18 Nanotechnology in Fish Health and Welfare: Recent Advancements and New Perspectives

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Abstract

Nanoscience, where objects are measured at one billionth of a meter, enables the particles with the unique properties that function as a unit within the size range. In aquaculture, nanotechnology has a wide range of applications, from the delivery of drugs, nucleic acids, peptides, feed, nutraceuticals, etc., to the water treatment system. In general, nanotechnology has started replacing the antiquated fish production systems especially in breeding, disease management, and postharvest technology. In the fish disease management system, the nanostructured materials are being used as immunomodulatory substances to more efficiently manipulate or deliver immunologically active substances to the target location. Starting from the disease-causing bacteria to the deadly viruses, nanomedicine at a lower dosage has been used to curb all kinds of infections at a faster rate in aquaculture species. In this chapter, the general outline of nanotechnology and its current use in fish production management is discussed. Further the current trends of the use of nanotechnology in fish disease prevention are thoroughly summarized. Successful applications of nanotechnology in the field of fish disease management enable to develop new effective vaccines, adjuvants, as well as immunomodulatory drugs to enhance clinical outcomes in response to a variety of noninfectious and infectious diseases.

Keywords

Nanotechnology · Breeding · Disease · Immunomodulatory substances · Vaccines

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S. K. Gupta, S. S. Giri (eds.), *Biotechnological Advances in Aquaculture Health Management*, https://doi.org/10.1007/978-981-16-5195-3_18

18.1 Introduction

Nanotechnology deals with the science of developing and using particles of nanometer size. In definition, the particles that fall in the size between 1 and 10 nm is categorized under nanoparticles (Nps). Broadly speaking, Nps can be in the form of ultrafine nano-sized particles that are typically found in nature or can be constructed and designed artificially for a specific purpose (Oberdörster et al. 2005). At the nanoscale, the properties (electrical, catalytic, magnetic, and thermal) of the substances change which allows them to have tremendous applications in the biomedical, environmental, and other scientific fields. The specific properties of Nps allow them to have novel applications that otherwise are not possible to harness from a substance when it is present on other measuring scales in nature. Due to unique applications, nanotechnology has become an extensive area of study. Nanotechnology has created a significant impact in almost every field of science. In the biological field particularly in medicine, Nps are being used in diagnostics, vaccines, drug, and gene delivery. This science is concerned with the design and utilization of biomedical uses of Nps and nanodevices (Cavalieri et al. 2014). Since the discovery of the unique properties of the Nps, scientists are trying to include more and more substances into the biological field for specific functions. The available Nps are classified based on their origin, formation, size, shape, and applications in different areas. The small size of the Nps makes them have a reasonably higher surface area to volume ratio than the usual forms. The nanomaterials can be produced as a surface film (one dimension), strand or fibers (two dimensions), or as particles (three dimensions) in various regular and irregular shapes such as rods, tubes, wires, etc. The Nps can be a sphere-shaped in which substances get adsorbed or attached on the surface, nanocapsules that encapsulate the drug, tubes (carbon nanotubes), liposomes, branched dendrimers, and polymeric Nps. The different types of Nps along with their applications are presented in Fig. 18.1.

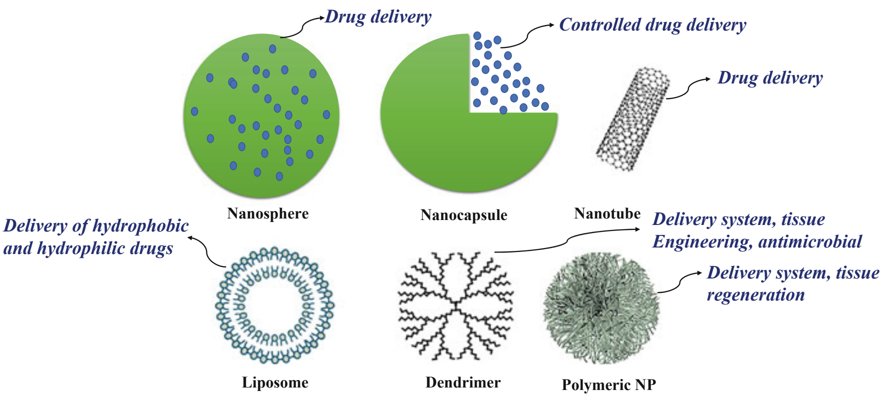


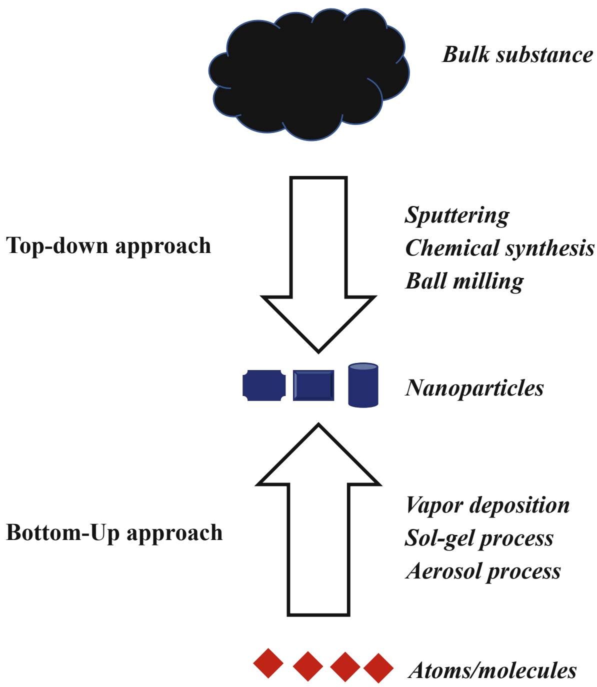
Fig. 18.1 Various types of Nps used in aquaculture and their applications

As far as fishery and aquaculture are concerned, Nps have a wide range of applications including wastewater treatment, sterilization of fishponds, fish packaging, barcoding, and tagging as indirect use and in feeding and healthcare as direct use. The different Nps are continuously used which poses a threat to the environment due to the nanotoxicity associated with them on biological species including plants, humans, fish, and other invertebrates. Many studies have been performed to know about the potentially harmful effects of Nps as well as to determine the dosage that is considered safe for the tissue or cells. It allows the establishment of a safe concentration limit to be applied. In this chapter, we are dealing with the applications of nanotechnology in fish healthcare and other fields related to increasing aquaculture production.

18.2 Synthesis of Nps

The key methods for nanoparticle synthesis are categorized into top-down or bottom-up approaches (Holmes et al. 2003). In the top-down approach, the bulk substance at macro or microscale is ground to transform it into the nanoscale, which is accompanied by the addition of stabilizing agents that protect the Nps from aggregation or instability. In the bottom-up approach, the Nps are created from atoms or molecules by different processes like vapor deposition, sol-gel process, etc., to convert them into nanomaterials (Fig. 18.2).

Fig. 18.2 Top-down and bottom-up approaches for the synthesis of Nps



18.2.1 Physical, Chemical, and Biological Synthesis

The physical methods to produce Nps involve the use of mechanical pressure, electrical energy, radiations, thermal energy, etc. These processes bring out the changes in the material like abrasions, evaporation, and melting which results in the Nps formation. The physical methods have the advantage of being fast without the use of toxic substances, but productivity is usually lesser compared to other methods. Physical synthesis using microwaves is being used to produce the silver Nps, and in comparison, with the thermal method, it is faster and produces a higher concentration of Nps at a given temperature and exposure. Laser ablation is one of the simple methods of production of Nps, but the high cost limits its usage. Ball milling is the process of reducing the higher size of the particles and blending them into new phases.

The chemical synthesis involves the bottom-up approaches, and the process involved is called nucleation where the use of water-soluble cations triggers the reduction of macro substances to metal monomers. The metals are allowed to remain in a chemical solution, and after chemical reactions, the Nps are formed (Stepanov et al. 2014).

Synthesis of nonmetallic Nps is on a trend nowadays, and different methods of their preparation have been validated. The chitosan Nps are prepared by the ionotropic gelation, with the addition of polyanion tripolyphosphate (TPP) which acts as a crosslinker between the chitosan molecules. The technique involves the usage of acidic solutions like acetic acid in which chitosan is dissolved, and the approach depends on electrostatic contact between the chitosan amine group and polyanion polymer groups. The TPP is added drop by drop to allow the proper cross-linking between the chitosan molecules (Bhat et al. 2018a). Other methods are the microemulsion method which utilizes the addition of surfactants, with the major disadvantage of the usage of organic solvents, prolonged preparation time, and the difficulty of the washing processes. The most common method for synthesis of Poly D, L-lactide-co-glycolic acid (PLGA) Nps is the precipitation method combined with the double emulsion solvent evaporation method.

The biological method also known as green synthesis is the most eco-friendly approach to produce Nps. Biologically synthesized Nps involve the usage of bacteria, fungi, and plants. This method comes under a bottom-up approach that mostly involves reduction/oxidation reactions (Prabhu and Poulose 2012). The enzymes present in the microbes and chemicals in plants have antioxidant or reducing properties that act on precursor compounds to produce the desired Nps. So, according to Prabhu and Poulose (2012) for the biosynthesis of Nps, there is a requirement of three systems. Viz., a solvent medium for synthesis, a reducing agent, and a stabilizing agent.

Antibacterial activity and cytotoxic effects against a human lung cancer cell line were demonstrated by silver Nps synthesized using *Origanum vulgare* leaf extract. Cashew nutshell liquid was used for the green synthesis of both silver and gold Nps, which showed bactericidal activity against several fish pathogens (Velmurugan et al. 2014). In juvenile *Feneropenaeus indicus*, silver Nps synthesized using tea leaf

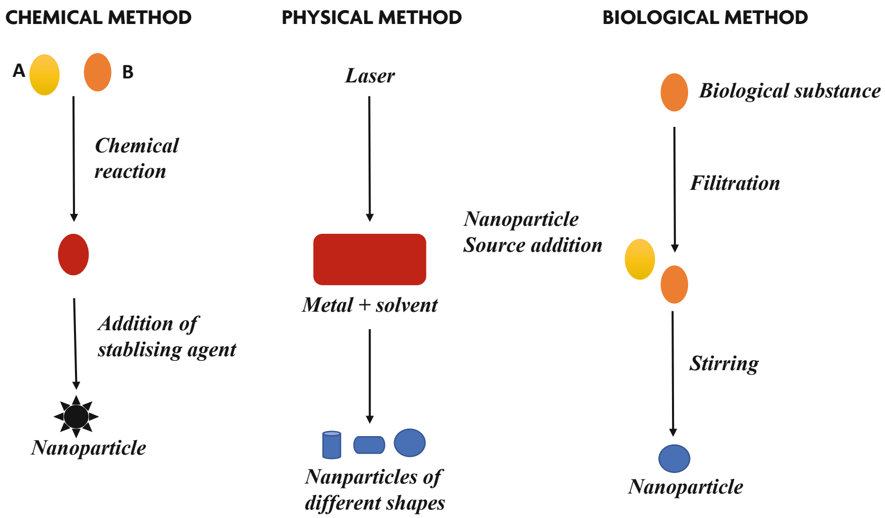


Fig. 18.3 Different methods for synthesis of Nps

extract (*Camellia sinensis*) exhibited bactericidal activity against *Vibrio harveyi* (Vaseeharan et al. 2010). For the green synthesis of zinc oxide Nps (ZnO Nps), a broth of aloe leaf extract was used, which have shown higher bactericidal activity than Nps by standard chemistry (Gunalan et al. 2012). A novel method for the biological synthesis of zinc oxide Nps uses *Aeromonas hydrophila* bacteria as the reducing agent. This method is environment-friendly and economically viable and produces ZnO Nps with antibacterial and antimycotic properties. The illustration of different methods used for NP synthesis are shown in Fig. 18.3.

18.3 Nanotechnology in Aquaculture

18.3.1 Application in Feed Technology

In fish feed technology, nanotechnology is emerging as a fast technology to be used for many processes including delivery, protection, and stability of the enriched feed particles. Also, some of the Nps act as growth promoters as well as immunostimulants and have been used in fish with significant results. Some of the examples include the usage of chitosan-coated calcium alginate to prevent shark liver oil leakage (Peniche et al. 2004). Similarly, chitosan prevented tuna oil from degradation (Klinkesorn and McClements 2009), and usage of carbon nanotubes prevented the leaching of nutrients from feed (Fraser et al. 2011; Bisesi et al. 2015; Ramsden et al. 2009). In Nile tilapia, the nanospheres loaded with nerolidol increased survivability and prevented the fish from oxidative brain damage when infected with *Streptococcus agalactiae* (Baldissera et al. 2020). The use of selenium (Se) NPs has boosted the growth and immunity of many aquaculture species. It increased the beneficial cellular responses in the crucian carp and promoted the growth in another fish species (Wang et al. 2013; Deng and Cheng 2003). Nano Se-supplemented fish diet prevented the particle dissolution, aggregation, and release of feed and was not toxic to fish compared to Se alone (Monikh et al. 2020). Se NPs along with riboflavin reduced the stress responses caused due to high temperature and arsenic pollution in *P. hypophthalmus* (Kumar et al. 2020). Zinc (Zn) in nano form improved growth and immune response in grass carp (Faiz et al. 2015) and increased growth performance in rainbow trout (Ramsden et al. 2009). Zn NPs supplemented through feed were found to increase the growth rate in fish compared to inorganic Zn (Mondal et al. 2020). Bhattacharyya et al. (2015) investigated the use of nanomaterials to increase the proportion of nutrients passing through the gut tissue.

18.3.2 Application in Fish Reproduction

In fish, the NPs have been used to deliver the peptides, hormones, and genes to sustain their effects for longer durations. Chitosan conjugated with Au NPs were used to deliver salmon GnRH in common carp which resulted in increasing the reproductive parameters (Rather et al. 2013). Similarly, chitosan-conjugated LHRH increased the transcript level of gonad-developing gene, Sox9, in fish and reproductive hormones in *Clarias batrachus* (Bhat et al. 2016). Chitosan NPs sustained the plant extract eurycomanone an extract of *Eurycoma longifolia* which resulted in increasing the gene expression as well as the reproductive parameters like fertilization rate, the hatching percentage, and survival rate in *C. magur* (Bhat et al. 2018, 2019). Chitosan-conjugated aromatase inhibitors were used to augment the gonadal development of male *C. magur* (Wisdom et al. 2018). In male Asian catfish, the chitosan was used to deliver methyltestosterone for increasing reproductive performance (Saha et al. 2018). In one study, the StAR gene construct which is important to start the steroidogenesis inside the cell was delivered through chitosan Nps in *C. batrachus*, and results indicated the increase in gonadal transcript of testes and reproductive hormonal profile (Kumar et al. 2017).

18.3.3 Nanotechnology in Water Treatment of Aquaculture Pond

The water contamination of pond due to excess feed or environmental conditions or intentionally (pesticides, chemicals, antibiotics) creates stress to the aquatic animals which result in their poor growth or mass death. So, the pond water must be either replaced or aerated continuously, which makes the management difficult. Nanotechnology has been applied to purify the water by using nanoengineered substances. The best example to remove harmful substances, heavy metals, and noxious gases from pond water through nanotechnology is the usage of metallic Nps and carbon nanotubes (Ren et al. 2011; Xu et al. 2012; Pradeep 2009; Rather et al. 2011; Chen et al. 2013). La (Lanthanum) oxide Nps are useful in treating water to make it free from disease-causing bacteria like *Chlorella vulgaris*, *Escherichia coli*, *Penicillium roqueforti*, and *Staphylococcus carnosus* (Gerber et al. 2012).

Nanocheck, a La-based device, is used to get rid of phosphates in pond water (Mohd Ashraf et al. 2011). Nanodevices improve the water quality in shrimp ponds thereby reducing the water exchange rate (Wen et al. 2003). Another problem related to cages, pens, nets, and ships is biofouling which results in the degradation of the surfaces by bacteria (Champ 2003). Antifouling paints rich in metallic Nps are being used, and they have been found more effective than the normal paints used to curb biofouling (Ashraf and Edwin 2016). The bacterial antibiofilm activity of Ag and Au Nps synthesized from *Turbinaria conoides* extracts was evaluated by Vijayan et al. (2008), highlighting that Ag in nanoform was successful in preventing the formation of biofilms.

18.3.4 Advances in Drug and Gene Delivery

Nps are an ideal delivery system for the transfer of drugs, vaccines, and peptides in the living cells or tissues. The Nps used for the delivery should be safe, biocompatible, and biodegradable and should protect the conjugated substance for a longer duration (De Jong and Borm 2008). Lots of Nps are being used in the process, but the polymeric Nps including chitosan and PLGA are mostly safe with least toxicity for the living cells. The chitosan Nps have the property to slowly release the drug and sustain it for longer durations (Bhat et al. 2018). It has been used for the delivery of peptides, plant extracts, vitamins, genes, etc. (Alishahi et al. 2011; Rather et al. 2013; Kumar et al. 2017; Bhat et al. 2018). Chitosan Nps coupled with vitamin C after administration in rainbow trout showed that the release of the vitamin was regulated up to 48 h. Further, the innate immunity, as well as nonspecific defense mechanisms, were upregulated in the treated fish which is mainly because of the synergistic effects of vitamin C and the chitosan polymer (Alishahi et al. 2011). All the studies indicated that the effect of the delivery agent remained for longer durations without any harmful effect in fish. Similarly, PLGA a copolymer of polylactic acid and polyglycolic acid has the same properties as chitosan and is used as a delivery agent in many fish species. In zebrafish, it was used to deliver rifampicin to treat *Mycobacterium marinum* infections, in rohu for the delivery of *A. hydrophila*, and DNA vaccine against lymphocystis in Japanese flounder (Behera et al. 2010; Tian and Yu 2011; Fenaroli et al. 2014). These studies indicate that PLGA can be effectively used in the delivery process in aquaculture species with a lower risk. Furthermore, Nps dependent on silica can be used for drug administration because of its porous structure and capacity to integrate at elevated doses (Strømme et al. 2009).

18.3.5 Advances in Aquaculture Species Health

The intensification of aquaculture has increased the production exponentially. The modern facilities for breeding, rearing, and feeding of aquaculture species have led to a boom in the aquaculture industry, but at the same time, it has led to the introduction of deadly disease-causing agents (Pulkkinen et al. 2010). Most of the aquaculture farms are either using traditional methods to eradicate harmful substances or using antibiotics to treat the diseases. The use of excess antibiotics has led to an increase in antibiotic-resistant microorganisms which is becoming very difficult to control. To limit the use of excess antibiotics, nanotechnology plays an important role. The use of Nps can decrease the dosage of antibiotics which will be effective for longer durations thereby minimizing the harmful effects. Nps act as a potential antimicrobial substance, and the antibiotic-resistant bacteria conjugated with nanoscale particles can be delivered as vaccines for disease prevention (Shaalan et al. 2016).

Silver Nps possess an antimicrobial property and has been tested for it (Mathur et al. 2014). The ions ($Ag+$) present in the silver Nps connect with the membranes and proteins of bacterial cells and destruct them which results in cell death (Lara et al. 2010; Huang et al. 2011). The *Azadirachta indica*-constructed Ag Nps resulted in the resistance against *A. hydrophila* in mrigal (Rather et al. 2016). In the study higher survival rate was detected compared to the control group suggesting that Ag Nps have an immunomodulatory and antibacterial role. Chitosan-Ag nanocomposites were tested for their antimicrobial property against fish pathogen *Aliivibrio salmonicida* (Dananjaya et al. 2016). The results indicated that it acts as a potential antibacterial agent against the disease. Antony et al. (2013) constructed Ag Nps using Aspera and suggested that it has an antimicrobial property against *A. hydrophila* infections in *Catla catla*. Ag NPs and Zn oxide Nps inhibited the growth of *A. salmonicida*, *A. invadans,* and *Yersinia ruckeri in fish* (Shaalan et al. 2017). Other than Ag and Zn, the other metallic Nps having antimicrobial activity is copper, and it has been tested in many fish species. Ag Nps have been used to treat fungal infections in rainbow trout eggs, whereas ZnO Nps disrupted the bacterial cell membrane (Johari et al. 2015; Pati et al. 2014).

18.3.6 Nanotechnology in the Diagnostics

Nanotechnology has strengthened its roots in the field of diagnosis in the biomedical field, and now in the aquaculture industry, there has been a shift towards the usage of nano-constructed diagnostic kits for pathogen detection. Gold Nps are the most used ones for the purpose and has been used to detect *A. salmonicida* and *Aphanomyces invadans* in fish (Saleh et al. 2011; Kuan et al. 2013). Guo et al. (2016) in a study used an immunomagnetic Nps-based microfluidic device for the detection of *Staphylococcus aureus* by creating a microfluidic indium tin oxide chip. It was better than the colony counting process, and the detection process took a shorter time without cultivation of the colony. A colorimetric approach using Au Nps and loop-mediated isothermal amplification was developed for the detection of the yellow head virus and white spot syndrome virus (WSSV) in shrimps (Jaroenram et al. 2012; Seetang-Nun et al. 2013). Similarly, a colorimetric approach was used to develop a sensor using Au Nps for detection of spring viremia of carp and DNA herpesvirus 3 in fish

(Saleh et al. 2012; Saleh and El-Matbouli 2015). Furthermore, nervous necrosis virus (NNV) detection was done by using magnetic Nps coated with rabbit anti-NNV antibody, and also the biosensor was developed using Au Nps for detection of the virus (Yang et al. 2012; Toubanaki et al. 2015). The examples of Nps used in diagnostics in fish are presented in Table 18.1.

18.3.7 Nanotechnology-Based Fish Vaccines

Nanotechnology has some exciting applications in delivering of vaccines in aquaculture species. Nps have been efficiently used either as adjuvants or as delivery vehicles for the vaccines used in fish and shellfish. The current trend of the use of nanotechnology in vaccines is continuously rising in world aquaculture including India. Nps prevent the drug, antigen, or other substances from enzymatic attack, guide it to reach the target site, slowly release it so that the effect could be felt for longer durations, as well as due to the smaller size can cross any barrier inside the body.

18.3.7.1 Polymeric Nps

Polymeric Nps are being used in vaccine delivery due to their lesser side effects and easy degradable nature. They also act as immunostimulants to protect the antigen as well as trigger the immune system during the need. Nps conjugated with antigens protect and guide it to reach the targeted immune cells (Zhao et al. 2014). Some other examples of Nps used in the vaccine delivery are particles identical to viruses, immunostimulant complexes, liposomes, metallic Nps, nanoemulsions, etc. (Zhao et al. 2014; Shaalan et al. 2016). Polymeric Nps can be of natural type of synthetically derived ones with examples as chitosan, hyaluronic acid, alginates of the former, and PLGA of the latter.

Chitosan Nps enhanced mucosal immunity when delivered orally in fish. Chitosan conjugated with inactivated virus vaccine against infectious salmon anemia virus (ISAV) increased the survivability up to 77 percent against this pathogen (Rivas-Aravena et al. 2015). The use of chitosan Nps as a delivery vehicle to deliver vaccines against viral hemorrhagic septicemia (VHSV) was successfully performed in zebrafish (Kavaliauskis et al. 2016). The oral DNA vaccine against *V. anguillarum* in Asian sea bass (*Lates calcarifer*) was developed with chitosan as a delivery agent and adjuvant (Vimal et al. 2014). Similarly, in black seabream (*Acanthopagrus schlegelii*), the recombinant nanovaccine containing chitosan elicited a defensive immune response against *V. parahaemolyticus* (Li et al. 2013). A Japanese flounder (*Paralichthys olivaceus*) oral DNA vaccine containing chitosan as a carrier was developed against the lymphocystis disease virus (LCDV) (Tian et al. 2008). Chitosan conjugated with DNA vaccines against *Philasterides dicentrarchi* in *Scophthalmus maximus* (León-Rodríguez et al. 2013), RNA in *Labeo rohita* (Ferosekhan et al. 2014), *V. parahaemolyticus* in *Acanthopagrus schlegelii* (Li et al. 2013), and hematopoietic necrosis virus in rainbow trout (Adomako et al. 2012) has been developed successfully. DNA-based vaccines conjugated with Nps generated the immunological proteins that protect shrimps

Table 18.1 Applications of different Nps in vaccine delivery and diagnostics in fish

Type of nanoparticle used	Delivery against disease	Fish	References
Chitosan	*Salmon anemia virus*	Atlantic salmon	Rivas-Aravena et al. (2015)
	Viral haemorrhagic septicaemia virus (VHSV)	Zebrafish	Kavaliauskis et al. (2016)
	Turbot reddish body iridovirus TRBIV	Turbot	Zheng et al. (2016)
	Nodavirus	Asian seabass	Vimal et al. (2014)
	Vibrio parahaemolyticus	Blackhead seabream	Li et al. (2013)
	Vibrio anguillarum	Asian seabass	Vimal et al. (2012)
	Vibrio anguillarum (Listonella)	Asian seabass	Rajesh et al. (2008)
	Edwardsiella tarda	Rohu	Kole et al. (2018)
	VHSV	Olive flounder	Kole et al. (2019)
Alginate	*Ichthyophthirius multifiliis*	Rainbow trout	Heidarieh et al. (2015)
	Infectious hematopoietic necrosis virus (IHNV)	Brown trout and rainbow rout	Ana et al. (2010)
Alginate + chitosan	*Yersinia ruckeri*	Rainbow trout	Dezfuly et al. (2020)
PLGA	*A. hydrophila*	Rohu	Dubey et al. (2016)
	Streptococcus agalactiae	Tilapia	Zhang et al. (2015)
	A. hydrophila	Rohu	Rauta and Nayak (2015)
	Infectious pancreatic necrosis virus (IPNV)	Atlantic salmon	Munanġandu et al. (2012)
	IHNV	Rainbow trout	Adomako et al. (2012)
	Uronema marinum	Flounder	Harikrishnan et al. (2012a)
	Lymphocystis disease	Flounder	Tian and Yu (2011)
PLGA+ chitosan	*Edwardsiella tarda*	Rohu	Leya et al. (2020)
Liposome	*Vibrio harveyi*	Grouper	Harikrishnan et al. (2012a, b)
	A. salmonicida	Common carp	Irie et al. (2005)
	Koi herpes virus	Common carp	Yasumoto et al. (2006a)
Carbon nanotubes	*Grass carp reovirus*	Grass carp	Zhu et al. (2014)
	Grass carp reovirus	Grass carp	Zhu et al. (2015)

(continued)

Table 18.1 (continued)

Type of nanoparticle used	Delivery against disease	Fish	References
	Rhabdovirus	Common carp	Zhang et al. (2020)
	Grass carp reovirus	Grass carp	Zhu et al. (2020)
	Grass carp reovirus	Grass carp	Qiu et al. (2020)
	Infectious spleen and kidney necrosis virus	Mandarin fish	Zhao et al. (2020)
Calcium phosphate	*A. hydrophila*	Rohu	Behera and Swain (2011)
Diagnostics			
Nanoparticle	*Antibody used/method*	*Detection in fish*	
Gold	*A. salmonicida*	Furunculosis	Saleh et al. (2011)
Gold	DNA based	*Aphanomyces invadans*	Kuan et al. (2013)
Gold	Not needed	Nervous necrosis virus (NNV)	Toubanaki et al. (2015)
Gold	Colorimetric assay, probe used	Spring viremia of carp virus (SVCV)	Saleh et al. (2012)
Gold	Colorimetric assay, probe used	Cyprinid herpes virus-3 (CyHV-3)	Saleh and El-Matbouli (2015)
Magnetic nanoparticles	Rabbit anti-NNV antibody	NNV	Yang et al. (2012)

from white spot syndrome virus (WSSV) for application up to 7 weeks (Rajeshkumar et al. 2009). The delivery of antisense nodavirus DNA was encapsulated with chitosan Nps and resulted in a higher survival rate in freshwater prawns (Ramya et al. 2014). Polyanhydride Nps have been used for vaccine encapsulation and release antigens that decide shrimp immunization through immersion or with feed (Ross et al. 2014). Bicistronic DNA vaccine encoding *Edwardsiella tarda* antigen glyceraldehyde-3-phosphate dehydrogenase and immune adjuvant IFN-γ was conjugated with chitosan Nps and delivered to rohu through oral and immersion methods which resulted in immunity against the infection (Kole et al. 2018). The same bicistronic DNA vaccine was conjugated with chitosan and PLGA Nps to develop immunity against the infection in rohu (Leya et al. 2020). Chitosan Nps were encapsulated with inactivated viral hemorrhagic septicemia virus (VHSV) antigen and administered via mucosal routes in olive flounder to develop protective antiviral immunity against the virus (Kole et al. 2018).

Alginate microparticles were used as a delivery vehicle for transferring plasmid DNA orally against IPNV in brown trout and rainbow trout (Ana et al. 2010). Similarly, they were used to deliver the antigens against *Flavobacterium columnare* in Nile tilapia and *Ichthyophthirius multifiliis* in rainbow trout (Leal et al. 2010; Heidarieh et al. 2015). In rainbow trout, the alginate particles produced by the spray method was conjugated with the antigens of *Lactococcus garvieae*, and an effective response against the disease was found (Ana et al. 2010). Alginate-chitosan micro/Nps conjugated with *Yersinia ruckeri* lipopolysaccharide enhanced the immunogenicity against the infection in rainbow trout (Dezfuly et al. 2020).

PLGA was used to deliver the vaccines against *A. hydrophila*, *Streptococcus agalactiae*, infectious pancreatic necrosis virus, infectious hematopoietic necrosis virus in rohu, tilapia, Atlantic salmon, and rainbow trout, respectively (Dubey et al. 2016; Zhang et al. 2015; Munanġandu et al. 2012; Adomako et al. 2012). Antigen from *Uronema marinum*, which is a protozoic pathogen that infects flounder and grouper, was encapsulated into PLGA and it sucessfully elicited the immune response against the opportunistic pathogen, and the effect remained for a maximum of 4 weeks (Harikrishnan et al. 2012a, b). In another study on flounder, a plasmid that codes for the major capsid protein (MCP) of lymphocystis disease virus was conjugated with PLGA Nps, and the results suggested that antibodies against the virus were peaked after 30 days of treatment and also the encapsulated treatment was more effective in treating the infection compared to naked group (Tian and Yu 2011).

18.3.7.2 Liposomes

Liposomes are composed of a phospholipid bilayer which is considered self-sealing and could deliver lots of hydrophilic and hydrophobic drugs (Ji et al. 2015). Liposomes were used to deliver formalin killed *V. harveyi* antigen in grouper, and results revealed that in the entrapped group, the mortality recorded was lowest compared to the naked group (Harikrishnan et al. 2012b). In common carp, antigens against *A. salmonicida* were administered orally by using liposomes as vectors, and the survival rate up to 83% was calculated in the liposome-treated group (Irie et al. 2005). Lipopolysaccharide extracted from *A. salmonicida* was entrapped into liposomes to enhance the immune response in rainbow trout (Yasumoto et al. 2006b). Similarly, the koi herpesvirus inactivated by formalin treatment was enclosed within liposomes for vaccination of common carp orally (Yasumoto et al. 2006a). Liposomes have been used to deliver cinnamaldehyde (cinnamon extract) and melittin, an antimicrobial peptide against different pathogens and viral infections in fish, and the liposome-entrapped group was found to have an upper edge in eliciting the immune response against the disease-causing agent (Faikoh et al. 2014; Falco et al. 2013).

18.3.7.3 Inorganic Nps

The most efficient inorganic Nps that is currently having a tremendous application is carbon nanotubes. These Nps have been used in every field ranging from physics, engineering, to biomedicine. The main issue associated with the use of carbon nanotubes is the toxicity, so before applying a safe dosage needs to be selected. Carbon nanotubes have two forms, viz., single-walled and multiple-walled, and both are used for drug delivery and have different properties. A single-walled carbon nanotube has been used to deliver plasmid that codes for VP7 protein of grass carp reovirus (GCRV) intramuscularly in grass carp. The immune response against the infection was high in the nanotube-conjugated group compared to naked treatment

(Zhu et al. 2015). In a recent study, single-walled carbon nanotubes were used to deliver GCRV VP7 antigen into fish through immersion against the viral disease, and a good immune response was reported (Zhu et al. 2020). Carbon nanotubes were successfully used to deliver the antigens against rhabdovirus infection in fish (Zhang et al. 2020). Single-walled carbon nanotubes were used to deliver antigen against infectious spleen and kidney necrosis virus (ISKNV) in mandarin fish, and successful results were obtained (Zhao et al. 2020). Qiu et al. (2020) employed carbon nanotubes to deliver antigen against grass carp reovirus, and a higher percentage of immunity was generated compared to the naked treatment.

Calcium phosphate Nps when adsorbed to S-layer protein of *A. hydrophila* resulted in stimulation of both innate and adaptive immune response in fish, and the complete protection against the infection was provided (Behera and Swain 2011). Table 18.1 presents the examples of Nps used in the vaccine delivery against the particular disease in fish.

18.4 Conclusions

In summary, the present chapter deals with the advancement of nanotechnology in the aquaculture sector with more emphasis on its applications in disease prevention and diagnosis. The Nps of all kinds have been proven to be sufficient to enhance the resistance against the disease in fish. Ranging from the nature of Nps, polymeric Nps still occupy the top position in the list in terms of the application in fish medicine. Carbon nanotubes due to their unique properties are now being used in the disease management of aquaculture species.

The awareness about the benefits of Nps in the different fields like biomedicine, food processing industry, as well in the fisheries sector has led to an increase in their usage. At the same time, the harmful effect of the Nps on the surrounding environment is a matter of concern. Even though polymeric Nps are considered biodegradable and safe, still care and attention are needed to use them in a better way. Metallic Nps and carbon nanotubes are considered extremely hazardous for the living tissues, developing embryos, and growth in general when used at high concentrations. So, before their usage, safer concentration needs to be calculated to prevent long-term harmful effects.

Conflict of Interest None of the authors declare any conflict of interest.

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