Chapter 2
Nanotechnologies in Controlling Aquatic Diseases

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2.1 Introduction

Aquaculture is the age-old practice of tending to confined water for growing aquatic organisms such as fish and shellfish and harvesting the production for human benefit. It is the human-controlled cultivation and harvest of freshwater and marine plants and animals. It includes fish farming, fish culture, mariculture, fish breeding, and ocean ranching. Throughout the world, aquaculture operations constitute an integral part of fisheries and aquatic resource management. Organisms as varied as trout, carp, and tuna (i.e., finfish), shrimps and oysters (i.e., shellfish), and seaweed are grown, using ponds, tanks, or nets, in salt, brackish, and fresh waters.

Aquaculture in India is one of the leisure activities among fishermen and farmers. With the passage of time, due to the limitations of the land-based food supply and the Malthusian fear, man has turned more seriously to water-based production systems. Earlier, it was easier to harvest wild fish stocks when compared to culturing fish, but now a stage of saturation in production through capture fisheries has been reached. Globally, annual water-based production has reached a plateau phase of 95–100 million tonnes during the current decade.

A vaccine is a biological preparation that improves immunity to a particular disease. The agent stimulates the body's immune system to recognize the agent as a foreign body. It destroys the foreign body and "reminisces" it so that the immune system can easily recognize and destroy any of these microorganisms that it later encounters. There are a lot of varieties of vaccines like DNA vaccines, recombinant vaccines, and many more. Whole-cell vaccine is a bacterial suspension of whole bacterial cells that have been killed. Whole-cell vaccine production is cheaper. It is

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of two types: killed vaccine and attenuated vaccine. Though attenuated vaccine provides both cellular and humoral immunity, there is always a chance of infection in immunocompromised individuals and also a possibility of reversion to pathogenic forms. Thus, whole-cell killed vaccines are preferred as they are able to provide potent immunization. Whole-cell vaccines are preferred over other types because they are cheaper than others and also very effective. Though others have been found effective, their approval is still an issue, and also their production is difficult as in the case of the pertussis vaccine (Halperin et al. 1992). Though this is a human vaccine, this issue still affects other vaccines intended for other living forms.

Zhao et al. (2014), Pankhurst et al. (2003), and Tissot et al. (2008) reported that nanotechnology has made a huge progress in the field of biomedicine. In addition, the application has been increasing in the area of vaccinology, which gave rise to "nanovaccinology" (Mamo and Poland 2012; Zhao et al. 2014). Thus, the nanovaccines developed contain nanoparticles formulated with antigens either absorbed on or encapsulated within the surface against which an immune reaction is elicited (Gregory et al. 2013; Zaman et al. 2013).

In the past few decades, nanoparticles have been used as delivery systems and adjuvants in vaccines. Nanotechnology has formulated various efficient vaccine delivery systems that have helped in protecting the encapsulated antigens from the belligerent gastrointestinal environment. They also maintained the sustained release, which was inducing the immunostimulatory properties of the vaccine. Moreover, nanotechnology has been applied in the development of several fish vaccines for mass vaccination by either incorporating them in feed or administering them via immersion. Thus, nanovaccines can be a potential alternative and possible solution for injection-free mass vaccination and its applications in the aquaculture industry (Vinay et al. 2016).

Poly (lactic-co-glycolic acid) (PLGA) and polymeric chitosan are the most investigated nanoparticles in the fish vaccine research area (Mohamed et al. 2016). PLGA is a synthetic polymeric nanoparticle, whereas chitosan is a natural polymeric nanoparticle (Liang et al. 2014; Nirmal et al. 2014). Chitosan nanoparticles have been reported in the development of various fish vaccines. A study was reported on a nanoparticle-based oral vaccine against infectious salmon anemia virus (ISAV) that incorporated an alphavirus replicon as an adjuvant (Aravena et al. 2015).

2.2 Fish Vaccine in Aquaculture

The fish immune system is exposed to part of a pathogen or the entire pathogen (antigen), allowing time for the immune system to develop a response. Fish vaccines are classified as killed and modified live vaccines. Killed fish vaccines consist of killed (heat-killed/formalin-killed) pathogenic bacteria that stimulate the immune systems of the water-based production organisms and generate an immune response.

In order to prepare the bacterins, each bacterial isolate was inoculated separately into tryptic soy broth (TSB) and incubated for 24 h at 25 °C. Formalin (40% w/v)

was added to the broth culture at a final concentration of 0.5% (V/V) and left for 48 h at room temperature. In the case of bivalent formalin-killed vaccine formulation, an equal portion of each bacterin was added to make one volume of the vaccine. Besides, the heat-killed vaccine was prepared by heating the broth culture for 30 min at 100 °C. The inactivated cells were counted with the hemocytometer (1×10^8 cells/ml) for all the isolates. After that, the bacterins were tested for their sterility (free from the living cells) by streaking them onto trypticase soy agar, which showed no growth (Dehghani et al. 2012).

Pridgeon and Klesius (2010) reported that the modified live vaccines are comprised of live microorganisms that are grown in culture. They cannot cause significant disease. Live attenuated vaccines work by stimulating both cell-mediated and humoral immune responses and conferring protection for a long time.

Other than PLGA and chitosan, numerous other kinds of nanoparticles have been used in fish vaccine delivery including nanoliposomes, calcium phosphate, carbon nanotubes, immunostimulating complexes, and biodegradable polymers that have the potential to develop new vaccines against various fish pathogens (Vinay et al. 2017).

2.3 Types of Vaccines and Their Applications in Nanotechnology

2.3.1 Fish Vaccines

With an increase in water-based products for human and animal consumption, a decline in fishery resources is also observed simultaneously. Hence, there is an additional need for alternate sources of water-based products, mainly through the aquaculture industry, which is claimed to be the fastest-growing segment of agriculture in the world (Hanfman 1993). The increased demand for aquatic animals, which is partly due to greater health awareness among consumers and the decline or stagnation of natural harvests, has largely contributed to this rapid growth.

Fish management, coupled with good hygiene practices, remains a major key factor in aquaculture-based production systems.

Recent researches are focusing on the importance of nanoparticles in terms of their application in the development of fish vaccines in aquaculture (Yildirimir et al. 2011). Nanoparticles are able to exhibit properties that are interesting as well as different compared to their parent compounds, including quantum size effects and increased relative surface area. Studies have proven that the application of nanoparticles has led to the enhancement of the stability, solubility, permeability, targeting, and biocompatibility of vaccines (You et al. 2012; Lai et al. 2013; Frohlich 2012; Doll et al. 2013).

2.3.1.1 Viral Fish Vaccines

Benmansour and de Kinkelin (1997), Lopez Doriga et al. (2001), and Ronen et al. (2003) reported that most of the available virus vaccines for aquaculture nowadays are based on inactivated viruses or recombinant subunit proteins. Inactivated or killed viral vaccines are generally not successful unless delivered by injection. High doses are required to achieve protection. Live viral vaccines have been tested with good results in fish. The first viral vaccine for fish was against a carp rhabdovirus, causing spring viremia of carp (SVC). Commercially available vaccines for IPNV are based on either inactivated cell culture-propagated viruses or recombinant structural proteins. However, DNA vaccines encoding the same viral glycoproteins are remarkably efficacious. Indeed, these DNA vaccines are protective when used in small doses and efficacious as early as 4–8 days and for up to 2 years post vaccination (Corbeil et al. 2000).

A study was carried out to improve the prophylactic efficacy of immersion vaccines against fish viral diseases by constructing a targeted single-walled carbon nanotube (SWCNT)-based immersion vaccine delivery system "CNTs-M-VP7." The surface of this delivery system was modified with mannose to allow the targeting of antigen-presenting cells (APCs). This monosylated nanoparticle-based immersion vaccine was able to enter into the fish body through mucosal tissues like gill, skin, and intestine and later present to immune-associated tissues. They could trigger robust immune responses by inducing the maturation as well as the presenting process of the APCs (Zhu et al. 2020).

2.3.1.2 Bacterial Fish Vaccines

The first commercially available bacterial vaccines were against enteric red mouth disease (ERM, yersiniosis) and vibriosis, introduced in the USA in the later 1970s (Evelyn 1997). These vaccines were based on inactivated whole cell formulations and were administered by immersion. Such vaccines have proven efficacious in preventing many of the major bacterial diseases. Vibriosis is an example of a disease against which the simple inactivated bacterin vaccine works well, but other bacteria have proven more difficult to control by vaccination.

Kuzyk et al. (2001) reported the development of new vaccines based on recombinant proteins. These new approaches might offer a solution for diseases where inactivated bacterins are inefficient, although the long-term performance of the vaccines remains to be documented.

The gram-negative bacteria were reported to hamper the growth of finfish in aquaculture. All gram-negative bacteria have surface-associated outer membrane proteins (OMPs), and they were known as potential vaccine candidates. A study revealed the applications of OMPs in designing certain vaccines based on subunit vaccines, DNA vaccines, chimeric proteins, and recombinant proteins as potential new-generation vaccine candidates for various bacterial pathogens in aquaculture. OMPs play a significant role in the adaptive responses of bacteria including iron

acquisition, solute and ion uptake, bile salt resistance, antimicrobial resistance, serum resistance, and also maintaining the integrity as well as selective permeability of the bacterial cell membrane. OMPs that have been found conserved across serotypes were used as potential candidates in the design of vaccines (Maiti et al. 2020).

2.3.1.3 Fish Vaccines Against Parasites

Parasitic diseases such as white spot disease, whirling disease, amoebic gill disease, proliferate kidney disease (PKD), and salmon live infestation create severe problems in fish farming, and no parasite vaccines are commercially available.

2.3.2 Need for New Fish Vaccines

Aasjord and Slinde (1994) and Mialhe et al. (1995) reported that outbreaks of infections can cause huge economic losses to fish farmers. There are no prophylactic or therapeutic measures available. Chemicals and antibiotics can be used to control bacterial and parasitic diseases, but these products often have undesirable side effects (Munn 1994).

In the case of viral infections that affect the water-based production systems of farms, there are no interventions available. Hence, a massive destruction of the infected stock is the only remedy. Current research in this field is focusing on disease prevention rather than treatment after infection. However, treatment can be opted for whenever the vaccine is unavailable. Vaccines provide herd immunity, but the currently available vaccines provide protection against bacterial diseases (Newman 1993; Schnick et al. 1997).

The high cost of new product development combined with the relatively small size of the industry and the low value of individual animals have largely contributed to this situation. There is a limited supply of many of the vaccines though tested and proven under laboratory conditions. This bias is due to the prohibitive cost of production, insufficient protection, or lack of safety (Leong et al. 1997; Munn 1994; Newman 1993). The lack of effective viral vaccines is one of the main problems in fish vaccinology.

2.3.3 DNA Vaccine

DNA vaccines are the most efficient vaccines established against viral diseases in fish to date only at an experimental level. DNA vaccines represent a powerful new approach to raising immune responses. The antigens are synthesized in transfected cells and obey the modification and antigen presentation rules of eukaryotic cells.

Very low levels of antigen (typically monogram levels) induce both antibody and cytolytic T-cell responses. Several reviews on DNA vaccines for fish are available (Anderson and Leong 2000; Heppell and Davis 2000; Jones 2001; Lorenzen et al. 2005).

Recently, research has diverted to the preparation of DNA vaccines that encode the pathogenic antigens and, when administered to fish, provide an immune response at an effective level (Boudinot et al. 1998; Lorenzen et al. 2000; Lorenzen et al. 2002; Mclauchlan et al. 2003; Pasnik and Smith 2005; Purcell et al. 2004; Vesely et al. 2004). Earlier, several research studies in fish involved the use of genes to study the magnitude of expression levels under different conditions with a focus on genes encoding proteins such as β-galactosidase, green fluorescent protein, and luciferase (Gomez Chiarri et al. 1996).

Advantages of DNA Vaccines

DNA vaccines offer several advantages to aquatic organisms over classical antigen vaccines (i.e., live attenuated, whole killed, and subunit vaccines). Practically. DNA vaccines are relatively inexpensive and easy to produce. Multivalent vaccines can also be easily prepared by mixing together different plasmids or including more than one antigen-encoding gene in a single vector for collinear expression, which will further reduce the cost of production. In addition, DNA is a very stable molecule at higher temperatures; therefore, shipment and storage need not be in a cold environment. All these factors contribute to making DNA vaccines very attractive for controlling fish diseases. DNA-based immunization also has immunological advantages over traditional methods of vaccination. It can induce strong and lasting humoral and cell-dependent immune responses without a boost, similar to that conferred by live vaccines, but without the risk of inadvertent infection (Davis and McCluskie 1999).

2.3.4 DNA Vaccines for Fish Pathogens in Nanotechnology

Gomez Chiarri et al. (1996) reported that one of the first bacterial fish pathogens for which DNA vaccines were tested was *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease in salmon and trout, but no protective effect has been reported. A more generic approach has been attempted for *Piscirickettisa salmonis*, against which fish were vaccinated with a full expression library of plasmid DNA. A pathogen-specific antibody response was subsequently detected, but the level of protection was relatively low. Sommerset et al. (2005) reported that the viral hemorrhagic septicemia virus vaccine induced a high level of protection against Atlantic halibut nodavirus in turbot when the challenge was performed shortly after vaccination, thus demonstrating early protection and is not limited to rhabdovirus infections in salmonid.

The DNA vaccine encoding the outer membrane porin protein of *Vibrio anguillarum* in sea bass also provides a good immune response in sea bass (Rajesh Kumar et al. 2007).

2.3.4.1 Oral Vaccine

Oral vaccines can be administered effectively to all fishes, regardless of their size. This method is a cheap way to either vaccinate or boost the immune status of any fish in any cultural environment. However, when administering oral vaccines to fishes, the attention is to be focused on overcoming degradation in the digestive environment (gut) of the fish so that an effective vaccine delivery is possible with an adequate immune response. Other delivery challenges include cost-effective production, shelf storage stability, and treatment to prevent leaching from feed upon contact with water.

An oral DNA vaccine was designed by loading the bacterial outer membrane protein K (ompK) gene of *Vibrio parahaemolyticus* onto the chitosan nanoparticles. They later elicited an immune response in black sea bream, *Acanthopagrus schlegelii*, against the pathogen *Vibrio parahaemolyticus* (Li et al. 2013). In another study, the pH-controlled release of dihydrolipoamide dehydrogenase (DLDH) antigens via a mesoporous silica nanoparticle (MSN) delivery system was used to develop an oral fish vaccine. The DLDH antigens of the bacteria, *Vibrio alginolyticus*, were loaded onto the MSN to design the vaccine delivery system. Moreover, hydroxypropyl methylcellulose phthalate (HP55) was coated to ensure the protection of the immunogen. They displayed the prepared MSN delivery system as a potential candidate carrier for fish vaccines through oral administration in aquaculture (Zhang et al. 2021).

2.4 Vibrosis: A Common Disease Pathogen in Aquaculture

A disease outbreak in aquaculture causes significant economic loss, but the use of antibiotics is not always preferable due to the development of drug-resistant strains. Antibiotic residue may also remain in the fishes, which can be toxic to them as well as humans. Sometimes, when the disease is not detected earlier, it finds its way into the human food chain, where the infection spreads to humans. Some of the diseases common in fisheries are furunculosis, dropsy, ergasilosis, lernaesosis, pike fly virus, viral hemorrhagic septicemia (VHS), and many more.

Vibriosis in aquaculture has been reported in various parts of the world. In India also, it is very much common. Thus, it has been a major concern. Vibriosis is caused by *Vibrio* spp. and has been a serious disease problem in prawns. Some of the *Vibrio* spp. include *V. harveyi*, *V. parahaemolyticus*, *V. anguillarum*, *V. vulnificus*, and *V. splendidus*, and some of the diseases they cause are tail necrosis, shell disease, red disease, loose shell syndrome (LSS), and white gut disease (WGD).

A combination of one or more of these can cause these diseases, and the virulence of the disease depends on the strain, its source, and its environment. The severity of infection depends on the species and strain of ***Vibrio*** involved, the stage of development and age of the prawn, and the ambient environmental conditions (Jayasree et al. 2006). Mass mortality is observed in different parts of the world in hatcheries and growing ponds of prawns (Couch 1978; Overstreet 1978; Lightner 1983, 1985, 1988, 1996; Sindermann 1990; Ruangpan and Kitao 1992; Chen et al. 1992; Yang et al. 1992; de la Pena et al. 1993; Jiravanichpaisal et al. 1994; Mohney et al. 1994; Lavilla-Pitogo and de la Pena 1998; Lavilla-Pitago et al. 1998). Over a dozen species have been isolated in case of implicated diseases (Overstreet 1978; Lightner 1988, 1996; Sindermann 1990). Vibriosis is also rampant in the Indian region, and infection from luminous vibriosis has caused many hatcheries to shut down (Couch 1978).

Vibrio parahaemolyticus, a marine bacterium, causes food-borne disease in humans, resulting in gastroenteritis. This is due to the possession of hemolysin genes (tdh. Trh. or both) that favor the progression of the disease (Kim et al. 1999). The mode of infection of *Vibrio parahaemolyticus* in fish is mainly via penetration of the bacterium to the host (chemotactic activity), followed by the deployment of an iron sequestering system, resulting in eventual damage to fish by means of extracellular products, i.e., hemolysin and proteases (Haldar et al. 2010).

Pathogenesis of *Vibrio* consists of gaining access to the host tissue, colonization, and invasion (Lee et al. 2008). During colonization, the composition of the organisms, such as outer membrane proteins (OMPs), plays an important role in their adhesion to host cells. OMP is a unique component of the gram-negative bacterial cell wall. OMPs are transmembrane proteins, accounting for about half of all the outer membrane. OMPs of gram-negative bacteria, which form channels for small hydrophilic molecules, are known as porins (Chakrabarti et al. 1996). However little information is known about the outer membrane protein of *V. parah*aemolyticus, so it is understandable that many studies have been focused on amplifying genes responsible for these porins, to obtain these recombinant properties and understand their role in pathogenicity (Ye et al. 2010).

Daniela Ceccarelli et al. (2013) reported that *Vibrio parahaemolyticus*, autochthonous to estuarine, marine, and coastal environments throughout the world, is the causative agent of food-borne gastroenteritis. More than 80 serotypes have been described worldwide based on the antigenic properties of the somatic (O) and capsular (K) antigens. Serovar O3:K6 emerged in India in 1996 and subsequently was isolated worldwide, leading to the conclusion that the first *V. parahaemolyticus* pandemic had taken place.

The most common fish disease caused by bacteria belonging to the genus *Vibrio* is vibrosis. Rajesh Kumar et al. (2007) reported that *Vibrio anguillarum* produces a 38-kDa major outer membrane porin protein (OMP) for biofilm formation and bile-protected activity caused by Vibriosis. They reported that the gene encoding the porin was used to construct a DNA vaccine. The evaluation in Asian seabass (*Lates calcarifer* Bloch) is a common species in the aquaculture industry of the Indian coast and a potential resource.

White spot syndrome virus (WSSV) causes severe economic loss in the shrimp culture industry worldwide. Sarathi et al. (2007) silenced the VP28 gene of the WSSV of shrimp by bacterially expressed dsRNA. This technology enabled them to produce a uniform quality of the VP28dsRNA, which was used to protect the shrimp against WSSV.

White tail disease (WTD) causes severe economic losses in prawn hatcheries and farms, and mortalities were observed at 100% within 2 or 3 days. *Macrobrachium rosenbergii* nodavirus (MrNV) and extra small virus (XSV) have been identified as pathogenic agents, which are 27 and 15 nm in diameter, respectively. Sudhakaran et al. (2008) cloned and sequenced the capsid protein of an Indian isolate of an extra small virus from *Macrobrachium rosenbergii* for gene silencing.

Rajesh Kumar et al. (2008) examines the potential efficacy of a DNA vaccine against *Vibrio anguillarum* through the oral route using chitosan nanoparticle encapsulation. The porin gene of *V. anguillarum* was used to construct a DNA vaccine using pcDNA 3.1, a eukaryotic expression vector, and the construct was named pVAOMP38. The chitosan nanoparticles were used to deliver the constructed plasmid.

2.5 Conclusion

Infectious disease in aquatic organisms leads to a drastic change in the biome, thereby causing a potential threat to mankind who are dependent on it. Also, it leads to a severe and catastrophic blow to the economy of a nation. Focusing on the growing human population and the need for alternative resources (water-based cultivation) for survival, several researches focus on the development of various interventions that cure infection among aquatic organisms. On a cost-based analysis of various therapeutic measures, vaccines have several advantages and have proven to be cost-effective.

From various experimental trials, it is evident that vaccines play a significant role in boosting immunity and providing an immune response against many fish pathogens. Hence, this study has focused on the area of vaccination towards preventing and eradicating various disease pathogens that affect aquatic life. Also, attention is drawn towards the benefit of the implementation of vaccination over a large scale to cure several communicable diseases of aquatic fauna, which ultimately serves as an alternate food resource to the land-based food supply.

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