Chapter 1
Nanotechnologies in Aquatic Disease Diagnosis and Drug Delivery

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Abbreviations

ALP	Alkaline phosphatase
CNTs	Carbon nanotubes
DDS	Drug delivery system
DNA	Deoxyribonucleic acid
EMA	European Medicine Agency
FAT	Fluorescence antibody test
GCRC	Grass carp reovirus
GO	Graphene oxide
HK	Head kidney
IFAT	Indirect fluorescence antibody test
IHC	Immunohistochemistry
IHNV	Infectious hematopoietic necrosis virus
IM	Intramuscular
IP	Intraperitoneal
IPNV	Infectious pancreatic necrosis virus
ISAV	Infectious salmon anaemia virus
KHV	Koi herpes virus
LCDV	Lymphocystis disease virus
LPS	Lipopolysaccharides
NP’s	Nanoparticles
PCR	Polymerase chain reaction
PLA	Poly lactic acid
PLGA	Poly (lactic-co-glycolic acid)
RAPD	Random amplification of polymorphic DNA
rcb-PCR	Reverse cross blot PCR
RNA	Ribonucleic acid
ROS	Reactive oxygen species

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RT-PCR	Reverse transcriptase polymerase chain reaction
SLN	Solid lipid nanoparticles
TLRs	Toll-like receptors
VHSV	Viral haemorrhagic septicaemia virus
WSSV	White spot syndrome virus

1.1 Introduction

Aquaculture represents the fastest-growing domain with prolific growth potential. It serves as the source of easily digestible protein, healthy fat, and a cache of various essential micronutrients for billions across the globe. Aquaculture provides a potential platform for rural employment and livelihood development, possesses high export earning potential, and can support national GDP through income generation. Recent years have seen development in the production of farmed fin and shellfish that has increased rapidly due to enhanced, intensive aquaculture practices (FAO 2018) and demand. Due to the increase in intensive farming systems, the need for wild seed stocks as well as fish supplies has declined (Naylor et al. 2006). A report from the Food and Agriculture Organization shows that the average annual consumption of fish increased by 3.2% globally from 1961 to 2016. In 2015, of the total animal protein consumed globally, fish food dominated at 17% and supplied almost 20% of the average consumption of animal protein for 3.2 billion populations (FAO 2018). However, environmental factors like excessive accumulation of nutrients result in the eutrophication of ponds due to intensive aquaculture practices, which are being looked at as a block in aquaculture growth dynamics (Herbeck et al. 2013). Along with eutrophication, climate change also poses a serious threat to aquaculture due to the temperature rise, increased methane and carbon dioxide emissions, etc. (FAO 2018). An increase in water temperature will have an impact on plankton communities, the survival of larvae and juveniles, and the reproductive ability of fishes. It has been also observed that contaminated water is being introduced into reservoirs and fish culture systems (Patel et al. 2019). Moreover, detecting pollution and toxic levels in water is a tedious and time-consuming process (Altenburger et al. 2019). In extensive aquaculture practices, nutritional deficiency is common among juvenile and broodfish populations, and health management is also another challenging problem. Depleting environmental quality and the impact of climate change have led to a surge in infectious diseases in aquaculture. Diseases like fin and gill rot and epizootic ulcerative syndrome (EUS) are still prevalent with no possible solutions and become incurable with antibiotics.

A white spot disease outbreak has inhibited the otherwise profitable *Penaeus monodon* culture system (Zhang et al. 2016). It has been estimated that the global shrimp industry has faced a loss of US$10 billion from white spot disease syndrome and the infectious myonecrotic virus alone since 1990. Meanwhile, the $15 billion global ornamental fish business also faced a problem with antibiotic resistance, which created uncertainty in its therapeutic practices. Conventional drug delivery models have low efficacy and bioavailability in aquatic ecosystems. Besides these, traditional disease detection methods are also not feasible in the long run.

At this point, there is a need for some innovative technological interventions to get rid of these problems in aquaculture. Nanotechnology has emerged as a promising solution, leading to new possibilities by developing and applying materials at nanoscale dimensions (1–100 nm) with unique properties, paving the way for novel therapeutic as well as diagnostic applications (Matteucci et al. 2018; Mohamed et al. 2015). The application of nanotechnology has become a part of human life with multiple value-added products (Stern et al. 2016; Vinay et al. 2018). With recent technologies, nanoparticles are being prepared by different physical and chemical methods. The main commercial nanoparticles include silver, gold, oxides of zinc, iron, calcium, titanium, and manganese, as well as carbon nanotubes (CNTs), mesoporous silica nanoparticles, quantum dots (Khan et al. 2019; Stone et al. 2010), etc. With complex three-dimension technology, 'Second-generation' nanomaterials are now being synthesized with highly functional surfaces (Sarkar et al. 2022; Jeevanandam et al. 2018; Ju-Nam and Lead 2008). Even the impact of toxic pollutants on aquatic species can be intervened by using nanotechnology. Besides conventional toxicological assays, scientists are also using cellular cytotoxicity, apoptosis as well as bioinformatics-based interactive tools to narrate nanotoxicity and its remediation by applying green nanoparticles in bacteria and fish models (Kumari et al. 2017; Husain et al. 2021; Verma et al. 2017, 2021). Hence, the efficient application of nanotechnology can be addressed for maximizing aquaculture productivity and a schematic diagram is presented in this regard as Fig. 1.1.

1.2 Fish Disease and Immune System

The disease is a primary cause of fish mortality, especially when fishes are in the larval stage. There are two types of fish diseases according to their infectious pattern, viz. pathogenic diseases and non-pathogenic diseases (Arfat et al. 2014; Vevers and Jha 2008). Different infectious diseases are the main cause of death that might be due to bacterial, viral, or parasitic infection, and sometimes pathogens cause high levels of toxicity. It is mainly related to poor water quality management, inadequate feed supply, etc. A high-nutrition diet will have a positive effect on fish health. In this way, nanoparticles serve as a main source of transportation as well as supplementation of materials across the membrane. Non-infectious diseases are mainly caused by gas bubble diseases due to extensive aeration, nutritional diseases due to deficiency of nutrients such as vitamins and minerals, disorders caused by pollutants in the form of agricultural and industrial wastes, and neo-plastic and genetic anomalies resulting in abnormal growth which makes organs lose their structure and function (Rajkumar et al. 2016; Gao et al. 2017; Vinay et al. 2018; Bacchetta et al. 2017; Verma et al. 2020).

The fish immune system plays an important role in the protection against pathogens. Under the infectious stage, the immune system can detect pathogens

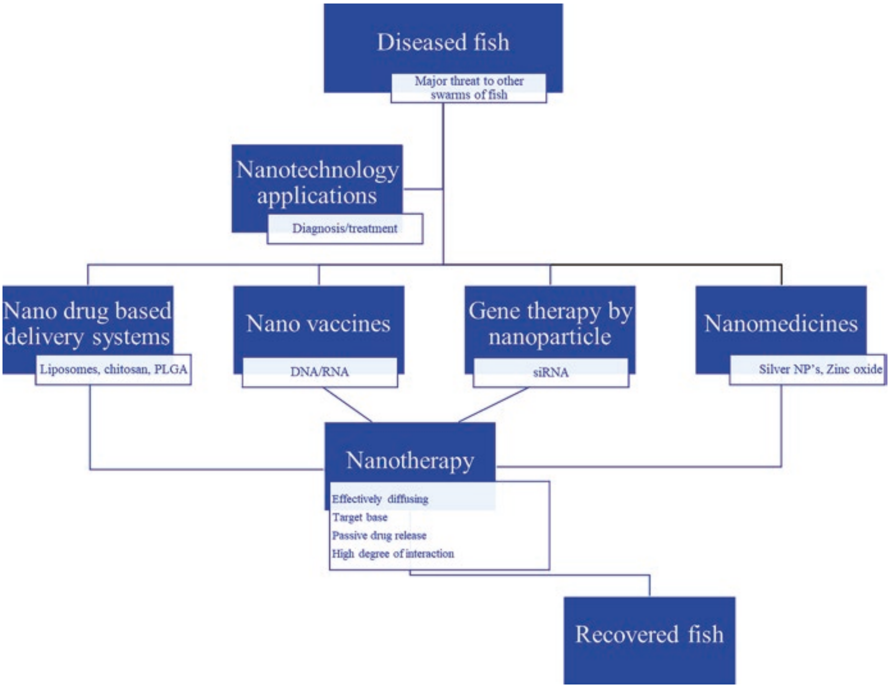

Fig. 1.1 Application of nanotechnology in aquaculture

(Firdaus-Nawi and Saad 2016). The immune system of fish is composed of two main components: innate and adaptive immunities (Firdaus-Nawi and Saad 2016). Innate immunity is nonspecific and serves as the primary defence against pathogen invasion, while adaptive immunity is much more specific to a particular pathogen. Innate immunity is composed of nonspecific cellular and humoral elements. The nonspecific cellular portion consists of toll-like receptors (TLRs), macrophages, neutrophils, eosinophils, and nonspecific cytotoxic cells, while the nonspecific humoral portion includes lysozyme, complement cells, interferons, C-reactive proteins, transferrins, and lectins, where they work together to prevent pathogen invasion at the primary stage of infection (Firdaus-Nawi and Saad 2016). On the other hand, the adaptive immune system comprises highly specific systemic cells and mechanisms that are divided into two major components: humoral and cellular (Firdaus-Nawi and Saad 2016). Three forms of antibodies, IgM, IgD, and IgT, are essential components of humoral immunity which operate on invading extracellular diseases. The cytotoxic T-lymphocyte cells are a significant component of cellular immunity that often destroys invading bacterial, viral, or parasite-infected cells and intracellular cells (Firdaus-Nawi and Saad 2016). Both innate and adaptive immune systems work together efficiently to protect the body from any diseases, whereas innate immunity responds to invading pathogens by identifying line-encoded molecules of the germ.

1.3 Key Factors to Improve Disease Diagnosis and Vaccine Development

The major underlying factors for improving disease diagnosis and vaccine development are the continued significant losses to the industry caused by pathogens. Bacterial diseases cause substantial economic inflation in the aquaculture industry, and although antibiotics and chemotherapeutics are extensively used to control disease outbreaks, there is increasing concern about their use because of drug residues in food, the development of antimicrobial drug resistance, and their detrimental effect on aquatic microbial ecosystems and populations (Thompson and Adams 2004).

1.4 Limitations of Current Diagnostic Methods

Most of the current techniques for the detection of pathogens and the diagnosis of diseases are reliable. On the other hand, it is difficult to identify certain pathogens, and some of the methods developed may be too complicated to apply or infer. Conventional pathogen isolation and characterization techniques, combined with histopathology, remain the methods of choice for the diagnosis of many diseases. However, these traditional methods are costly, labour-intensive, slow, and time-consuming, and might not always lead to a definitive diagnosis being made. The rapid progress made in biotechnology since the 1990s has enabled the development and improvement of a wide range of immunodiagnostic and molecular techniques (Cunningham 2004; Adams and Thompson 2006, 2008), and reagents and commercial kits have become more widely available. These rapid methods both complement and enhance the traditional methods of disease diagnosis.

1.5 Advances in Methods of Disease Diagnosis

Disease diagnosis is currently made using a variety of methods, as reviewed by Adams and Thompson (2008). Traditional bacteriological methods, where the pathogen is isolated and identified biochemically (e.g. using API strips), and observation of histological sections from diseased fish are widely used. Rapid methods that specifically identify the pathogen using antibodies (immunodiagnostics) or by amplifying specific sequences of DNA or RNA using polymerase chain reaction (PCR) (i.e. molecular diagnostics) are also being used in many laboratories. In some cases, recent advances in molecular diagnostics have completely replaced other methodologies.

Immunodiagnostic methods such as immunohistochemistry (IHC), the fluorescence antibody test (FAT), and the indirect fluorescence antibody test (IFAT) enable rapid and specific detection of pathogens in tissue samples without the need to first isolate the pathogen. The use of molecular technologies for the detection of fish bacterial pathogens is rapidly increasing, and a vast array of methods has already been developed (Karunasagar et al. 1997; Cunningham 2004; Adams and Thompson 2006, 2008; Wilson and Carson 2003). Molecular methods generally have the highest sensitivity and are particularly useful for detecting microorganisms that are present in low copy numbers or those that are unculturable. The PCR is the best-known method, although there are many different kits, including nested PCR, random amplification of polymorphic DNA (RAPD), reverse transcriptase-PCR (RT-PCR), reverse cross blot PCR (rcb-PCR), and RT-PCR enzyme hybridization assay (Puttinaowarat et al. 2000; Wilson and Carson 2003; Cunningham 2004).

1.6 Nanotechnology Tool for Enhancing Aquaculture Operations and Fishing System

For any aquaculture practice, the pond is the core unit, and its professional management is the primary criterion for developing a successful aquaculture system. To design a perfect pond structure, multiple construction materials, as well as fabrication items, are required. Moreover, water supply and management of quality water are of primary importance. Sometimes, natural water bodies like the extensions of reservoirs and lakes, commonly called creeks, can be used for cage or open culture systems. The use of nanoparticles like silver, zinc oxide, copper oxide, titanium oxide, etc. can be mixed and coated with fabrication materials to increase the shelf life of the pond. Carbon nanotubes are approximately a hundred times stronger than steel and could be employed as an additive in boat and gear making or in designing cages and nets. ULVAC Inc., a corporate leader in vacuum equipment manufacturing, has designed a fishing lure coated with several hundred-nanometre-thick polyimide films that have a natural shining effect. Using this lure, better fishing practices can be achieved (Handy 2012).

1.7 Nanotechnology in Fish Disease Control

Disease occurrence is one of the major perils in intensive aquaculture practices, (Toranzo et al. 2005), and management of pond health is also important for the prevention and curing of fish species from pathogenic infestation. Nanotechnology can contribute significantly through novel methods as well as restructuring conventional technology (Kaul et al. 2018).

1.8 Nanomaterials as a Diagnostic Tool

In the modern era, aquaculture scientists are looking for an easy and reliable method for early detection and routine diagnostics of important fish diseases. An antibody-based, highly sensitive immunodiagnostics protocol incorporating nanoscale gold with alkaline phosphatase (ALP) conjugated secondary antibody was developed to measure titre against white spot syndrome virus (WSSV) in shrimp (Thiruppathiraja et al. 2011). Moreover, it has been reported that immuno-targeted gold nanoparticles are being conjugated with antibodies targeted to a particular biomolecule of interest, such as immunoglobulin G-capped gold nanoparticles, that bind specifically to antibodies produced against *Staphylococcus pyogenes* and *S. aureus*, among others (Roy et al. 2012).

Similarly, nanosensors also serve as an effective and easy method to identify pathogens. Nano biosensor systems are currently being developed for detecting very low concentrations not only of parasites, bacteria, and viruses but also of polluting elements in the water (Chen et al. 2016). Different nanosensors are being used to detect important aquaculture viruses like aquabirnavirus, salmonid alphavirus, and betanodavirus (Crane and Hyatt 2011). Currently, graphene oxide (GO) is used for designing electrochemical biosensors due to its unique chemical constitution and biocompatibility. A simple, sensitive, real-time, and rapid detection method of white spot syndrome virus (WSSV) in shrimp samples was demonstrated by applying a GO-based electrochemical immunosensor. The detection limit was found to be very low, at 1.36×10^{-3} copies μL^{-1}. This is a unique and alternative qualitative and quantitative method, unlike PCR amplification-based detection techniques (Natarajan et al. 2017). This is particularly important in outbreaks at commercial aquaculture systems since conventional methods take too much time to identify the etiological agent causes, delaying the treatment to control the pathogen and creating an important economic impact. In this regard, nanotechnology holds the key to overcoming the potential threat through early detection and eradication of pathogens. Currently, nanosensors can detect a wide range of pathogens, be it bacteria, viruses, or parasites. For instance, electrical nanosensors are feasible to detect single virus particles (Patolsky et al. 2004). Nanobiosensors are also used for fishpond cleaning and stock inspections, such as those based on carbon nanotubes, which are highly sensitive for the detection of traces of pathogens like viruses, parasites, and bacteria, as well as heavy metals, in both food and water (Husain et al. 2021).

1.9 Nanomaterials as Nanomedicine

Nanomedicine, another rapidly growing area in nanotechnology, has a wide opportunity to use the intrinsic properties of different forms of nanoparticles for improving fish health. The antimicrobial and prophylactic actions of nanomaterials like nanosilver and zinc oxide nanoparticles are already exploited to reduce the pathogenic load in the aquaculture system (Siddiqi et al. 2018). This unique phenomenon is nonspecific, universal, and widely applicable. Antibacterial properties of nanoparticles such as titanium dioxide and copper oxide are being investigated as a potential nanomedicine for fish. Graphene appears as a commercially attractive, cheap, and renewable nanomaterial when, in its oxidized form, it is easy to process and dispersible in water (Brisebois and Siaj 2019). Graphene oxide (GO) exhibited an inhibitory effect against important aquatic pathogens like *S. aureus*, *P. aeruginosa*, *E. coli*, and *Vibrio harveyi*, both individually and in combination with metal/metal oxide and polymeric nanoparticles. Graphene oxide causes mechanical enfolding followed by cell membrane damage and lysis upon interacting with pathogens (Kumar et al. 2019).

1.10 Phyto-Nanocomposites as Nanomedicines

Different herbal and phytoextracts are currently being used as potential drugs in treating fish diseases. Different nanoparticles are being synthesized using medicinal plant and herbal extracts at optimized hydrodynamic conditions, and a composite of the phyto-nanoformulation is delivered as drugs with synergistic effects on improving fish health. Mahanty et al. (2013a, b) described some methods of phytoextract-synthesized nanosilver composite showing effective inhibition against the important fish pathogen, *Aeromonas hydrophila*.

1.11 Nanomaterials as Drug Delivery Systems (DDS) and Therapeutic Tools

Nano-delivery of drugs is attributed to novel properties like the sustained release, regulation, and control of size, shape, dispersity, and surface charge of targeted materials; location-specific, multi-route delivery processes; and regulated degradability of nanocarriers (Patra et al. 2018).

1.12 Nanovaccine Delivery

Nanotechnology, in conjunction with biotechnology, has made tremendous progress in the biomedicine field (Zhao et al. 2014) and has expanded its portfolio in the field of vaccinology, thereby giving rise to a new scientific field called nanovaccinology (Mamo and Poland 2012; Zhao et al. 2014). Vaccination plays a pivotal role in large-scale, commercial pisciculture and has been a major factor in the success of salmon and trout cultivation (FAO 2018) and partially in Indian major carp farming.

In the last 20 years, fish vaccinations have become a highly recognized, cost-effective method for preventing certain pathogenic infections in aquaculture (Assefa and Abunna 2018; Vinitnantharat et al. 1999). Most of the vaccines produced are kept and preserved in liquid form at low temperatures and are usually injected through blood networks due to their short life span. These bottlenecks have reduced the chances of vaccines being widely used, particularly in certain finfish and shellfish. 'Nanovaccine' is an emerging mass vaccination method in aquaculture (Assefa and Abunna 2018). The USDA demonstrated ultrasound-driven tools for mass vaccination of fish. Short strands of DNA-loaded nanocapsules are placed in the fish pond, where they adsorb onto the surface of fishes. Then ultrasound is triggered to rupture the capsules for releasing the DNA eliciting an immunological response. This technology has been examined on rainbow trout and commercialized by 'Clear Springs Foods', USA (Mongillo 2007). Similarly, oral delivery of vaccines and targeted release of active ingredients for vaccination will reduce the cost of fish farming (Aravena et al. 2013).

DNA nanovaccines, comprising short strands of DNA within nanocapsules, have been used in the aquaculture industry to induce an immune response in fishes. Iron nanoparticles have been shown to accelerate fish development when combined with drug delivery via programmed release (Hussain et al. 2021). For instance, Bhattacharyya et al. (2015) successfully showed nanoencapsulated vaccines against the bacterium *Listonella anguillarum* in Asian carp and also in rainbow trout (*Oncorhynchus mykiss*), as shown in studies by (Mongillo 2007; Ogunkalu 2019; Zheng et al. 2016).

1.13 Nanoparticle-Based Gene Therapy

Gene therapy is another well-practiced method used in the aquaculture sector (Xiang 2015). In contemporary times, the European Medicines Agency (EMA) has recommended the use of 'Clynav', a DNA vaccine for protecting Atlantic salmon against acute infections. In another report, an efficient carrier vehicle was designed using chitosan-dextran sulfate and silica nanoparticles to deliver dsRNA into *Penaeus monodon* post-larvae for silencing the Monodon baculovirus (MBV) structural gene, p74. This carrier system exhibited a significant survival rate (86.63%) after successful gene silencing in *P. monodon* (Ramesh Kumar et al. 2016).

1.14 Different Nanocarriers for Drug Delivery

Chitosan

Chitosan is a processed by-product of the shrimp industry and has been developed as a well-known polymer-based nanocarrier. Chitosan nanoparticles were orally delivered to black tiger shrimp (*Penaeus monodon*) loaded with a DNA construct comprising the VP28 gene of white spot syndrome virus (WSSV). A significant survivability rate was monitored in WSSV-challenged shrimp compared to the 100% mortality in the control group (Rajeshkumar et al. 2009). Chitosan microspheres exhibited positive trends as an orally delivered plasmid vaccine carrier for immunization in Japanese flounder (*Paralichthys olivaceus*) (Tian et al. 2008). Many studies have successfully implemented encapsulation and delivery by chitosan-based systems in aquaculture, viz. treatment methods have been developed against *Vibrio parahaemolyticus* in the blackhead seabream (*Acanthopagrus schlegelii*) (Li et al. 2013), *Philasterides dicentrarchi* in the turbot (*S. maximus*) (Leon-Rodrıguez et al. 2013), and *Vibrio anguillarum* in Asian sea bass (*Lates calcarifer*) (Kumar et al. 2008). Both DNA and RNA have been successfully encapsulated and delivered using this system. Studies by Ferosekahn et al. (2014) showed dietary RNA has been used in rohu (*Labeo rohita*), as well as inactive particles of the viral hemorrhagic septicemia virus (VHSV) in Japanese flounder (*Paralichthys olivaceus*), as reported by Kole et al. (2019). Recently, Kitiyodom et al. (2019) reported a chitosan-coated mucoadhesive nanovaccine with enhanced efficacy by immersion vaccination in tilapia (*Oreochromis* sp.) against columnaris disease. Additionally, chitosan-coated membrane vesicles (cMVs) derived from the pathogen *Piscirickettsia salmonis* were injected into adult zebrafish (*Danio rerio*), which conferred significant protection with an increased survival rate and induced an increased immune response (Tandberg et al. 2018).

1.15 Liposomes

Another commonly applied product is liposomes, which are artificially designed nanostructures comprising a lipid bilayer and have natural properties for application as carriers for drugs, vaccines, etc. It is nontoxic, biodegradable, and nonimmunogenic, and several nanoliposomal products are approved by the FDA. Moreover, it serves as an efficient delivery system, as it possesses the capacity to control and target the release of hydrophobic and hydrophilic compounds that are easily permeable through cell membranes. Nanoliposomal encapsulated Omega-3 fatty acids synthesized from seafood and fish oil are used in food fortification for efficient delivery and protection (Hadian 2016). It is also used to deliver important vitamins like folic acid via a dermal route that paved the way for delivering nutraceuticals (Kapoor et al. 2018). Liposome-encapsulated *Aeromonas salmonicida* antigen exhibited effective immunization in carp (*Cyprinus carpio*) (Irie et al. 2005). Similarly, an immunostimulant cocktail was applied as a nonspecific nanocarrier of the vaccine to multiple fish species. A liposomal lipopolysaccharide (LPS)-dsRNA cocktail was observed entering into the hepatocytes of zebrafish and trout macrophage plasma membranes, triggering pro-inflammatory and antiviral reactivity (Ruyra et al. 2013). Positive results were recorded after encapsulating curcumin into liposomes, prepared from natural sources (salmon lecithin), and examining them under primary cortical neuron culture (Hasan et al. 2018).

1.16 Poly (Lactic-*Co*-Glycolic) Acid (PLGA)

Polymeric nanoparticles of poly (lactic-*co*-glycolic) acid (PLGA) are examples of well-established drug delivery vehicles having adjuvant properties. It was found that an antigenic (TNPLPH) and immunostimulant (β-glucan) candidate encapsulated within the PLGA nanoparticles had the potentiality to enhance the expression of pro-inflammatory markers in salmon (Fredriksen et al. 2011). PLGA nanoparticles have also been monitored as a potential carrier of DNA vaccine for oral immunization against the lymphocystis disease virus, as evaluated in Japanese flounder (*Paralichthys olivaceus*) (Tian and Yu 2011). Surfactant-free PLGA nanoparticles displayed optimal biocompatibility and targeted dendritic cells in adult zebrafish after mucosal administration. This experiment opens a powerful platform for mucosal vaccine delivery in aquaculture (Resseguier et al. 2017). Shah and Mraz (2019) showed the use of encapsulation and delivery of different compounds in fish. For example, a DNA vaccine encapsulated in PLGA showed an improved immunological response against lymphocystis (Tian and Yu 2011). Additionally, Behera et al. (2010) showed the potential of PLGA encapsulated antigens from *Aeromonas hydrophila*, used as a vaccine in Rohu, to produce an enhanced immune-stimulatory and antibody response, both at 21 and 42 days post-immunization. Similarly, Yun et al. (2017) synthesized PLGA microparticles to carry *A. hydrophila* cells that were previously killed by formalin treatment as a way to deliver antigens to pond loaches (*Misgurnus anguillicaudatus*) and common carps (*C. carpio*). Upon being challenged with *A. hydrophila*, the fishes were treated with PLGA-*A. hydrophila*, which showed a higher survival rate with increased innate and adaptive immune responses than the group treated just with the inactivated *A. hydrophila*, which could potentially induce higher and more lasting immune responses than the antigens alone. Additionally, mass vaccination can be achieved using nanocapsules resistant to digestion and degradation. Both oral administration and site-specific release of the active agent will reduce the effort and cost related to disease management, leading to more sustainable practices (Rather et al. 2011).

1.17 Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) are investigated due to their potential topical delivery and controlled release in the skin. Pandita et al. (2011) investigated the potential applications of solid lipid nanoparticles (SLNs) in improving the oral bioavailability of paclitaxel, a hydrophobic drug. Experiments were conducted to evaluate the delivery of solid lipid nanoparticles (SLN) encapsulating potential antioxidant glutathione (GSH) on immunocompetent fish cells of a seawater teleost, the gilthead seabream (*S. aurata L.*). It was observed that the phagocytic function of HK (head kidney) leucocytes incubated with encapsulated glutathione was significantly enhanced. A better *in vitro* antioxidant efficacy of SLN was also monitored (Trapania et al. 2016). Fin rot, dropsy, black and white spot disease, gill rot, columnaris, anchor worms, hole in the head, and *Argulus* infestations are some of the important external fish diseases that can be administered for recovery.

1.18 Microbial Disinfection

Nanoparticles synthesized from silver, titanium, and copper, among others, have been used for disease prevention and treatment. They have different modes of action against bacteria, and the most important function is acting on the cell membrane and cell wall by adhering to the cell wall through electrostatic interaction and disrupting the stability of the membrane. They can also disrupt the ion transport chain by interacting with ions and ion channels (Fajardo et al. 2022). It also generates double-strand breaks in the DNA, interfering with the ribosome assembly and enzymatic activity via electrostatic interactions. They are also known to trigger a higher oxidative stress state by increasing the number of reactive oxygen species (ROS) that can damage proteins, lipids, other cellular contents, and DNA. Colloidal silver nanoparticles are one of the most widely used nanotechnology products against a wide spectrum of pathogens, including viruses, parasites, fungi, and bacteria. Silver is considered to be one of the most effective nanomaterials among the oligo-dynamic metallic nanoparticles due to its wide-range effects on different microbial species, different application forms, crystallographic structure, high surface exposure compared to volume, and compatibility with several compounds, making it a go-to use candidate for any treatment (Nangmenyi and Economy 2014). It triggers the oxidation of DNA and proteins with highly damaging effects, thus exerting antimicrobial activity. For example, silver nanoparticles are effective against methicillin-resistant *S. aureus* (Jeong et al. 2005). Eliminating microbes using visible light photocatalysts mediated by nanoparticles of metal oxides, as well as nano-porous fibres and foams, were also found to be effective (Li et al. 2014). Their effect is not limited to microbial elimination but also includes the elimination of organic pollutants from the pharmaceutical and cosmetics industries (Chena and Yadab 2011). Titanium dioxide nanoparticles, for example, have a strong anti-bacterial activity and are capable of destroying fish pathogens *in vitro* (Cheng et al. 2009), and their actions have been confirmed as a strong immune modulator of fish neutrophil function (Jovanovic 2011). NanoCheck, a commercially available nanotechnology product that is currently used for cleaning fishponds with lanthanum-based particles (40 nm in size), functions by inhibiting algal growth by absorbing phosphates from the water (Rather et al. 2011). Korni and Khalil (2017) showed that ginger-derived nanoparticles control the infection by motile *Aeromonas septicaemia* in the Asian carp fingerlings. Rather et al. (2017) reported that silver nanoparticles synthesized by *Azadirachta indica* (neem) showed antibacterial and immunomodulatory activity in mrigal (*Cirrhinus mrigala*) fingerlings when challenged with *A. hydrophila*. More recently, Erdem et al. (2018) demonstrated the antibacterial efficacy of silver nanoparticles synthesized by *Aeromonas sobria* against *A. hydrophila*. This raised the possibility to improve sanitary management through the use of more eco-friendly and economically based antimicrobial agents (Shah and Mraz 2019). Recent studies by Kepiro et al. (2020) explain the conceptual design of protein pseudocapsids exerting broad-spectrum antimicrobial activity. In contrast to conventional antibiotics, these pseudocapsids are highly effective against diverse bacterial strains. This method can eliminate antibiotic-resistant bacteria *in vivo* without causing toxicity. Pseudocapsids, which are nothing but an icosahedral structure that is polymorphic in size, but not in shape, are available in both D and L epimeric forms. It has to be noted that pseudocapsids can cause fast and irreversible harm to bacterial cells.

1.19 Conclusions and Future Prospects

There are some significant disadvantages to the classical methods of treatment and remedy that have been observed. But those classical approaches have given a path for modern technology, without which science can’t flourish. One such field is nanotechnology, which has given rise to numerous options with its wide field of application. Nowadays, disease in the aquaculture sector is bringing huge losses, and methods are being developed for rapid diagnosis and treatment. One such method is nanovaccination, which is a new attempt to enhance the immunogenicity of vaccines using nanoparticles as carriers and/or adjuvants. The immune system can very well be triggered because of the similar scale (size) between the nanoparticles and the pathogens, resulting in triggered cellular and humoral immunity responses. Thus, using nanotechnology in aquaculture has become a comprehensive tool for solving a lot of problems, not only in disease diagnosis and treatment but also in water quality control, fish nutrition, environmental management, etc. Currently, various nanotechnological applications have been implemented to improve the aquaculture industry, which could play an important role in the development and sustainability of this industry in the future. Nowadays, there are many potential applications for nanomaterials in the fisheries and aquaculture industries. Some of the most promising areas in this field are applications related to fish health management, nanoscale ingredient incorporation, the use of nanotechnology in aquaculture feeds and food packaging, as well as applications linked to value-added products, stress reduction, and health management. Sustainable development of nanotechnology in the fisheries and aquaculture industries will require a comprehensive assessment of its potential negative impacts; therefore, a careful analysis of the life cycle and shelf life of new nanomaterials, combined with an assessment of potential health and environmental risks, including exposure, release, and deposition, must be performed. However, considering the negative sides of this method, we should not refrain from trying to implement nanotechnological applications in the aquaculture sector, which should be linked to careful monitoring and controlled use, thus favouring efforts to minimize risks and maximize benefits. In the future, nanoparticles can be used in the detection of different diseases caused by bacteria as well as viruses in fishes (Tables 1.1 and 1.2).

Table 1.1 Merits and demerits of nanoparticles

Type of nanoparticles	Merits	Demerits
Polymeric nanoparticles	Better immunogenicity can be obtained by easy modification of surface properties, biodegradable and targeted antigen delivery	Low aqueous solubility and synthesis require the use of organic solvents, low antigen loading, premature release of antigens, insufficient antigen protection
Inorganic nanoparticles	Easy to modify, less chances of premature release, and better protection of adsorbed antigens	Low aqueous solubility and low biodegradability
Nanoliposomes	Possess intrinsic adjuvant properties, accommodate both hydrophilic and lipophilic antigens and relatively stable in gastrointestinal fluids when modified	Low mucus penetration, limited antigen loading, and poor gastrointestinal stability of naked liposomes
Virus-like particles (VLP's)	Possess self-adjuvant properties, mimic original virus and high gastrointestinal stability	Lack of reproducibility
Nanoemulsions	Possess self-adjuvant properties, encapsulate both hydrophilic and lipophilic antigens	Premature release of antigens and poor gastrointestinal stability

Table 1.2 Progress in nanoparticle-based fish vaccine delivery

Nanoparticle	Pathogen	Fish species	Mode of administration	Vaccine formulations with nanoparticles	References
Chitosan	VHSV	*Danio rerio*	IP	NPrgpG pICrgpG	Kavaliauskis et al. (2016)
Chitosan	ISAV	*Salmo salar*	Oral	NP-V/ NP-Ad+NP-V	Aravena et al. (2013)
Chitosan/ TPP	Nodavirus	*Lates calcarifer*	Oral	pFNCPE42-CS/TPP	Vimal et al. (2014)
Chitosan	*Vibrio anguillarum (Listonella)*	*Lates calcarifer*	Oral	Chitosan-pVAOMP38	Rajesh et al. (2008)
PLGA	*Aeromonas hydrophila*	*Labeo rohita*	Oral	Np-rOmpW (HiAg) 8 µg/g Np-rOmpW (LoAg) 4 µg/g	Dubey et al. (2016)
PLGA, PLA	*Aeromonas hydrophila*	*Labeo rohita*	IP	PLGA-Omp	Rauta and Nayak 2015
PLGA	IPNV	*Salmo salar*	IP	PLGA nanoparticle-TA PLGA nanoparticle-PT	Munangandu et al. (2012)

(continued)

Table 1.2 (continued)

Nanoparticle	Pathogen	Fish species	Mode of administration	Vaccine formulations with nanoparticles	References
PLGA	IHNV	*Oncorhynchus mykiss*	Oral	High dose PLGA(10WPV) Low dose PLGA-pCDNA-G high dose PLGA-pCDNA-G high dose PLGA	Adomako et al. (2012)
PLGA, PLA	–	*Salmo salar*	IP	PLGA-NP50L PLGA-NP50H PLGA-NP75H PLA-NP100L	Fredriksen and Grip (2012)
PLGA	LCDV	*Paralichthys olivaceus*	Oral	pEGFP-N2-MCP PLGA PLGA- pEGFP-N2-MCP	Tian and Yu (2011)
PLGA	–	*Salmo* saliar	IP	NP NP/TNP-LPH, NP/β glucan, NP/TNP-LPH/β glucan	Fredriksen et al. (2011)
Liposome	*Vibrio harveyi*	*Epinephelus bruneus*	IP	Liposome-*V. harveyi*	Harikrishnan et al. (2012)
Liposome	*Aeromonas salmonicida*	*Cyprinus carpio*	Oral	Liposome-T1031	Irie et al. (2005)
Carbon nanotubes	GCRV	*Ctenopharyngon idellus*	IM Bath	SWCNTs-pEGFP-vp5 1 μg SWCNTs-pEGFP-vp5 2.5 μg SWCNTs-pEGFP-vp5 5 μg SWCNTs-pEGFP-vp5 1 mgL-1 SWCNTspEGFP-vp5 10 mg L-1 SWCNTs-pEGFP-vp5 20 mg L-1	Wang et al. (2015)

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