Nanoparticles-based technologies for cholera detection and therapy

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ABSTRACT

Cholera is a waterborne disease caused by Vibrio cholerae bacteria generally transmitted through contaminated food or water sources. Although it has been eradicated in most Western countries, cholera continues to be a highly transmitted and lethal disease in several African and Southeast Asian countries. Unfortunately, current diagnostic methods for cholera have challenges including high cost or delayed diagnoses that can lead to increased disease transmission during pandemics, while current treatments such as therapeutic drugs and vaccines have limited efficacy against drug-resistant serogroups of Vibrio cholerae. As such, new solutions that can treat cholera in an efficient manner that avoids Vibrio cholerae's adaptive immunity are needed. Nanoparticles (NPs) are a suitable platform for enhancing current theranostic tools because of their biocompatibility and ability to improve drug circulation and targeting. Nanoparticle surfaces can also be modified with various protein receptors targeting cholera toxins produced by Vibrio cholerae. This review will address recent developments in diagnostics, therapeutics, and prevention against cholera particularly focusing on the use of metal-based nanoparticles and organic nanoparticles. We will then discuss future directions regarding nanoparticle research for cholera.

1. Introduction

Vibrio cholerae (V. cholerae) is a gram-negative bacteria that contains both pathogenic and non-pathogenic serogroups based on the surface antigen lipopolysaccharide composition. Although the majority of V. cholerae serogroups are not harmful, pathogenic serogroups of V. cholerae induce cholera, a diarrheal disease contracted by ingesting contaminated foods or liquids [1]. Cholera affects up to 4 million people and causes up to 143,000 deaths worldwide each year, primarily in underdeveloped countries and regions lacking clean water and sanitation facilities, including South Asia, Africa, and Haiti [2,3]. The diagnosis of cholera is based on clinical observation of patients afflicted with severe acute watery diarrhea. Some infected with V. cholerae do not demonstrate symptoms, which increases the risk of infecting others if fecal matter is left behind in the environment [2].

Upon ingestion, V. cholerae adheres to the intestinal epithelial cells and secretes cholera toxin (CT), which contains an enzymatically active A subunit (CT-A) and five B subunits (CT-B). Endocytosis of cholera toxin by small intestine epithelial cells via the GM1 ganglioside activates the enzyme adenylate cyclase [3,5]. This causes an increase in cyclic AMP (cAMP) concentrations within the intestines, activating protein kinase A, and generating an increased secretion of chloride ions along with water and bicarbonate ions (Fig. 1) [3,6]. The abrupt shift in the ionic concentration across the cell membrane induces fluid secretion from the epithelial cells, resulting in severe watery bowel excretion [3]. Additionally, V. cholerae contains lipopolysaccharides that contribute to its immunogenicity and survivability in hostile aquatic and intestinal environments by forming a biofilm, [7]

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Further contributing to its pathogenicity.

1.1. Current diagnostic, therapeutic, and preventive solutions

*V. cholerae* serogroups mainly differ in their outer (O) side chains, the presenting antigen on the outer surface of the bacteria, which highly vary because of differences in structure, length, and monosaccharide composition across serogroups [8,9]. Although multiple serogroups induce milder diarrheal symptoms, O1 and O139 are the two pathogenic *V. cholerae* serogroups that induce cholera [10]. Since O1 and O139 present different antigens, diagnostic technologies for the detection of pathogenic *V. cholerae* are required to test for both serogroups [8]. Diagnosis for cholera is commonly carried out by testing for *V. cholerae* serogroups O1 or O139 in the stool using antibody-based immunochromatographic rapid diagnostic tests (RDTs), while positive tests are confirmed using cell culture or PCR [2]. Despite being widely used, RDTs vary in sensitivity and tend to generate false positives more frequently than traditional laboratory methods like real-time polymerase chain reaction (RT-PCR) or an enzyme-linked immunosorbent assay (ELISA), which require longer testing times, are labor-intensive, and are costly [11].

On the therapeutic side, cholera has various established treatment options, including oral rehydration therapy (ORT), antibiotics, and vaccines [10]. ORT involves the administration of an oral rehydration solution—typically a glucose or rice-based solution containing vital ions lost in cholera-induced diarrhea [12]. Antibiotic treatment includes the administration of medication that shortens recovery time, diarrheal duration, and the amount of rehydration fluid required. Antibiotics are often reserved for severe cases of cholera because of the possible development of antibiotic tolerance by *V. cholerae* due to evolutionary genetic resistance mechanisms [3,10].

Oral vaccinations are recommended to be used in endemic areas with a high risk for cholera and in conjunction with other cholera control strategies [2]. There are three commercially available vaccines against cholera approved by the FDA: Dukoral (Valneva), Euvichol (EubioLogics), and Vaxchora (PaxVax) [13]. These vaccines stimulate mucosal immunity mediated by antibodies that target O1-specific polysaccharide antigens and CT antigens [10]. However, these vaccines are ~76 % effective on average for three years and require refrigerated storage between 2 °C–8° C [12,14]. Furthermore, Euvichol is the only vaccine that also incorporates O139-specific antigens, with effectiveness ranging from 39–65 % after two years [15]. Thus, improvements in cholera vaccine efficacy and stability without increasing toxicity against patients are needed.

1.2. Nanoparticle potential against cholera

To combat antibiotic resistance, nanoparticles (NPs) have emerged as a potential therapy because NPs can directly modify or degrade bacterial cell walls through reactive oxygen species formation and inhibit bacterial replication without having to internalize within the cell. Bacteria can develop drug resistance by decreasing their membrane permeability to prevent drug entry or changing their metabolic pathways to improve their drug efflux, but NPs, which can act as direct therapeutic agents or drug carriers to improve internalization, can circumvent these issues [16,17]. Additionally, NPs are candidates for cholera detection both clinically and environmentally because of their versatility in carrying specific targeting molecules on their surfaces that enhance binding to CT [18–20]. This allows NP-based methods of detection to have a lower limit of detection (LOD) compared to current methods without compromising specificity and sensitivity [21–23]. NP technology could also improve oral vaccines by enhancing immune response to cholera antigens and increasing shelf stability [24,25].

This review article will provide an overview of existing NP-based technologies for cholera diagnostics, treatment, and disease prevention. Specifically, NP-based detection methods against CTs using electrochemical sensors to test natural water sources prone to contamination and biological fluids from patients will be outlined first, followed by NP-derived therapeutic methods using NPs derived from metal and organic substances to inhibit bacterial proliferation and an overview of NP-based vaccine technology against cholera. Lastly, we will examine future directions regarding NP research on cholera.

2. NP-based diagnostics against cholera toxin

Numerous commercial products are capable of testing for CT, but none of them use NP platforms which can improve the limit of detection (LOD) or detection time. Immunoassays like the BengalScreen Lateral Flow Assay kit have demonstrated an LOD of 10³ colony forming units (CFU)/mL, whereas NP-based methods can achieve an LOD as low as 10⁴ CFU/mL.
NP-based methods of cholera detection have a detection time of 15 minutes, which exceeds current detection methods ranging from 1 to 7 h [21–23, 27–30]. With higher specificity and shorter testing times, NP platforms are a promising candidate to outperform existing commercial products for diagnostics.

2.1. NP-based diagnostics using GM1 ganglioside

To avoid inaccuracies from RDTs that attempt to confirm V. cholerae in stool samples, an alternative approach towards cholera detection is to target secreted CT from the bacteria. The high affinity of GM1 ganglioside for CT makes it a favorable binding agent for receptor-mediated detection (Fig. 2) [6]. Since GM1 can be extracted from the small intestine, GM1-NPs are also highly sustainable and manufacturable. Thus, GM1-NP-based sensors serve as a promising platform for CT detection.

Caco-2 cells, a human intestinal epithelial cell line, exhibit natural GM1 receptors [31]. Kim et al. presented a biosensor that took advantage of sustainable sources with GM1 by extracting those cell membranes to form liposomal structures that could bind to CT-B [11]. Caco-2 cell membranes (CCMs) were collected using sequential centrifugation, which were then seeded onto a carbon electrode that was activated using sulfuric acid. Successful attachment of the CCMs was confirmed through electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV), which detected changes in sensor impedance and current after CCM liposomes were added. Fluorescently labeled CT-B suspended in either water or artificial intestinal fluid was seeded onto the electrode, and EIS and CV were used to confirm the binding of CCM-GM1 NPs and CT-B (Fig. 3). In addition to a 3-week shelf life and 30 min detection period, the resulting sensor had a LOD of roughly 11.46 nM with an effective range between 1 nM and 100 nM, which is comparable to commercial dipsticks and an RDT for cholera detection in stool samples that takes up to 4 h [11, 26, 32].

Alternatively, Viswanathan et al. presented a sandwich antibody-based detection method using GM1-coated liposomes. After fixing an anti-CT antibody onto a carbon electrode, GM1-tagged DPPC liposomes were formed via extrusion. PBS-CT and water-CT solutions were placed onto the electrode to permit adhesion. The liposomes, loaded with ferrocyanide as an electrical marker, were placed onto the electrode and...
lysed to release the ferrocyanide and perform voltammetric analyses. The sensor was even more precise than the CCM sensor presented earlier, reporting that the LOD was around 1 fg/mL while only using 40 ug/mL of antibody for sensor fabrication. Although mass production of liposome-based sensors is underdeveloped because of difficulties with obtaining exact counts of NPs for each sensor, this technology has potential as a diagnostic tool for environmental cholera detection that could potentially move towards clinical trials using stool samples [33].

2.2. Cholera diagnostics using gold NPs

Gold NPs are the most commonly used method for disease diagnostics in research because of their modifiable optical properties that allow for highly specific detection [34]. Khan et al. demonstrated the efficacy of gold NP (AuNPs)-based colorimetric for CT detection. Cholera antibodies were conjugated onto the surface of AuNPs using EDC and NHS as linking reagents. Cholera detection is based on the aggregation of AuNPs due to recognition between the cholera B-subunit and the conjugated cholera antibody. The specificity of the AuNPs to CT was confirmed using DLS and TEM, which observed an increase in NP size in the presence of CT but not with other competing toxins. The change to NP aggregation can be visually discerned by a color shift from red to blue in the sample solution, and UV-Vis spectroscopy confirmed the binding of CT since the absorbance spectrum of the AuNP with CT exhibited a higher wavelength [27].

Ahn et al. took a similar approach in a bioassay utilizing beta galactose-conjugated AuNPs and amine-terminated CdTe quantum dots (QDs) to detect changes in fluorescence resonance energy transfer (FRET) when CT is present [35]. Absorption and fluorescence analysis used UV-Vis spectrophotometry and spectrofluorometry. When galactose-AuNP conjugates and CdTe QDs were mixed in a solution, the fluorescence emissions of the QDs were quenched by AuNPs. This phenomenon was attributed to hydrogen bonding between the hydroxyl groups of beta galactose and the amino groups on the outer shell of CdTe QDs (Fig. 4). The QDs-AuNP complexes formation resulted in a strong FRET signal, but this signal was attenuated in the presence of CT. The basis of detection in sample solutions thus depended upon the specific binding of beta galactose AuNPs to CT, which introduced steric hindrance and disallowed binding of the QDs to AuNPs. The LOD was 280 pM and lower compared to other carbohydrate-stabilized AuNPs colorimetric methods at 54 nM and was far lower than electrochemical sensors like the GM1-based CCM sensor outlined by Kim et al. at 11.46 nM [11,35,23]. The galactose-stabilized NPs maintained stability for up to 6 months and did not experience as much signal loss compared to anti-CT antibody immunoassays [35].

Although the previously outlined methods were shown to be effective, the authors still required advanced equipment for analyzing results which makes it difficult to diagnose in resource-poor regions that cannot afford such equipment. As a solution, Wang et al. developed a method that used AuNPs coated in streptavidin paired with multiple cross-displacement amplification (MCDA) which was testable on 31 V. cholerae serogroups including O1 and O139 [19,25]. The streptavidin-coated NPs were assembled onto a lateral flow biosensor dipstick and MCDA was used to amplify the extracted DNA signal before placement on the test strip. MCDA products could then be quantified to determine the presence of CT, which was further confirmed through colorimetric reactions, gel electrophoresis, and lateral flow assays. The test had an LOD of 4.1 CFUs/mL and took 65 minutes, which was suitable for rapid testing and had a similar accuracy as PCR [19]. This cost-effective and easily manufactured test strip shows similarities to current rapid antigen tests like those used for SARS-CoV-2 detection, which demonstrates its commercial potential.

2.3. Other metal-based NP diagnostics

Although gold remains the most commonly used metal NP because of its wide commercial application, many other metals with conductive properties share similar favorable traits like biocompatibility and low
toxicity [37]. Metal oxides have frequently been used as a component of nanomaterial research because of their ability to immobilize biological molecules like antibodies through covalent binding and their ability to attach to electrode surfaces through electrostatic interactions [38]. Using gadolinium oxide for its biocompatibility, high manufacturability, and ease in creating different physical shapes, Kumar et al. conjugated antibodies for cholera onto gadolinium oxide NPs using 3-(Aminopropyl) triethoxysilane to create an immunosensor [39]. These NPs were immobilized onto an indium-tin-oxide glass electrode and gadolinium nitrate was added as an electrolyte to improve conductivity between the NPs and the electrode. The gadolinium oxide NPs allow for the unique detection of cholera through a change in current. When monoclonal antibodies bind to CT in solution, electrons can no longer freely float between the electrolytes and the electrode because the antibodies no longer have a free site to quickly diffuse electrons towards the electrode [40]. Thus, as CT concentration increased, current through the sensor decreased, detecting cholera at an LOD of 1.48 ng/mL and a range from 5 to 700 ng/mL. Considering this was also tested using urea, glucose, and other potentially interfering bodily fluids mixed with CT, its high specificity demonstrates promising potential as a clinical diagnostic tool [39].

Kaittanis et al. demonstrated another type of metal NP used for CT detection in iron oxide NPs (IONPs) [20]. The NPs acted as magnetic relaxation nanoswitches (MRNs) that could conjugate to carbohydrates like dextran and galactose with high CT-B affinity. The binding of CT-B and the IONPs in solution was detected using magnetic relaxation measurements through changes in the spin-spin relaxation time ($\Delta T_2$) using an NMR analyzer. The $\Delta T_2$ of low-valent galactose and dextran MRNs exhibited a linearly inverse relationship with the concentration of CT-B in solution and were reported to be useful in detecting CTB concentration at 40 pM and 16 nM LOD, respectively [20]. Huy et al. similarly utilized chitosan-coated IONPs, which were then conjugated to Protein A-linked glutaraldehyde and IgG antibodies specific to the O1-CT. These IONPs are bound only to the bacterial wall and flagellum, thus serving as a capture mechanism of the cholerae bacteria via magnetic separation or as an additional component of conventional methods like immunochromatographic strip tests to detect and separate CT in bodily fluid samples [41].

Nonetheless, these strategies still required advanced analytical equipment that may not be available in low-resource communities. As an alternative, Thiramanas et al. combined PCR to amplify cholera bacteria DNA signals with colorimetric reactions for an easy-to-read test for their cholera detection test for water samples. The authors used a magnetic polymeric NP (MPNP) made of iron oxide because it binds to DNA using specific probes, creating a magneto-PCR-colorimetry system. DNA primers were attached to the MPNPs so cholera DNA PCR could be performed directly on the MPNP surface. A magnet aggregated the MPNPs, and the solution was mixed with hydrogen peroxide and ABTS. A colorimetric reaction occurred if cholera DNA was amplified in the solution, and the concentration of cholera could be quantified with a spectrometer. The LOD was $10^3$ CFU/mL, and the reaction took one hour to complete, which proves its potential to be used in point-of-care testing with biological fluids [22].

3. NPs as a platform for therapeutics

Beyond diagnostics, there has also been growing interest in using NPs to treat cholera. Although diagnostic tools typically rely on sensors for detection, therapies require higher standards of biocompatibility, safety, and targeting ability that could allow NPs to directly target cholera while avoiding damage to other tissues. NPs would also circumvent any potential for V. cholerae to metabolize antimicrobial drugs from developed immunities.

3.1. Metal-based NP therapies

One approach is to develop NPs using inorganic microbial agents such as metals and metal oxides. Bacteria are less likely to develop resistance to metal NPs since they make direct contact with the bacterial cell wall rather than act on a specific target/receptor-like antibiotics and fabricating the metal as NPs increases surface area and antimicrobial targeting. [16] Metal agents are also beneficial because they can target and prevent biofilm structures formed by V. cholerae serogroups that lead to higher virulence factors [42,43].

Metal NPs are often formed using green synthesis, a technique that utilizes plant or microbial extracts from organisms like algae and fungi. Metal salts such as silver nitrate or zinc oxide have been used as a substrate to cultivate whole plants prone to metal ion accumulation like mustard greens or alfalfa, where metals tend to be stored as NPs. However, this process is largely inefficient, since metal accumulation widely varies across different regions of each plant, while harvesting the NPs tends to be a high-loss recovery process [44]. Instead, biological extracts from such plants are more commonly used because they often contain terpenoids that are capable of reducing metal salts into an NP particle structure [45-47].

Silver NPs (AgNPs) have been highly investigated due to their toxicity against several microorganisms, biocompatibility with major organs like the kidney and liver, and low cost [48]. AgNPs have been shown to inhibit enzyme activity in V. cholerae and prevent DNA replication. Gahlawat et al. performed a study in vitro where AgNPs were functionalized using a glycolipid protein polymer extracted from microbes. These NPs were around 10 nm in size to maximize the surface-to-volume ratio and were incubated with V. cholerae. Using SEM, the authors observed cell lysis and changes in the morphology of the bacteria incubated with the AgNPs (Fig. 5). The researchers hypothesized this was due to the AgNP binding to the cell membrane and

Fig. 5. SEM images of A) untreated V. Cholerae and B) V. Cholerae treated with GL-AgNPs for 5 hours. Arrows indicate cell lysis. Reprinted with permission from Gahlawat et al. [45] Microbial Cell Factories 15. © 2016 Springer Nature.
disruption in the bacterial membrane structure which causes cellular leakage and cell death [53]. Although this study used chitosan microspheres to target the small intestines, attack the bacteria, and prevent the biofilm formation within mouse intestinal epithelium by inhibiting bacterial growth [43,49]. After infecting mice with V. cholerae, which were previously mentioned as diagnostic tools for cholera, there have also been studies testing AgNPs in vivo; Salem et al. used a mouse model where immunodeficient mice were infected with V. cholerae and treated orally with AgNPs 6 h post-infection. After 24 h, the mice were sacrificed, and their small intestines were removed and incubated in LB broth before the colonization levels were measured. Colonization levels reduced 50-fold in the mice treated with the AgNPs compared to a control group treated with saline [46].

AuNPs, which were previously mentioned as diagnostic tools for cholera, have also been proposed for therapeutic applications because of their ability to carry biomolecules like DNA, proteins, and drugs. [49] AuNPs are highly stable and can be easily synthesized through green synthesis techniques that allow for broader NP applications because of their environmental sustainability [43,49]. Shikha et al. developed AuNPs using sorphorolipids (SL), a glycolipid subclass known for their biosurfactant properties. SLs were extracted from Stenella bombicola cultures and added to an Au-NP-chloride salt solution to form self-assembling NPs of around 40 nm in diameter. SL antimicrobial properties allowed AuNPs to completely inhibit the growth of V. cholerae, inhibited biofilm formation, and killed non-multiplying cells more than commercially available AuNPs, proving that green synthesis could produce viable AuNPs for therapeutics [49].

AuNPs also proved to be disruptive against V. cholerae in vivo. In addition to the study by Shikha et al. that demonstrated that their AuNPs could both inhibit biofilm formation and eradicate mature biofilm in vitro, another study by Chatterjee et al. demonstrated that AuNPs disrupted biofilm formation within mouse intestinal epithelium by inhibiting bacterial growth [43,49]. After infecting mice with V. cholerae intragastrically, AuNPs were orally administered, and the mice were incubated for 12 h before sacrifice. By harvesting their intestines, the authors concluded that AuNPs were able to localize towards the small and large intestines, attack V. cholerae colonies, and inactivate CT to prevent fluid buildup within the intestinal lumen that is characteristic of diarrheal symptoms. Not only were the AuNPs able to alter CT structure to prevent binding to GM1, but AuNPs were also able to reduce CT production from V. cholerae itself. [43] Although this study showed promising results, more studies need to be conducted on how the AuNPs affect biofilm formation in vivo and how the AuNPs are cleared from the mice’s intestinal tract after treatment.

3.2. Therapeutic strategies using organic NPs

Organic NPs such as liposomes have also been investigated for treating cholera. Organic materials are widely regarded as having low toxicity and biodegradability, and they are far more biodegradable and less subject to aggregation compared to metal NPs that cannot be cleared through the kidney or liver because of their large size. Organic NPs also avoid bacterial development of drug resistance because of their ability to conjugate to stealth molecules that allow them to avoid deactivating enzymes that target antibiotics [50]. Additionally, larger organic NPs have a higher chance of retention within diseased sites compared to smaller NPs that fail to target the diseased site and are excreted into the urine [51,52].

NPs can take advantage of the unique properties of the bacterial outer membrane, such as utilizing the membrane surface charge for improved targeting. As a delivery platform, chitosan is advantageous because the positively-charged amine groups disrupt the negatively-charged outer membrane of V. cholerae via electrostatic interactions, reducing cell viability and increasing permeability. Weppelmann et al. demonstrated the efficacy of chitosan as a biomaterial using microparticles that were able to both inhibit V. cholerae growth and exhibit dose-dependent toxicity against V. cholerae. The positive charge leads to the disruption in the bacterial membrane structure which causes cellular leakage and cell death [53]. Although this study used chitosan micro-particles instead of NPs, it outlined chitosan’s advantages as a biomaterial with the potential to act as both a delivery system and therapeutic agent on its own.

Saberpour et al. loaded chitosan NPs with mesenchymal stem cell (MSC)-conditioned media because MSCs would internalize within V. cholerae and produce peptides that silence gene expression. Through an in vitro experiment, the authors concluded that chitosan NPs decrease biofilm formation and toll-like receptor gene expression in multidrug-resistant V. cholerae, reporting approximately a 91 % and 90 % decrease, respectively [54]. Tan et al. also proved chitosan NPs to be carriers for antibiotics like oxacillin and deoxyribonuclease, an enzyme that catalyzes DNA degradation, against S. aureus, a gram-positive bacteria that is also known for biofilm formation [55]. Although more in vitro studies need to be explored to better understand what therapies chitosan NPs can deliver, their biocompatibility and versatility as carriers are highly favorable factors for in vivo and preclinical studies.

An alternative approach to treatment is biological mimics that compete to bind CT before CT reaches epithelial cells. Similarly to Kim et al., Das et al. developed a GM1-NP composed of a polymeric core and lipid shell and observed significantly decreased levels of secreted CAMP in the intestinal lumen with GM1-NP treatment compared to control PEG-NPs after V. cholerae infection [6]. Less CAMP means ion channels are not as activated between the epithelial cells and intestinal lumen, so diarrheal symptoms are less likely to occur. One disadvantage of this approach is that GM1 is expensive to synthesize, thus Heggelund et al. investigated potential low-cost GM1 mimics. Considering galactose is an essential carbohydrate in GM1 function, the authors concluded that bidentate ligands and C-galactosides bind in the primary binding site of CT, making them ideal candidates for a sustained NP delivery platform [13].

4. NP-based vaccines against cholera

In addition to developing diagnostic and treatment options, vaccine development would prevent cholera transmission. Cholera is commonly transmitted through pandemic events. For example, during a cholera outbreak in 2017, the number of reported cholera cases across all six WHO regions significantly increased to a total of over 1.2 million recorded cases with a roughly 4.2 % fatality rate. Although this number has significantly decreased, previous studies still estimate the global burden of cholera to be much higher than recorded numbers at nearly 3 million cases worldwide [4].

Vaccines condition the immune system to fight V. cholerae rather than directly inhibiting proliferation or killing the bacteria locally which could allow bacteria to develop drug resistance. NPs could improve vaccines by improving the targeted delivery of vaccine antigens through surface moieties, prolonging circulation by protecting vaccine antigens from degradation, and utilizing controlled release mechanisms to maintain vaccine effectiveness over long periods [56].

Chitosan is a suitable vaccine material since vaccine antigens can enter through bacterial membranes more efficiently as a result of the positive amino groups [53]. Tabrizi et al. conjugated chitosan to a PLGA NP and loaded it with CT-B. The authors tested their treatment via oral, oral-subcutaneous, and subcutaneous administrations in vivo. In addition to confirming that vaccine treatment results in higher IgA and IgG antibody levels in serum, their immunized animals neutralized CT to a greater extent than a PBS control which was characterized by less inflammation in a rabbit ileal loop test in vivo 18 h after CT injection. In addition, mice that were vaccinated before exposure to CT led to an average survival rate of 50 %, while mice with no treatment had a 0 % survival rate [57]. While this study proved the efficacy of the NPs in vivo, additional characterization such as toxicity and safety profiles will be needed before moving toward clinical studies.

Liposomes have also emerged as a popular drug delivery tool because of their biocompatibility, versatility in carrying lipid and aqueous substances, and customizability in altering size, charge, and lipid composition [58]. Chaicumpa et al. presented a patient study comparing liposome-associated and free antigen oral vaccines against serogroup O1

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N. Ho et al.  SLAS Technology xxx (xxxx) xxx

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made from secreted lipopolysaccharides (LPS), which make up the bacterial outer membranes and contain virulent factors like the presenting O-antigen. LPS was extracted from O17SR, a variant of O1, and liposomes were prepared using sphingomyelin and cholesterol. Both vaccines were loaded with 5 mg of LPS and volunteers were administered vaccines, along with boosters on days 14 and 28. Serum and intestinal aspirates were collected before immunization, and on day 14 and 56 after immunization, and analyzed for anti-CT properties using ELISA. Treatment with the liposome-associated vaccine resulted in higher IgA and IgG production against LPS, CT, and fimbriae, another V. cholerae antigen, within intestinal fluid compared to the free antigen vaccine formulation (19–32%). Additionally, neither vaccine had any harmful side effects. One drawback to this study was that there was no difference in anti-LPS and anti-fimbriae antibody levels in serum, meaning the liposomes did not improve the degradation of cell-cell adhesions that maintained V. cholerae proliferation compared to free antigen. This could be due to the anti-fimbriae and anti-LPS antigens trapped within or on the liposome surface as the liposome traveled through mucosal membranes which also occurred in previous rat studies [24]. Although this study showed promising results, more research needs to be performed on the release mechanism of the antigens from the liposomes and regarding how LPS affect the immune response to improve antibody production in serum. With the continuing development of low-cost cholera vaccines like Hillchol®, that use LPS as antigen carriers, there is high potential for future products that utilize NP platforms to produce vaccines with controlled delivery that prolong their effectiveness and shelf life [24,59].

5. Future directions

Cholera has been a focus of much NP research, including contamination detection, patient diagnosis, and treatment. Several factors contribute to the demand for cholera NP technologies. Traditional toxin detection methods are hindered by cost, time, and accuracy, especially at the point of care. Antibiotic treatments of cholera are becoming unfavorable as antibiotic-resistant serogroups emerge, and current vaccines are limited in effectiveness and accessibility. Therefore, there is a need for alternative treatment strategies. NP methods utilizing CT can also be applied outside of cholera, including therapies against other pathogenic bacteria like H. pylori, Group A Streptococcus, and S. pneumonia [60–62]. Considering CT has also been studied as an immune adjuvant in viral infections and novel drug delivery, further NP research on cholera could have broader applications on numerous other diseases, including cancer, influenza, and motor neuron diseases in western countries that have already abolished cholera [63–65].

NP approaches for cholera include colorimetric detection assays, biological mimic treatments, and vaccines, each with benefits and drawbacks as delineated above. For example, many investigated treatments involve metal-based NPs. Traditional metal-based NPs are usually more shelf-stable and have more product uniformity, but they come from unsustainable sources. The metal NPs presented in some of these studies are synthesized with green synthesis using plant extracts to improve the sustainability and biocompatibility of the product. However, this process is still in development for high-quality, large-scale production, and additional in vivo studies will be needed to compare efficacy to traditional NPs [25].

Before NP-related technologies can be applied in the clinic, standardization regarding the reporting of detection method efficacy among studies will also need to be addressed. Some sensors focus on the detection of V. cholerae itself, while others prioritize the detection of the CT. Studies on sensors for V. cholerae generally count bacterial colonies based on concentration using CFU in Table 1, but this can be inaccurate since it is difficult to distinguish between bacterial colonies from a single bacterium [66]. Others quantify cholera toxin based on density or concentration using mass as shown in Table 2, which makes it difficult to compare sensor efficacy considering some studies only test for specific CT subunits as opposed to CT as a whole. Units of sensitivity, specificity, and limit of detection are also dependent on the type of sensor that is used for detection, where electrical sensors generally use metrics like resistance and impedance to confirm samples while other assays rely on colony concentrations. These sensors also used different mediums including water and salt buffers, many of which were not biological fluids that could alter sensor efficacy in a clinical setting. Further improvements and studies to clarify these discrepancies and standardize such measurements are recommended. Measuring the relationship between chemical and electrical signals from the detection of cholera toxin and V. cholerae bacteria would also provide additional data for sensor efficacy.

Regarding therapeutic applications, there is potential for NP-based treatments to transition from preclinical to clinical studies. However, additional studies will be needed to predict pharmacokinetic and pharmacodynamic properties for patients from animal studies. Some studies have shown that NPs are prone to degradation in biological fluids as proteins, peptides, lipids, electrolytes, and other metabolites can bind

<table>
<thead>
<tr>
<th>Device</th>
<th>LOD</th>
<th>Specificity*</th>
<th>Sensitivity*</th>
<th>Testing Time</th>
<th>Shelf Life</th>
<th>Sample Medium Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>BengalScreen SMART Kit</td>
<td>2*10^7 CFU/mL</td>
<td>95–100 %</td>
<td>Up to 6*10^7 CFU/mL</td>
<td>5 min</td>
<td>1 yr</td>
<td>Stool [26]</td>
</tr>
<tr>
<td>Gold NP based sensors for V. cholerae</td>
<td>410 CFU/mL</td>
<td>100 %</td>
<td>Up to 4.5*10^6 CFU/mL</td>
<td>65 min</td>
<td>N/A</td>
<td>PBS, shrimp homogenates [19]</td>
</tr>
<tr>
<td>Iron oxide NPs with PCR</td>
<td>10^6 CFU/mL</td>
<td>80–90 %</td>
<td>10^6–10^7 CFU/mL</td>
<td>60 min</td>
<td>N/A</td>
<td>Tris buffer, salt buffer [22]</td>
</tr>
</tbody>
</table>

* Specificity refers to the true negative rate of a sample with no cholera-related substance.

* Sensitivity refers to the optimal concentration of CT for the sensor’s detection efficacy.
to the surface of the NP and disrupt their stability and structure [67,68]. Additionally, although NPs are known to restrict the development of antibiotic resistance against NP-based therapies. More studies into the mechanisms behind bacterial gene transfer during NP treatment would help to elucidate methods that minimize bacterial NP resistance [50,67]. Nonetheless, recent research into NP technologies against cholera showcases the potential to advance existing commercial sensors and current treatments, providing a promising outlook for future research on cholera and other anti-bacterial NP-based technologies.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: E. coli cholera and other anti-bacterial NP-based technologies.

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