Nanovaccines: a Possible Solution for Mass Vaccination in Aquaculture

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The production intensity of aquaculture production systems is increasing and this intensification is leading to increased prevalence of diseases. Poor health management in aquaculture costs roughly US$ 50 billion annually (Rather *et al*. 2011). In aquaculture, the possibility of occurrence of disease is unpredictable. Emerging or existing diseases may lead to severe economic losses in aquaculture. Diseases in aquaculture have a direct impact on the livelihood of millions of people as well as food security.

Nanotechnology has helped to formulate efficient vaccine delivery systems that protect the encapsulated antigen from the hostile gastrointestinal environment and maintain sustained release that helps to induce the immunostimulatory properties of the vaccine. Nanoparticles can be used as adjuvants and delivery systems in vaccines. Nanotechnology can be applied to develop fish vaccines for mass vaccination — incorporated in feed or through immersion — making application of these vaccines relevant to field conditions.

Disease prevention is a main issue to have sustainable aquaculture. Indiscriminate use of antibiotics leads to the development of antibiotic-resistance in many bacterial species and the accumulation of antibiotics causes pollution (Karunasagar *et al*. 1994).

Fortunately for several species in aquaculture, vaccination can provide protection against pathogens present in the water. Vaccination is one of the best health management practices in aquaculture and has helped control several major diseases in aquaculture. Historically it is based on the principle of "isolate, inactivate and inject" (Liang *et al*. 2014).

In brief, a vaccine is a preparation of a weakened or inactivated pathogen such as a bacteria or virus or of a portion of the pathogen's structure produced using recombinant DNA technology. Upon administration, the vaccine stimulates antibody production or cellular immunity against the pathogen but is incapable of causing severe infection, thereby reducing the risk of disease outbreak.

Nanovaccines

Nanovaccines are vaccines designed with a suitable nanoparticle with an antigen or group of antigens. They are emerging as a new class of vaccines that directly targets the infection site in the body by using the body's immune system and prevents infections and diseases from spreading.

Although oral vaccination is most preferred, it comes with numerous hurdles for effective delivery to immune cells. Firstly, an oral vaccine has to suffer exposure to acidic pH, proteolytic enzymes and bile salts, leading to its degradation in the gastrointestinal tract. Encapsulating antigenic materials using several polymeric and lipid-based nanoparticle carriers could be an effective approach because such particles can reduce degradation of antigens in the GI system (Nirmal *et al*. 2014). Nanotechnology has helped to formulate efficient vaccine delivery systems that protects the encapsulated antigen from the hostile gastrointestinal environment and maintain sustained release that helps to induce the immunostimulatory properties of the vaccine (Akagi *et al*. 2012, Kim *et al*. 2014).

Nanoparticle systems also possess adjuvant properties that can enhance the efficacy of the antigens. An ideal vaccine carrier is expected to protect the structural integrity of the antigen and effectively deliver it to the desired mucosal surface in order to produce sufficient mucosal, humoral and cellular responses. Due to their very small size, nanoparticles enter living cells through cellular endocytosis (Treuel *et al*. 2013, Liang *et al*. 2014).

Nanotechnology presents an opportunity to design particles with different composition, surface properties, shapes and sizes. Nanotechnology is the study and application of extremely small things (about 1 to 100 nanometers), and it is used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering.

New-generation vaccines thus produced are generally safe but they are often less immunogenic and need an adjuvant or delivery system to enhance immunity (Vinay *et al*. 2013). Nanoparticles can be used as adjuvants and also as delivery systems in vaccines. Nanoparticles such as dendrimers, polymeric nanoparticles, metallic nanoparticles, magnetic nanoparticles and quantum dots have emerged as effective vaccine adjuvants.

Injection vaccination is very effective but not practical and economical for aquaculture species. Oral or immersion vaccines are best suited for mass vaccination. Nanotechnology can be applied to develop fish vaccines for mass vaccination – incorporated in feed or through immersion – making application of these vaccines relevant to field conditions. The use of nanotechnology in vaccinology has increased in the last decade, leading to a new field of science called nanovaccinology.

Nanoparticles

Nanoparticles can be grouped into several categories based on their functional properties, shape and size. Further, nanoparticle based oral delivery system are categorized as polymeric nanoparticles, inorganic nanoparticles, nanoliposomes, immunostimulating complexes, virus-like particles, and nanoemulsions. There are merits and demerits in using each of these nanoparticles for developing vaccines (Table 1).

Polymeric nanoparticles have the capacity to encapsulate, adsorb or conjugate any foreign material within itself or on its surface. Nanoparticles used in vaccine development usually range from 2-1000 nm. There are synthetic and natural polymers that are biocompatible and usually biodegradable, an essential property in safe vaccine development. Poly (d, l-lactide-co-glycolide) (PLG), Poly (d, l-lactide-coglycolic acid) (PLGA), Poly (g-glutamic acid) (g-PGA), Poly (ethylene glycol) (PEG), Polystyrene are some of the synthetic polymeric nanoparticles. Chitosan and inulin are natural polymeric nanoparticles. (Liang *et al*. 2014, Nirmal *et al*. 2014).

THE MOST INVESTIGATED NANOPARTICLES IN FISH VACCINE RESEARCH ARE POLYMERIC CHITOSAN AND PLGA NANOPARTICLES. CHITOSAN NANOPARTICLES HAVE BEEN USED FOR THE DEVELOPMENT OF FISH VACCINES, FOR EXAMPLE THE INACTIVATED VIRUS VACCINE AGAINST INFECTIOUS SALMON ANEMIA VIRUS (ISAV) THAT INCORPORATES THE DNA CODING FOR ISAV REPLICASE AS AN ADJUVANT.

Inorganic nanoparticles are potential oral vaccine carriers due to their attractive physical and chemical properties. Nanoparticles used in vaccine development usually range from 2-1000 nm. These nanoparticles are biocompatible, however not biodegradable. The advantages of inorganic nanoparticles are the rigidity and controlled synthesis (Liang *et al*. 2014), better colloidal stability, higher antigen encapsulation and better targeted delivery. Gold nanoparticles, Carbon nanotubes (CNT's), Silica nanoparticles (SiNP's) and calcium phosphate nanoparticles are examples of inorganic nanoparticles.

Nanoliposomes have been well documented for their diverse ability to deliver various hydrophilic and lipophilic antigens. These are formed by nontoxic and biodegradable self-assembled structures of phospholipids consisting of an internal aqueous core entrapped by a lipid bilayer. The size usually ranges from 100-400 nm. Liposome-polycation-DNA nanoparticles (LPD) and Interbilayer-crosslinked multilamellar vesicles (ICMVs) are some of the liposome nanoparticle formulations.

Immunostimulating complexes (ISCOMs) are self-assembling spherical cage-like structured particulate complexes, formed by saponin adjuvant Quil A, phospholipids, cholesterol and antigens. Immunostimulating complex used in vaccine development usually are 40 nm. ISCOMATRIX is a common immunostimulating complex lacking antigen.

Virus like particles are self-assembling nanoparticles, formed by biocompatible capsid proteins. They are the ideal nanovaccines because they lack the infectious nucleic acid but have the evolved structure of the virus to induce immunity. Nanoparticles used in vaccine development usually range from 20-800 nm.

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TABLE 1. MERITS AND DEMERITS OF NANOPARTICLES.

NANOPARTICLE SYSTEM	*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 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 • Accommodates both hydrophilic and lipophilic antigens • Relatively stable in gastrointestinal fluids when modified	• Low mucus penetration • Limited antigen loading • Poor gastrointestinal stability of naked liposomes
ISCOMS	• Easy to encapsulate • Built-in adjuvant property of Quil A	• Do not form depot • Difficult to incorporate hydrophilic antigens
Virus-like particles	• Possess self-adjuvant properties • Mimics original virus and high gastrointestinal stability	• Lack of reproducibility Premature release of antigens and poor gastrointestinal stability
Nanoemulsions	• Possess self-adjuvant properties • Encapsulates both hydrophilic and lipophilic antigens	

Nanoemulsion is an isotropic system of two immiscible liquids (water and oil), which stabilizes with the addition of an appropriate amount of surfactant. Nanoemulsions usually range from 20-200 nm. They can exist as water in oil or oil in water forms and carry vaccine in their core or can just simply be mixed with antigens for delivery. MF59, Montanide and Tailorable nano-sized emulsion (TNE) are some of the emulsion-based nanoparticles.

The most investigated nanoparticles in fish vaccine research up to now are polymeric chitosan and PLGA nanoparticles (Mohamed *et al*. 2016). Chitosan nanoparticles have been used for the development of fish vaccines, for example the inactivated virus vaccine against infectious salmon anemia virus (ISAV) that incorporates the DNA coding for ISAV replicase as an adjuvant (Andrea *et al*. 2015). Another oral DNA vaccine was developed by loading the outer membrane protein K (ompK) gene of *Vibrio parahaemolyticus* onto chitosan nanoparticles. This recombinant nanovaccine elicited a protective immune response in black sea bream *Acanthopagrus schlegelii* against *V. parahaemolyticus* (Li *et al*. 2013).

The efficacy of PLGA nanoparticles as a DNA vaccine carrier and adjuvant has been reported (Wang *et al*. 1999, Tinsley *et al*. 2000, Holvold *et al*.2014). Examples include:

- An oral DNA vaccine in Japanese flounder *Paralichthys olivaceus* against lymphocystis disease virus (LCDV) (Tian *et al*. 2008, Tian *et al*. 2011).
- An orally administered DNA vaccine was used to immunize rainbow trout against infectious hematopoietic necrosis virus, and an immune response was observed (Adomako et al. 2012).
- Oral delivery of liposome nanoparticle based Carp Herpes Virus-3, *A. hydrophila, A. salmonicida,* was attempted (Irie *et al*. 2005, Yasumoto *et al*. 2006a, 2006b, Miyazaki *et al*. 2008).
- Oral administration with PMMMA-PLGA/Trx-SIP nanoparticles stimulated robust immunity in tilapia, an animal with a relatively simple immune system (Lei *et al*. 2015).
- An oral DNA vaccine based on chitosan nanoparticles has been developed against reddish body iridovirus recently (Zheng *et al*. 2016).
- An oral DNA vaccine against *Vibrio anguillarum* in Asian sea bass *Lates calcarifer* was developed using chitosan and chitosan/tripoly phosphate nanoparticles. The nanovaccine conferred only moderate protection against the pathogen (Rajesh *et al*. 2008, Vimal *et al*. 2012).
- The effectiveness of recombinant DNA-chitosan nanoparticles in providing protection against white spot syndrome virus (WSSV) in shrimp was investigated. When administered orally, the vaccine enhanced shrimp immunity, providing a protective response against WSSV (Rajesh *et al*. 2009, Vimal *et al*. 2013).

Conclusion

Nanoparticle-based vaccines have tremendous advantages and applicability as fish vaccines. The article has mentioned several research attempts and success stories in eliciting good immune response in aquaculture species. However, nanovaccine research in aquaculture health management is in its infancy and more research and development is required. Nanoparticle-based vaccines represents a potential option for injection-free, mass vaccination in aquaculture.

Note

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References

Adomako, M., S. St-Hilaire, Y. Zheng, J. Eley, R.D. Marcum and W. Sealey, B.C. Donahower, S. LaPatra and P.P. Sheridan. 2012. Oral DNA vaccination of rainbow trout, *Oncorhynchus mykiss* (Walbaum), against infectious hematopoietic necrosis virus using PLGA [poly (D, L-lactic-co-glycolic acid)] nanoparticles. Journal of Fish Diseases 35:203-214.

Akagi, T., M. Baba and M. Akashi. 2012. Biodegradable Nanoparticles as Vaccine adjuvants and delivery systems: Regulation of immune responses by nanoparticle based vaccine. Advances in Polymer Science 247:31-64.

Andrea, R. A., F. Yazmin, C. Julio, B. Tania, P. Veronica, L.T. Jose, M. Hegaly, G. Fernando, M.S. Ana and S. Eugenio. 2015. Development of a nanoparticle-based oral vaccine for Atlantic salmon against ISAV using an alphavirus replicon as adjuvant. Fish and Shellfish Immunology 45:157-166.

FAO (Food and Agriculture Organization of the United Nations). 2014. The State of World Fisheries and Aquaculture. Food and Agriculture Organization of the United Nations, Rome, Italy.

Hølvold, L.B., A.I. Myhr and R.A Dalmo. 2014. Strategies and hurdles using DNA vaccines to fish. Veterinary Research 45:21-31.

Irie, T., S. Watarai, T. Iwasaki and H. Kodama. 2005. Protection against experimental *Aeromonas salmonicida* infection in carp by oral immunisation with bacterial antigen entrapped liposomes. Fish and Shellfish Immunology 18:235-242.

Karunasagar, I., R. Pai, G.R. Malathi and I. Karunasagar. 1994. Mass mortality of *Penaeus monodon* due to antibiotic-resistant *Vibrio harveyi* infection. Aquaculture 128:203-209.

Kim, M.G., J.Y. Park, Y. Shon, G. Kim, G. Shim and Y.K. Oh. 2014. Nanotechnology and vaccine development. Asian Journal of Pharmaceutical Sciences 9:227-235.

Lei, Z., Z. Zhanzhuang, H. Chaohua, L.B. Susan, Y. Wendi, S. Yintao, Z. Xinyan and W. Yunkun. 2015. Controlled and Targeted Release of Antigens by Intelligent Shell for Improving Applicability of Oral Vaccines. Biomaterials doi: 10.1016/j. biomaterials.2015.11.009.

Li, L., S.L. Lin, L. Deng and Z.G. Liu. 2013. Potential use of chitosan nanoparticles for oral delivery of DNA vaccine in black sea bream *Acanthopagrus schlegelii* Bleeker to protect from *Vibrio parahaemolyticus*. Journal of Fish Diseases 36(12):987-995.

Liang, Z., S. Arjun, W. Nani, X.Z. Chun, M. Neena, Y. Chengzhong, Anton, P.J. and Middelber. 2014. Nanoparticle vaccines. Vaccine 32:327-337.

Miyazaki, T., S. Yasumoto, Y. Kuzuya and T. Yoshimura. 2008. A primary study on oral vaccination with liposomes entrapping Koi Herpesvirus (KHV) antigens against KHV infection in carp. In: R.M.G. Bondad, C.V. Mohan, M. Crumlish, R.P. Subasinghe, (Editors), Diseases in Asian Aquaculture. Asian Fisheries Society, Manila, pp. 99-184.

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Mohamed, S., S. Mona, E.M. Magdy and E.M. Mansour. 2016. Recent progress in applications of nanoparticles in fish medicine: A review. Nanomedicine, NBM2016, 12:701-710.

Nirmal, M., S. Mariusz and T. Istvan. 2014. Oral delivery of nanoparticle-based vaccines. Expert Review of Vaccines doi: 10.1586/14760584.2014.936852.

Rajesh, K.S., A. Ishaq, V. Parameswaran, R. Sudhakaran, B.V. Sarath and A.S.S Hameed. 2008. Potential use of chitosan nanoparticles for oral delivery of DNA vaccine in Asian sea bass (*Lates calcarifer*) to protect from *Vibrio (Listonella) anguillarum*. Fish and Shellfish Immunology 25:47-56.

Rajesh, K.S., C. Venkatesan, M. Sarathi, V. Sarathbabu, J. Thomas, K.A. Basha and A.S.S. Hameed. 2009. Oral delivery of DNA construct using chitosan nanoparticles to protect the shrimp from white spot syndrome virus (WSSV). Fish and Shellfish Immunology 26:429-437.

Rather, M.A., R. Sharma, M. Aklakur, S. Ahmad, N. Kumar. M. Khan and V.L. Ramya. 2011. Nanotechnology: A Novel tool for Aquaculture and Fisheries Development, A prospective Mini-Review. Fisheries and Aquaculture Journal FAJ-16.

Tian, J. and Y. Juan. 2011. Poly (lactic-co-glycolic acid) nanoparticles as candidate DNA vaccine carrier for oral immunization of Japanese flounder (*Paralichthys olivaceus*) against lymphocystis disease virus. Fish and Shellfish Immunology 30:109-117.

Tian, J., X. Sun, X. Chen, J. Yu, L. Qu, L. Wang. 2008. The formulation and immunisation of oral poly (DL-lactide-co-glycolide) microcapsules containing a plasmid vaccine against lymphocystis disease virus in Japanese flounder *(Paralichthys olivaceus*). International Journal of Immunopharmacology 8:900-908.

Tinsley, B.A.M., R. Fretwell, A.B. Dowsett, S.L. Davis and G.H. Farrar. 2000. Formulation of poly (D, L-lactic-co-glycolic acid) microparticles for rapid plasmid DNA delivery. Journal of Control Release 66(2-3):229-241.

Treuel, L., X. Jiang and G.U. Nienhaus. 2013. New views on cellular uptake and trafficking of manufactured nanoparticles. Journal of Royal Society Interface DOI: 10.1098/rsif.2012.0939

Vimal, S., M.S. Abdul, G. Taju, K.S. Nambi, R.N. Sundar, N. Madan, M.A. Farook, T. Rajkumar, D. Gopinath and A.S.S. Hameed. 2013. Chitosan tripolyphosphate (CS/TPP) nanoparticles: preparation, characterization and application for gene delivery in shrimp. Acta Tropica 128:486-493.

Vimal, S., G. Taju, K.S.N. Nambi, M.S. Abdul, B.V. Sarath, M. Ravi and A.S.S. Hameed. 2012. Synthesis and characterization of CS/TPP nanoparticles for oral delivery of gene in fish. Aquaculture 358-359:14-22.

Vinay, T.N., Y.J. Kim, M.H. Jung, W.S. Kim, D.H. Kim and S.J. Jung. 2013. Inactivated vaccine against viral hemorrhagic septecemia (VHS) emulsified with squalene and aluminum hydroxide adjuvant provides long term protection in olive flounder (*Paralichthys olivaceus)*. Vaccine 31:4603-4610.

Wang, D., D.R. Robinson, G.S. Kwon and J. Samuel. 1999. Encapsulation of plasmid DNA in biodegradable poly (D, L-lactic-co-glycolic acid) microspheres as a novel approach for immunogene delivery. Journal of Control Release 57:9-18.

Yasumoto, S., Y. Kuzuya, M. Yasuda, T. Yoshimura and T. Miyazaki. 2006a. Oral immunization of common carp with a liposome vaccine fusing Koi Herpesvirus antigen. Fish Pathology 41:141-145.

Yasumoto, S., T. Yoshimura and T. Miyazaki. 2006b. Oral immunization of common carp with a liposome vaccine containing *Aeromonas hydrophila* antigen. Fish Pathology 41:45-49.

Zheng, F., H. Liu, X. Sun, Y. Zhang, B. Zhang, Z. Teng, Y. Hou and B. Wang. 2016. Development of oral DNA vaccine based on chitosan nanoparticles for the immunization against reddish body iridovirus in turbots (*Scophthalmus maximus*). Aquaculture 452:263-271.