPA).[96] Alternatively, the presence of acid cleavable linkage in the polymer backbone (e.g., hydrazone,[39] schiff base,[97] oxime,[98] acetal/ketal moieties,[99] and ortho esters[100]) can accelerate the hydrolysis of the polymer to release drugs at acidic pH.

As shown in **Figure 6A**, a pH-responsive DDS P(ManAm-*co*-DAAm-hydrazone-INH-*co*-DPAEMA) was synthesized through a polymerization of three monomers, hydrazone bond-linked INH, diisopropylaminoethyl methacrylate (DPAEMA) and acrylamided mannose (ManAm).[101] THP-1 macrophages could specifically endocytose the DDS via mannose receptors. The hydrazone bond in the DDS was stable at extracellular pH (7.4), while it would be hydrolyzed to release INH in acidic phagolysosomes after the DDS was specifically endocytosed by THP-1 macrophages. The DDS increased intracellular INH concentrations, showing an increased antimicrobial activity against intracellular BCG *M. bovis* bacteria compared to free INH. Convertine et al. constructed a polymer brush with pH-responsive properties by RAFT polymerization.[94] The antibiotic ceftazidime was loaded into the polymer brushes and self-assembled into a DDS to clear intracellular *Burkholderia thailandensis*.[102] Upon internalization by RAW264.7 macrophages, the tertiary amine of DEAEMA residues protonated and increased the positive charge density inside the DDS within the phagolysosomes. This led to electrostatic repulsion between adjacent polymer chains. When sufficient charge density was reached, the DDS destabilized and thus collapsed to release ceftazidime to kill bacteria. Similarly, polymer-augmented liposomes (PALs) were developed by binding poly((DEAEMA-*co*-BMA)-*b*-ManEMA) to the liposomes via hydrophobic interaction. PALs were used to deliver streptomycin to alveolar macrophages.[103] They first entered into RAW264.7 macrophages via mannose receptor-mediated endocytosis. Under acidic lysosomal conditions, poly(DEAEMA-*co*-BMA) detached from PALs and disrupted liposome integrity, allowing streptomycin to be released. *In vitro* antibacterial results showed that PALs exhibited about 13−16-fold increased bactericidal efficiency than free streptomycin at doses of ~15 μg mL^{-1} against intracellular *Francisella novicida*.

(2) Enzyme-responsive strategy

(2-1) Cellular enzyme as a trigger for drugs release

Cathepsin B is an intracellular lysosomal cysteine protease. It plays a key role in protein synthesis and degradation within the lysosomes.[104] There is evidence cathepsin B can also recognize and degrade specific amino acid sequences such as Phe-Lys, Val-Cit, Glu-Val-Cit, GFLG, and GGFG.[105-107]

Accordingly, these amino acid sequences are frequently introduced into DDSs to trigger the release of drugs in the intracellular lysosomal environment.

An antibody-antibiotic coupling (AAC) containing cathepsin B-sensitive linkers (MC-VC-PAB-OH) was proposed to effectively kill intracellular MRSA.[108] AAC has no antimicrobial activity when bound to planktonic MRSA; but when AAC was internalized by infected host cells, intracellular proteases in the phagosome cleaved the linker and released the antibiotic in an active form to kill MRSA. Similarly, Stayton et al. synthesized a Cip prodrug monomer by coupling Cip with a protease-sensitive linker valine-citrulline dipeptide linker (VC), and then further developed an inhalable macromolecular prodrug platform (Man-*co*-VC) using RAFT polymerization technique.[109] Man-*co*-VC could specifically release Cip after enzyme cleavage by intracellular cathepsin B. In an alveolar pulmonary infected mouse model, this protease-responsive release of the prodrug platform provided a higher cure efficiency (75% survival) than a similar but slower-releasing prodrug platform. He et al. synthesized an amphiphilic mannosylated pillar[5]arene (Man@AP5) polymer and encapsulated Van to form Man@AP5-Van. Man@AP5-Van could target macrophages via mannose receptors. Following that, Man@AP5-Van was protonated in acidic lysosomal conditions. At the same time, the Phe-Lys linker was cleaved by cathepsin B in the lysosome, leading to the DDS degradation and release of Van. Man@AP5-Van successfully eradicated intracellular MRSA.[110]

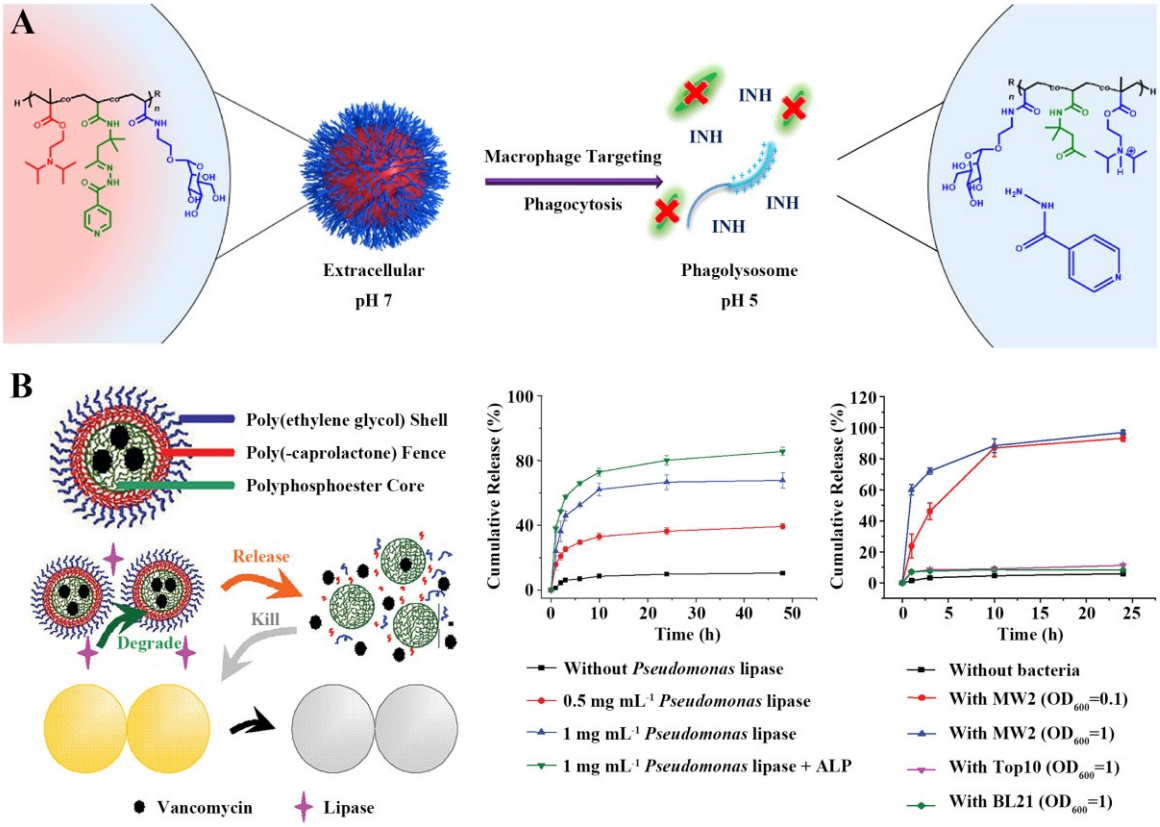

Figure 6. Stimuli-responsive targeting strategies. A) Schematic illustration of the dual pH-responsive macrophage-targeted DDS for intracellular tuberculosis therapy. Reprinted with permission.[101] Copyright 2021, American Chemical Society. B) Schematic illustration of DDS for the on-demand delivery drug triggered by bacterial lipase to treat bacterial infections and the cumulative release curves of Van from TLN-V under various conditions. Reprinted with permission.[111] Copyright 2012, American Chemical Society.

(2-2) Bacterial enzyme as a trigger for drugs release

Intracellular bacteria can produce a variety of enzymes as virulence factors to evade the damage from host cells,[112, 113] such as lipases,[114] proteases,[115] esterase,[113] hyaluronidase,[116] phosphatases, and phospholipases.[117] Therefore, stimuli-responsive DDSs for intracellular infections can also be created using bacterial-derived enzymes. Lipases, for instance, can selectively destroy poly(ε-caprolactone) (PCL). Liposomes can be selectively degraded by phosphatases, lipases, and phospholipases.

A triple-layer nanogel (TLN) DDS was developed for the on-demand delivery of antibiotics to intracellular bacterial sites (**Figure 6B**).[111] TLN was constructed using polyphosphoester as a hydrophobic core, PCL as a sandwich layer, and PEG as a hydrophilic shell. Van was additionally

loaded into the hydrophobic core to form TLN-V. After TLN-V was internalized by *S. aureus* MW2-infected RAW264.7 macrophages, the PCL fence of TLN-V was hydrolyzed by bacterial lipase and released Van to kill bacteria. TLN-V demonstrated a concentration- and time-dependent function on intracellular bacteria growth inhibition. Another innovative approach of mesoporous silica NPs (Gen@MSNs) was developed to treat intracellular *S. aureus* infections.[118] Gen@MSNs contain antibiotic Gen as the core, bacterial toxin-sensitive liposomes, and bacterial targeting peptide ubiquitin (UBI 29-41). First, Gen@MSNs were delivered to the infected tissue on account of UBI 29-41 in a *S. aureus*-induced peritonitis model. At the site of infection, the outer layer of liposomes was degraded by bacterial enzymes (e.g., phosphatases, lipases, and phospholipases). Gen was subsequently released to eliminate intracellular *S. aureus*. Li et al. coupled Cip with desferrioxamine (DFO) to obtain an iron carrier-antibiotic coupling (DFO-Cip), which then generated DFeC with Fe^{3+}. The DFeC was then encapsulated in mannose-PEG grafted with poly(ethyl-bis[2-(acryloyloxy)-ethyl]phosphate-*r*-2-propenoic acid-tetraphenylethylene ester) P(EPE-*r*-TPE) to form mPET@DFeC DDS. [119] mPET@DFeC could be efficiently internalized into macrophages by mannose-mediated endocytosis. Next, lipase and phospholipase triggered the degradation of P(EPE) to release DFeC and Cip. DFeC was then actively taken up by bacteria accompanied by the topical high concentration of Cip, achieving a synergistic bactericidal impact against *S. aureus* in RAW264.7 macrophages. At the same time, degradation of mPET@DFeC mitigated the fluorescence resonance energy transfer (FRET) effect and generated TPE fragments to restore aggregation-induced emission (AIE) activity, which could reflect the presence of bacteria in the host cell and intracellular drugs release through fluorescence changes. These strategies maximized drugs delivery and decreased premature drugs leakage.

(3) Redox-responsive strategy

Intracellular redox regulation is a key process of phagocytosis for killing bacteria in phagocytes. It involves multiple chemical species such as NADPH/$NADP^+$, glutathione/GSSG, and cysteine/cystine.[120, 121] Despite there being only a few DDSs using redox response to release drugs against intracellular bacteria, this mechanism could be adopted as a new strategy. In one example, a DDS was prepared with dextran as the hydrophilic shell and poly(*β*-amino ester)-guanidine-phenylboronic acid (PBAE-G-B) as the hydrophobic core to deliver the Rif to eliminate intracellular bacteria.[122] The hydrophobic PBAE-G-B could be converted to a hydrophilic polymer due to the protonation of tertiary amines and the oxidation of the B-C bond will happen at low pH and high reactive oxygen species (ROS), respectively. Thus, after being internalized by RAW264.7 macrophages, the DDS would be collapsed because of the core transition from hydrophobic to hydrophilic under low pH and high ROS in the phagolysosome, leading to the release of cationic

polymer and Rif. There is a synergistic bactericidal effect against intracellular *S. aureus* infection. A red blood cell membrane nanogel (RBC-nanogel) is another example that using redox to trigger the release of antibiotics. RBC-nanogel was prepared by coating the RBC membrane on a hydrogel that contained cystine dimethacrylate (CDA) as the redox-responsive cross-linker.[123] In the extracellular environment, the RBC membrane spontaneously absorbed and neutralized *α*-toxin secreted by bacteria, promoting bacterial uptake by phagocytic cells. Once inside the THP-1 macrophages, the disulfide bond of RBC-nanogel was cleaved under the intracellular redox conditions, and Van was rapidly released to inhibit intracellular MRSA USA300.

5.2. Active targeted strategy

Active targeted is another modality to enhance drugs selectivity and efficacy for combating intracellular bacteria based on the recognition of a specific structure with a targeting ligand.[124] For example, bacterial lectin, a carbohydrate-binding protein, can specifically bind to sugar chains,[125] such as fucose[126] lactulose,[127] maltose,[128] and trehalose[129]. Similarly, antibodies,[130] peptides,[131] and some molecules (e.g., phenylboronic acid[132]) have an affinity to external structures of bacterial cells. These structures are widely studied for targeting planktonic bacteria,[133] but fewer strategies have been used to target intracellular bacteria to date. These active targeted strategies can significantly increase local drug concentrations at the infected site without compromising off-target sites.[134]

CARGGLKSC (CARG), a cyclic 9-amino-acid peptide isolated from an enriched phage pool, was biopanning *in vitro* on cultured *S. aureus* and further screened in the *S. aureus*-induced pneumonia model (**Figure 7A**).[135] CARG was modified further on the surface of Van-loaded porous silicon nanoparticles (pSiNPs). CARG-coated pSiNPs bonded specifically to *S. aureus* and accumulated in *S. aureus*-infected mouse lungs and skin but not in non-infected tissue. As a result, CARG-coated pSiNP increased Van efficacy by at least 10-fold while decreasing the systemic drug burden. Zhang et al. designed an all-in-one therapeutic DDS with dual-targeted properties for host cells and bacteria (**Figure 7B**).[66] The DDS was made up of three layers: a shell layer of mannosyl- and galacrosyl-decorated hydrophilic chains, an interlayer of dynamic boronic esters composed of glycosyl and phenylboronic acid (PBA) groups, and a core layer of hydrophobic polycaprolactone (PCL), and further encapsulated antibiotic clarithromycin (CLA) formed T-r/40@CLA. The T-r/40@CLA were first internalized into macrophages via mannose and galactose receptors. The PBA moieties on the T-r/40@CLA could then trigger bacterial aggregation by interacting specifically with the diol moieties of polysaccharides found in bacterial cell walls. Finally, the lipase secreted by the bacteria degraded the PCL core and CLA was released, resulting in the efficient elimination of intracellular *S. aureus*.

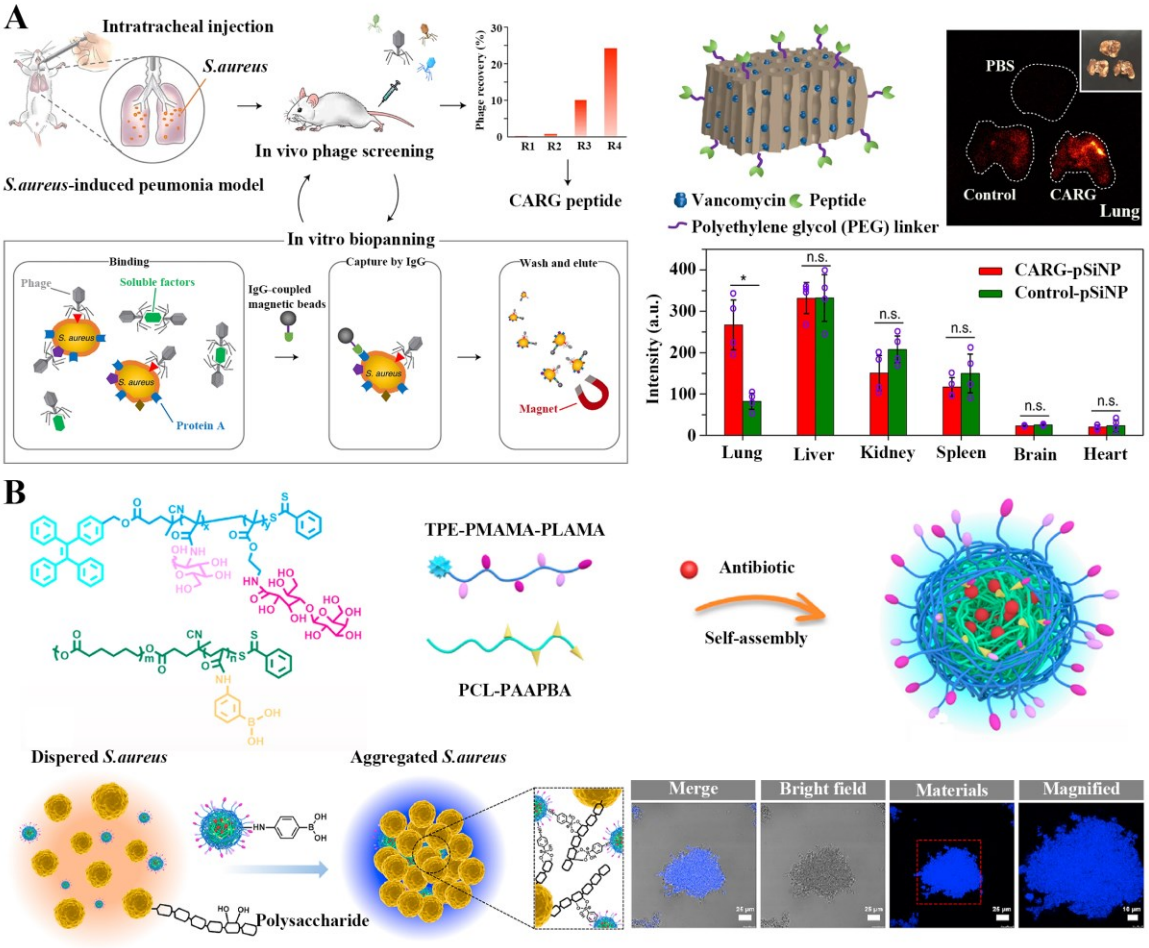

Figure 7. Active targeting intracellular bacteria strategies. A) Schematic illustration of the peptide library screening by *in vivo* phage display in an *S. aureus*-induced pneumonia model (Left). Schematic illustration of the CARG-pSiNPs DDS and their biodistribution in each organ of mice (Right). Reprinted with permission.[135] Copyright 2018, Springer. B) Schematic illustration of the targeted DDS and the aggregation of *S. aureus* induced by the PBA of DDS via multivalent PBA-polysaccharide interactions with the bacteria. Reprinted with permission.[66] Copyright 2022, Elsevier.

Poly(amino acids) have been extensively studied as DDSs due to their excellent stability and biocompatibility.[136-138] It is well established that D-amino acids can be efficiently incorporated into the peptidoglycan of bacterial cell walls.[139, 140] As a consequence, D-amino acids were also been used as an active ligand to target bacteria. A cascade-targeted DDS based on mannose-modified poly(*α*-*N*-acryloyl-phenylalanine)-*block*-poly(*β*-*N*-acryloyl-D aminoalanine) was developed and encapsulated antibiotic Rif (Rif@FAM, **Figure 8A**).[141] The DDS could go through a cascade-targeted process that targeted macrophages and intracellular bacteria in a sequential manner. First, Rif@FAM preferentially entered macrophages via a mannose receptor endocytosis. Subsequently, the

mannose was detached in an acidic phagolysosome and free D-aminoalanine was exposed. Then D-aminoalanine drove the DDS to specifically anchor the intracellular bacteria by peptidoglycan-specific binding. Last, the loaded antibiotic Rif was released on-site to eliminate the bacteria. Intracellular MRSA targeting of Rif@FAM was fully demonstrated via in-situ/ex-situ co-localization analysis (**Figure 8B**). This novel DDS could tolerate acidic environment, protect the activity of antibiotics, reduce the off-target probability, and release antibiotics on-site because of its abundant non-covalent interactions and high-density targeted groups. Rif@FAM shows great potential in dealing with intracellular bacteria.

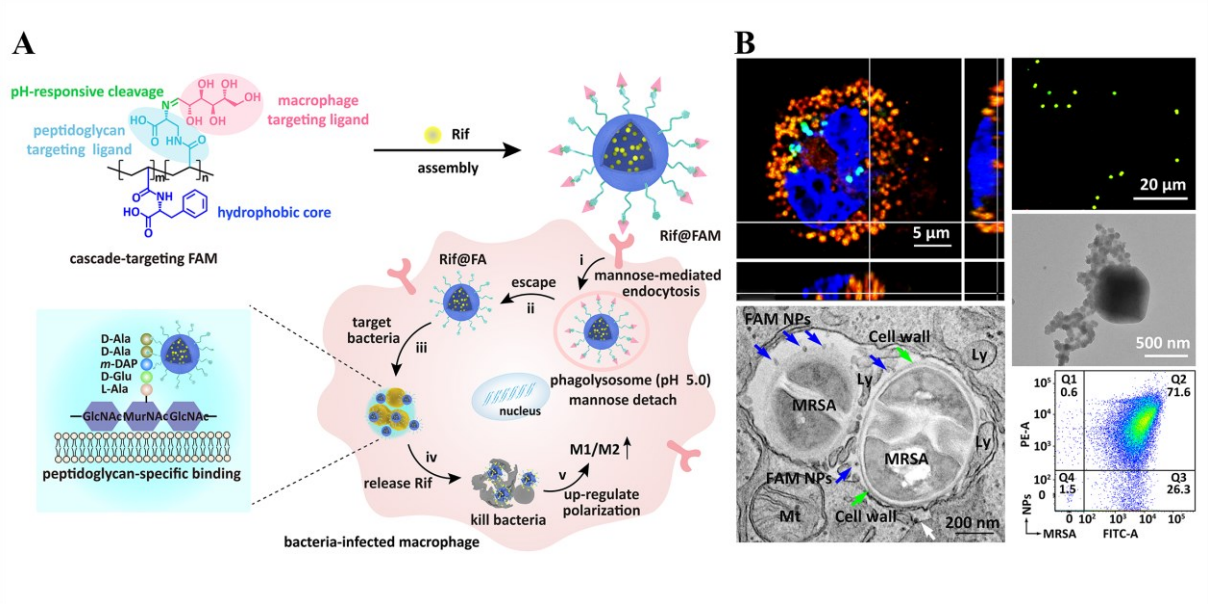

Figure 8. Active targeting intracellular bacteria to deliver antibiotics on-site. A) Schematic illustration of the cascade-targeted DDS for the eradication of intracellular MRSA. B) *In vitro* results for intracellular bacteria targeting. Reprinted with permission.[141] Copyright 2022, Wiley-VCH.

5.3. Immune therapy

There are innate immune responses of phagocytes to pathogenic bacteria, but they can be compromised by bacterial virulence factors through impairing signaling downstream or inducing the autophagy of phagocytes.[142] The DDSs designed to improve the immune response have also been tested in the context of intracellular bacterial infection. As such, some DDSs were designed for increasing or remodeling the immune capacity of host cells.[143, 144] Sailor and co-workers presented a DDS for the delivery of small interfering RNA (siRNA) to enhance the clearance capability of macrophages via exerting high gene knockdown efficacy (**Figure 9A**).[145] The DDS was fabricated by encapsulating siRNA into porous silicon NPs that contained an outer sheath of homing peptides and fusogenic liposomes, namely F-siIRF5-CRV. siIRF5 was introduced directly into the cytoplasmic matrix by specifically membrane fusion between F-siIRF5-CRV with J774A.1 macrophage. The delivery

bypassing endocytosis remarkably reduced the probability of siRNA being excreted from the macrophages. siIRF5 exerted high gene knockdown efficacy in cells, inhibiting inflammatory cytokine excretion from macrophages, and enhancing bacterial phagocytosis. The F-siIRF5-CRV DDS increased the survival rate in a mouse model of *S. aureus* pneumonia. The study is the first successful *in vivo* demonstration of gene silencing for immunotherapy of deep-tissue infection.

In addition, Chen et al. reported a mannose decorated Se NPs and further encapsulated INH (Ison@Man-Se NPs) against intracellular *M. tuberculosis* (**Figure 9B**).[56] Ison@Man-Se NPs were preferentially endocytosed by THP-1 macrophages through mannose receptors and then accumulated in lysosomes. Thereafter, INH was released to kill *M. tuberculosis*. Interestingly, Ison@Man-Se NPs could promote the fusion of *M. tuberculosis* into lysosomes, showing synergistic destruction of bacteria with INH. Ison@Man-Se NPs can additionally induce autophagy sequestration of *M. tuberculosis* in macrophages associated with ROS-mitochondrial and PI3K/Akt/mTOR pathways. This novel nanomaterial-assisted antibacterial strategy through manipulating antimicrobial immunity promises to be a more effective treatment.

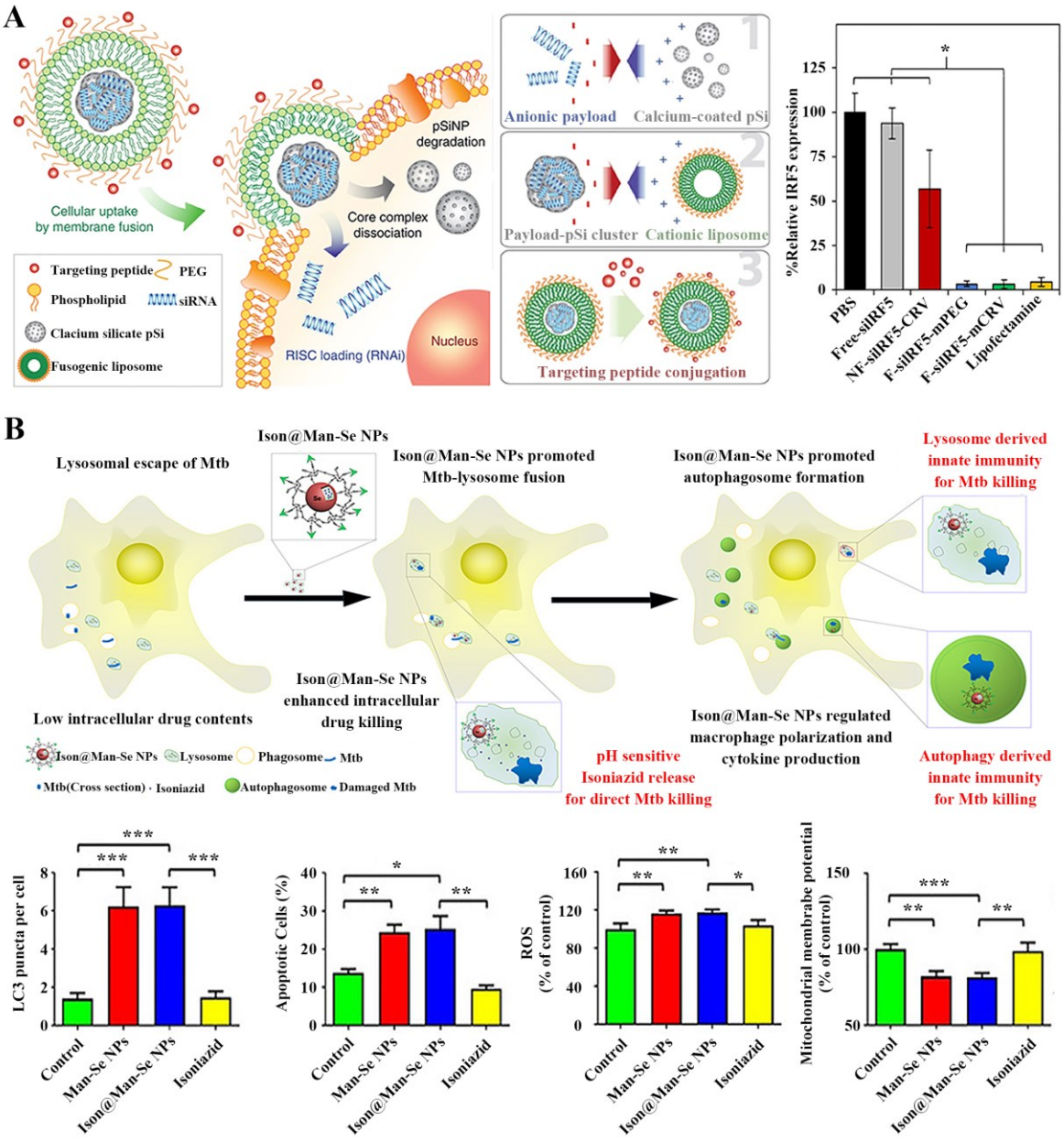

Figure 9. Strategies for improving host immunity to eradicate intracellular bacteria. A) Schematic illustration of the action of the fusogenic pSiNP DDS and siRNA knockdown results from RAW264.7 macrophages incubated with nanoparticles. Reprinted with permission.[145] Copyright 2018, Springer. B) Schematic illustration of Ison@Man-Se DDS-assisted anti-TB strategy for the synergistic killing of intracellular *M. tuberculosis* and the results for enhancing autophagy and apoptosis through PI3K/AKT/mTOR signaling pathway in *M. tuberculosis*-infected THP-1 macrophages. Reprinted with permission.[56] Copyright 2019, Wiley-VCH.

6. Outlook and Future perspective

In this review, we have elaborated on the classification and mechanisms of intracellular bacteria and given a comprehensive overview of the DDSs-based strategies for eradicating intracellular bacteria. According to the whole journey of the DDSs, the involved strategies were summarized as i) specific cellular internalization, including non-receptor-mediated strategies, receptor-mediated strategies, and biomimetic strategies; ii) targeted eradicating intracellular bacteria, including stimuli-responsive targeted drugs release, active targeted drugs release, and immune enhancement.

With a focus on intracellular bacterial eradication, the review highlights the full cycle design and modular construction of DDSs. Designing and constructing these DDSs is not just the combination of materials or ingredients to realize multi-functions. The cascade process of cellular internalization, intracellular drugs release, and bacterial killing needs to be carefully considered to achieve maximum clearance efficacy of intracellular bacteria.

Additionally, the macrophage is the most commonly used host cell type in these studies. The development of DDSs targeting neutrophil and non-professional cells related to intracellular infection is still in the early stages. Recently, Evi et al. created a nanomedicine platform that uniquely utilizes an α_1-antitrypsin-derived peptide to confer binding specificity to neutrophil elastase on activated neutrophils.[146] This innovative approach of cell-specific and activation-state-specific targeting can be applied to several neutrophil-driven pathologies. As such, we believe more and more successes can be achieved accompanied by the development of DDSs in the future.

Herein, we provide some future development directions for improved DDSs for eradiating intracellular bacteria:

(1) On-site drugs delivery

Multiple physiological barriers hinder the delivery of antibiotics, resulting in the attenuated efficacy for killing intracellular bacteria. To maximize the utilization of antibiotics, an ideal situation will be that they are released on the intracellular bacterial site regardless of the physiological barriers.[7, 147, 148] In this case, DDSs are requested to sequentially enter into the host cell, resist the complex intracellular microenvironment, and precisely release antibiotics at the intracellular bacterial site. Accordingly, the design and construction of these classes of DDSs are challenging. These DDSs need to overcome many obstacles, including i) achieving long circulation and overcoming elimination; ii) achieving specific targeting of local sites and infected host cells; iii) tolerating harsh acid and ROS conditions; iv) lysosomal escape and specific arrival at the sites of intracellular bacteria; v) controllable antibiotic release on-site and on-demand.

(2) DDSs-assisted drugs combination

The incidence of multidrug-resistant bacteria is increasing due to the overuse of antibiotics.[149] The combination of multiple drugs therapy may reduce the development of drug resistance by decreasing the dosage of a single antibiotic.[150] In addition, it is possible to combine antibiotics and other drugs for the synergistic treatment of drug-resistant intracellular bacteria, such as cell-penetrating peptides (CPPs),[151-154] anti-inflammatory drugs,[155] and photo/thermodynamic drugs.[156, 157] This combination of drugs for efficient clearance of intracellular bacteria is considered a feasible strategy. However, the multiple physiological barriers described above can alter the intracellular formulation of drug combinations. Its synergistic bactericidal effect, therefore, is hard to fully play in this situation. To address this, DDSs can specifically be internalized and release multiple drugs together, showing great potential for exerting synergistic bactericidal effects in host cells. To achieve this goal, two key problems that need to be considered, including i) how to use DDSs to load different doses of drugs to exert synergistic antibacterial effects; ii) how to release multiple drugs intracellularly at the same time or how to release multiple drugs in synergistic doses for a long duration.

(3) Persistent bacteria

Apart from resistant bacteria, the bacteria that survive high antibiotic concentrations are described as "persistent bacteria".[158] It is reported that persistence is a potential key trigger for treatment failure.[159] Persistence is present in a variety of bacterial species, including *S. aureus*,[160] *M. tuberculosis*, *S typhimurium*, and *Escherichia coli*.[161, 162] In addition to antibiotics, acidification and/or nutritional deficiency of bacteria within the host cell activates various toxin-antitoxin mechanisms, awakening the persistent properties of the intracellular bacteria. This physiological change in the bacteria reduces the susceptibility to antibiotics. For example, most clinically relevant antibiotics kill bacteria by acting on active targets (e.g., *β*-lactams), while these antibiotics are ineffective against persistent bacteria. Nevertheless, a recent and limited success indicated antibiotic-killing effects on persistent bacteria can be reconstructed by altering intracellular bacterial metabolism.[163] The study provides a proof of concept that alkalinizing phagocytosed lysosomes or changing the composition of environmental metabolites by DDSs may be a potential strategy to reverse bacterial persistence and further kill these stubborn intracellular bacteria with the presence of antibiotics.

(4) Multiple tissue barriers

Some bacterial infections, such as those caused by *M. tuberculosis*, result in the recruitment of additional macrophages and other immune cells from the bloodstream to form granulomas.[164]

Furthermore, some bacteria can cross the blood-brain barrier (BBB) and colonize the brain, such as *Streptococcus pneumonia*,[165] *Escherichia coli*,[166] and *L. monocytogenes,*[4] causing diseases like meningitis. It was recently shown that intra-tumor bacteria can metabolize anticancer drugs and attenuate their ability to kill cancer cells.[167-169] Besides, these intra-tumor bacteria mainly colonize cancer cells.[170-172] Antibiotics have difficulty penetrating the granuloma, BBB, and tumor tissue, resulting in increased morbidity and mortality. Therefore, these diseases necessitate the ability of DDSs to penetrate deeper barriers in order to deliver drugs to the site of infection.

In conclusion, the review serves as a resource for the design and construction of novel DDSs specifically against intracellular bacteria. It presents the wide variety of methods used to create DDSs and the specific targeted potential of these as applied to many different intracellular pathogens. These DDSs offer great promise in the fight against intracellular bacterial infection, even in the face of highly antibiotic-resistant, persistent infection.

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Conflict of Interest

The authors declare no conflict of interest.

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The table of contents entry

The systematic design strategies of drug delivery systems (DDSs) to eliminate intracellular bacteria are presented. These targeted strategies are described in terms of the entire DDSs journey, including specifically passing through host cell membranes, sequentially targeted arriving at the intracellular bacterial site, and releasing antibiotics on-demand to eradicate bacteria.

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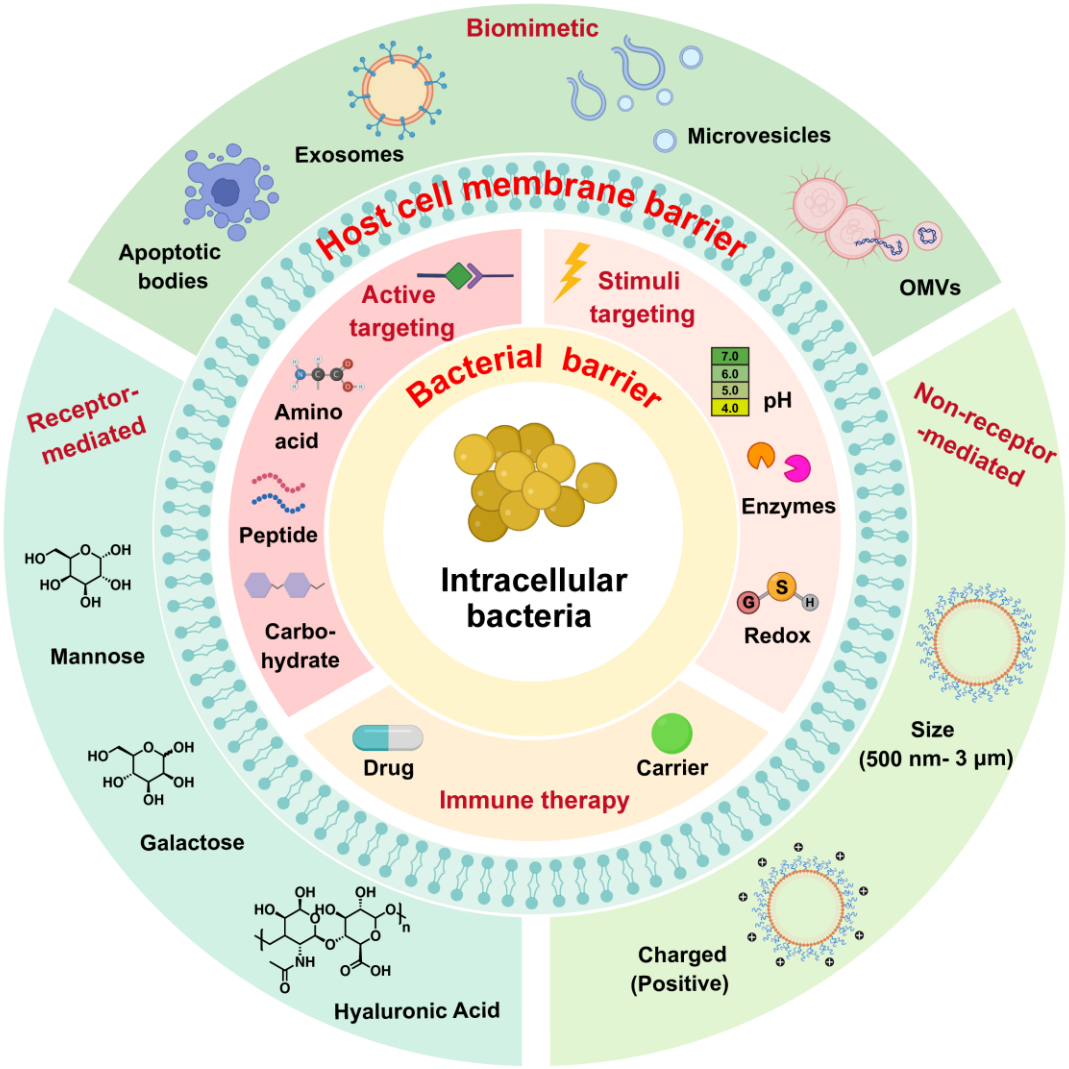
Title: Targeted Drug Delivery Systems for Eliminating Intracellular bacteria



Wenli Feng is a Ph.D. student at the College of Life Science and Technology, Beijing University of Chemical Technology. She completed her B.S. in Biological Engineering at Beijing University of Chemical Technology in 2017. Her research interests include the controlled synthesis of poly(amino acid)s and their biological applications. She is currently a visiting student at the AO Research Institute Davos, Switzerland, where she focuses on the application of biomaterials in orthopedic device-related infection.

Marco Chittò is a Post Doc in the Infection Biology group at the AO research institute Davos, Switzerland. He received his bachelor's and master's degrees from the University of Padua (Italy) in Biology and Evolutionary Biology and his Ph.D. in Microbiology, Molecular Biology and Biotechnology from the University Hospital Münster (Germany). Since August 2021 he focused his research on (i) developing new treatments against S. aureus fracture related infections and (ii) understanding the impact of different drugs on the bone healing process.

Thomas Fintan Moriarty is leader of the Infection Biology group at AO Research Institute (ARI) in Davos, Switzerland. Dr. Moriarty has received his Ph.D. degree from the Queen's University of Belfast. He is a visiting lecturer at the ETH in Zurich and the Bern University of applied science, and is a scientific editor of the eCM journal. His research in ARI has focused upon developing preclinical models of bone and implant infection. More recently, work has focused on the prevention and treatment of infection in models that resemble more closely the clinical situation of fracture related infection.

Guofeng Li received his Ph.D. degree from Beijing University of Chemical Technology in 2017. After working as a visiting fellow in the Chemical Engineering Department at UNSW, Australia, he first worked as a Post Doc and then joined College of Life Science and Technology, Beijing University of Chemical Technology as an associate professor in 2021. His current research is centered on

controlled synthesis and assembly of poly(amino acid)s and their potential antibacterial properties against stubborn bacteria.

Xing Wang received his Ph.D. degree in polymer chemistry and physics at Jilin University. After postdoctoral research at Münster University of Germany, he joined Beijing University of Chemical Technology with the talent honor. Now he is a full professor and doctoral supervisor in College of Life Science and Technology. His research interests mainly focus on biomedical polymer materials, including antimicrobial polymers, hemopstasis and drug delivery system. He has published more than 70 peer reviewed articles (h-index, 31) and 13 patents of China.