Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.13.01.Art059

Biodegradable Nanoformulations for Enhanced Recombinant Protein Drug Delivery: A Promising Approach for Controlling *Vibrio harveyi* in Aquatic Models

Vidya Saranya S[1], Vimal S[2]*, Arockia Alex[2], Debalina Sen[2], Deepak Sharma[3]
[1]*Department of Anaesthesiology, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India*
[2]*Department of Biochemistry, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India*
[3]*Center for Global Health Research, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India*

Abstract

Biodegradable nanoformulations have developed as an innovative method to improve the delivery and effectiveness of recombinant protein therapeutics in aquaculture. This research examines their efficacy in managing "Vibrio harveyi," a widespread disease responsible for considerable economic detriment in the aquaculture sector. Nanoformulations, made from eco-friendly and biocompatible substances, provide regulated and targeted medication administration, hence minimizing off-target effects and decreasing environmental pollution. These technologies improve the stability and bioavailability of recombinant proteins, addressing issues such as fast breakdown in aquatic conditions. The use of modern nanotechnology in aquaculture medicine provides a sustainable and efficient approach to address bacterial infections, enhance fish health, and guarantee ecological safety. This study shows the current progress, problems, and possible future paths of biodegradable nanoformulations for recombinant protein drug delivery, highlighting how they could completely change sustainable aquaculture operations.

***Keywords**: Biodegradable Nanoformulations, Recombinant Protein Therapeutics, Sustainable Aquaculture, Targeted Drug Delivery Vibrio Harveyi.*

Introduction

Aquaculture, or aquafarming, refers to the cultivation of aquatic creatures, including fish, crustaceans, molluscs, and aquatic plants. Aquaculture has become one of the world's fastest-growing food production industries in recent decades [1]. In 2020, the Food and Agriculture Organisation (FAO) projected that global aquaculture production amounted to around 122.6 million metric tonnes, valued at an estimated $281.5 billion. This industry currently accounts for over 50% of the global fish supply for human consumption, a statistic that has consistently increased as wild fish supplies have become depleted due to overfishing, habitat destruction, and environmental changes. Asia leads global aquaculture production, accounting for over 89% of total output, with China contributing over 60% of the worldwide aquaculture yield. Additional significant contributors comprise Southeast Asian countries, including Vietnam, Thailand, and Indonesia [2]. Norway holds a prominent position in Europe's aquaculture sector, chiefly due to its extensive salmon farming industry [3]. The FAO projects that by

Received: 26.01.2025 Accepted: 05.02.2025 Published on: 28.03.2025
Corresponding Author: vimals.smc@saveetha.com

2030, aquaculture must provide an additional 32 million metric tonnes of fish to satisfy increasing global demand, highlighting the necessity of sustainable practices for long-term output expansion. The swift growth of aquaculture has introduced various issues, especially with disease management. As the business increases output to meet global demand, fish are frequently cultivated in high-density systems, increasing the risk of disease outbreaks. These circumstances may facilitate the swift dissemination of infectious agents, encompassing bacteria, viruses, and parasites. Effective disease management is widely acknowledged as a critical challenge in aquaculture, necessitating novel methods to guarantee sustainable output [4].

Impact of Diseases in Aquaculture

Diseases provide a considerable risk to the sustainability and profitability of global aquaculture. The FAO estimates that fish illnesses account for approximately 40% of losses. In annual global aquaculture production. Such losses can be especially catastrophic in areas that depend significantly on aquaculture for food security and economic stability. Disease outbreaks in Southeast Asia, an area where aquaculture is essential for both subsistence and food production, have resulted in billions of dollars in losses over the previous decade. *Vibrio harveyi* is one of the most virulent and detrimental bacterial diseases impacting aquaculture. *V. harveyi* is a Gram-negative, bioluminescent bacterium belonging to the Vibrionaceae family. It is chiefly linked to vibriosis, a disease impacting several marine species, such as fish, prawns, and molluscs. The bacteria flourishes in both temperate and tropical marine ecosystems, rendering it a persistent issue for global aquaculture systems [5].

Vibrio harveyi and Vibriosis in Aquaculture

Vibrio harveyi induces vibriosis in fish, impacting the skin and gills, and may result in systemic infections with elevated mortality rates, particularly in juvenile fish with immature immune systems. This illness affects commercially significant species, such as Asian sea bass, groupers, and tilapia. In Southeast Asia, namely in Thailand and Vietnam, outbreaks lead to annual financial losses ranging from $100 to $300 million. In Norway's salmon farming sector, bacterial infections caused by Vibrio species result in significant expenses for disease control and management.

Ecological Impacts of Bacterial Diseases

Bacterial infections like vibriosis exert ecological effects that extend beyond the economic detriments in aquaculture. The overuse of antibiotics results in antibiotic-resistant microorganisms, including *Vibrio harveyi*, impacting both aquaculture and wild fish populations. The accumulation of antibiotics in adjacent marine ecosystems can disturb microbial communities, resulting in extensive ecological imbalances. Furthermore, the reduction in biodiversity in aquaculture due to monoculture practices creates favourable conditions for disease outbreaks, thereby intensifying the imbalance in the ecosystem.

The Need for Sustainable Disease Management Practices

The rapid expansion of aquaculture and the increase in bacterial diseases, such as vibriosis induced by *Vibrio harveyi*, necessitate sustainable disease management strategies. Traditional approaches, including antibiotics and vaccinations, have drawbacks such as resistance, environmental repercussions, and ineffectiveness in immature fish. Biodegradable nanoformulations provide an effective solution by facilitating targeted, controlled drug administration, minimising treatment frequency and ecological impact, and promoting more sustainable aquaculture methods [6].

Current Treatment Challenges in Aquaculture

Limitations of Antibiotics

The overapplication of antibiotics in aquaculture has been a major issue for decades. Historically, antibiotics have been the primary method for managing bacterial infections in aquaculture, including vibriosis caused by Vibrio harveyi. The widespread and often indiscriminate use of antibiotics has led to a variety of problems.

Overuse of Antibiotics and Development of Resistant Strains

The primary drawback of relying on pharmaceuticals for disease management is the rapid development of antibiotic-resistant bacterial strains. The extensive application of antibiotics, especially at subtherapeutic levels, promotes the persistence of resistant germs while eradicating susceptible strains. Over time, this selective pressure enables resistant bacteria to dominate, complicating the treatment of future illnesses. The rise in antibiotic resistance is a substantial obstacle to sustainable aquaculture production, as outbreaks of *Vibrio harveyi* and other pathogenic bacteria become increasingly difficult to control. The presence of multidrug-resistant (MDR) strains jeopardizes both cultured species and human health due to the potential transfer of resistance genes to human illnesses [7].

Environmental Impact of Antibiotic Residues

The use of antibiotics in aquaculture not only promotes resistance but also adversely affects ecosystems. Unmetabolized antibiotics infiltrate adjacent waters, accumulating in sediments and affecting vital microbial activities such as nutrient cycling. Research conducted in Norway and Asia indicates that antibiotics like oxytetracyclines generate resistance hotspots in sediments, jeopardizing biodiversity and polluting wild fish populations. Moreover, antibiotic residues can bioaccumulate in non-target organisms, infiltrating the human food chain and presenting health hazards. These residues pose enduring risks to ecological stability and human health.

Vaccine Limitations

Vaccination is a prevalent method for preventing bacterial infections in aquaculture, providing a more sustainable alternative to drugs. Nonetheless, current vaccine methods include significant drawbacks that impede their efficacy, especially against organisms such as *Vibrio harveyi* [8, 9].

Slow Immune Response in Fish

In aquaculture, fish, particularly in colder climates, exhibit a delayed immune response post-vaccination, frequently requiring weeks or months to achieve complete immunity. This postponement renders fish susceptible to ailments such as vibriosis, especially during their juvenile phases. In intensive aquaculture systems, disease outbreaks can rapidly arise because of elevated stocking numbers and environmental stressors [10, 11].

Difficulty in Administering Vaccines, Especially in Juvenile Fish

Fish vaccination poses more challenges than it does for terrestrial species because of the complexity of treating large populations, especially juvenile fish. Injectable vaccinations, though productive, are labour-intensive and may induce stress in fish, adversely affecting productivity. Immersion and oral vaccines serve as alternatives nonetheless, they possess limitations submergence offers only transient protection, while oral vaccines may degrade during digestion, leading to variable immunity.

Short Duration of Efficacy

In aquaculture, fish, particularly in colder climates, exhibit a delayed immune response post-vaccination, frequently requiring weeks or months to achieve complete immunity. This postponement renders fish susceptible to ailments such as vibriosis, especially during their juvenile phases. In intensive aquaculture systems, disease outbreaks can rapidly arise because of elevated stocking numbers and environmental stressors [10, 12, 13].

Nanoformulation Technology Overview

Nanoformulations provide an innovative method for medication delivery that has garnered considerable interest in medicine, biotechnology, and aquaculture. These technologies utilise nanoparticles—minuscule particles of 1 to 100 nanometres—to enhance the transport, stability, and bioavailability of medicinal drugs. Nanoformulations in aquaculture possess the potential to transform the administration of medications, vaccines, and other bioactive substances to improve fish health and address diseases, such as *Vibrio harveyi* [6, 14].

Advantages of Nanoformulations in Drug Delivery

Nanoformulations improve drug bioavailability, safeguarding them against degradation and facilitating controlled, targeted release at infection sites. This diminishes the necessary dosage, mitigates negative effects, and enhances therapeutic success, especially for ailments such as vibriosis in fish. Nanoformulations facilitate prolonged medication release, diminishing the necessity for repeated treatments; hence, they alleviate fish stress and mitigate environmental effects [15–17].

Recombinant Protein Therapies

Recombinant protein technology has revolutionized biomedical research and medicinal development, and it is currently being investigated in aquaculture as an innovative method of disease management. Recombinant proteins are synthesised via the genetic engineering of microorganisms, including bacteria, yeast, or mammalian cells. TPLGAhese proteins can be engineered to replicate natural proteins or to behave as effective bioactive agents with targeted roles, such as augmenting immune responses or directly suppressing harmful bacteria [18–20].

Potential of Recombinant Proteins in Aquaculture

Recombinant proteins can enhance fish immune responses or function as antimicrobial agents in aquaculture, addressing illnesses like *Vibrio harveyi*. Recombinant interferons and interleukins enhance the innate immune system of fish, augmenting their resistance to bacterial and viral diseases. Moreover, recombinant antimicrobial peptides (AMPs) can directly eliminate germs by altering cell membranes or critical bacterial processes, providing a precise and efficient alternative to conventional antibiotics [21–25].

Relevance to Aquaculture Health Management

Aquaculture has become an essential option to satisfy the escalating global demand for seafood; yet, the industry faces increasing threats from disease outbreaks, especially bacterial infections such as those induced by *Vibrio harveyi*. Conventional treatment approaches, particularly the prevalent application of antibiotics, are diminishing in efficacy due to increasing antibiotic resistance, necessitating the development of innovative therapies. Nanoformulated drug delivery technologies present a viable alternative that can improve therapeutic efficacy while reducing the environmental and public health hazards linked to traditional approaches [26, 27].

Economic Importance

Aquaculture is essential for numerous economies, offering employment, food security, and export revenue. China dominates worldwide aquaculture, with over 60 million metric tons per year, whereas Norway specializes in Atlantic salmon cultivation, and Indonesia ranks as a leading exporter of prawns. Nevertheless, outbreaks of bacterial infections such as *Vibrio harveyi* can significantly affect these industries [28]. Vibriosis in Southeast Asian prawn farms has resulted in billions of dollars in economic losses, with mortality rates reaching 70%, adversely impacting local people dependent on aquaculture [29]. Similarly, bacterial infections in Norwegian salmon farms lead to millions of fish losses, adversely affecting exports and the seafood industry. Efficient disease management is essential for aquaculture species' well-being and the impacted areas' economic viability. Novel therapies, including nanoformulated drug delivery devices, may substantially mitigate disease outbreaks, enhancing both financial stability and public health [30].

Advantages of Nanoformulated Drug Delivery Systems

Nanoparticle-based drug delivery systems signify a revolutionary method for addressing the constraints of conventional antibiotics and vaccines in aquaculture. These methods provide numerous benefits, such as precise and prolonged medication administration, enhanced bioavailability, and diminished environmental effects, which are pertinent for disease management in aquatic ecosystems [31].

Targeted and Sustained Delivery

Nanoformulations improve targeted and prolonged medication delivery in human health and aquaculture. Aquaculture enables the precise targeting of infection locations, such as the gills or digestive tract, facilitating controlled drug release, diminishing the necessity for multiple dosages and alleviating fish discomfort. This is especially advantageous for extensive operations; it reduces labour and expenses while enhancing fish health [32].

Biodegradable polymers, including poly(lactic-co-glycolic acid) (PLGA), chitosan, and alginate, are frequently used in these formulations. PLGA, a copolymer of lactic and glycollic acid, can be tailored for specific medication release characteristics. Chitosan, sourced from crustacean exoskeletons, is biodegradable and mucoadhesive, decomposing into non-toxic glucosamine in aquatic organisms. Alginate, derived from brown seaweed, creates hydrogels that deliver medications in reaction to environmental alterations. These polymers facilitate efficient drug delivery while reducing environmental impact via non-toxic breakdown [33].

Fish-Specific Considerations

Providing medication to fish presents unique challenges due to their physiological differences from mammals. Fish can assimilate pharmaceuticals via their gills, skin, or intestines, contingent upon the composition. Nanoformulations are advantageous for aquatic species as they enhance drug absorption and bioavailability via many mechanisms. In contrast to mammals, medicine delivery systems for fish must endure aquatic settings and release their contents progressively. Furthermore, fish have slower immunological responses, and nanoformulated therapies can augment immune protection by administering therapeutic chemicals or antigens in a regulated fashion, hence enhancing the effectiveness of vaccines and treatments.

Novelty of Research

Application of Biodegradable Nanoparticles in Aquaculture

Nanotechnology is swiftly progressing in aquaculture, providing novel options for disease management and pharmaceutical delivery. Despite extensive research in human health, aquaculture is still developing its utilization of nanotechnology, particularly through the use of biodegradable nanoparticles for therapeutic administration. Polymers such as chitosan, alginate, and PLGA have demonstrated potential in the delivery of antibacterial agents, vaccinations, and immunological modulators to fish. Chitosan nanoparticles improved the immune system of rainbow trout and PLGA nanoparticles improved the delivery of antibiotics to carp. These biodegradable nanoparticles disintegrate into non-toxic byproducts, mitigating environmental effects and averting antibiotic resistance. This study aims to fill in the gaps in knowledge about how nanotechnology can be used to deliver recombinant proteins to fish by making biodegradable nanoformulations that work well for giving fish recombinant protein medicines (Fig.1).

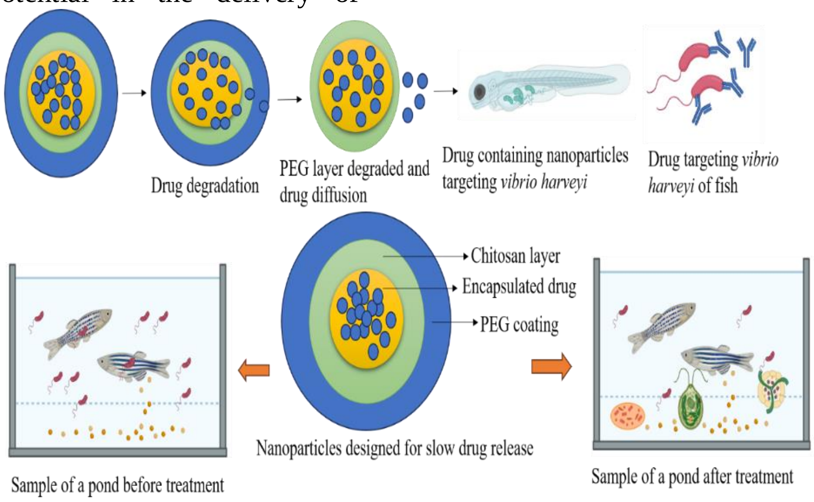

Figure 1. PLGA Microspheres Vaccine Delivery

Innovation in Combining Recombinant Protein Delivery

The combination of recombinant protein treatments with nanoformulation technology offers a viable approach to disease management in aquaculture. Recombinant proteins, although efficacious in human therapy, encounter difficulties in fish due to instability, degradation, and inadequate delivery through traditional means such as injection and oral administration. Nanoformulations safeguard these proteins from degradation, improve bioavailability, facilitate regulated release, and provide targeted delivery to infection locations such as gills or intestines (Fig.2). This novel method can enhance illness management in fish through the utilization of recombinant proteins such as antimicrobial peptides and cytokines. Nonetheless, obstacles such as protein stability, delivery efficacy, and immunological regulation persist. This research seeks to create biodegradable nanoformulations to tackle these challenges, providing a sustainable alternative to conventional therapies for illnesses such as *Vibrio harveyi*.

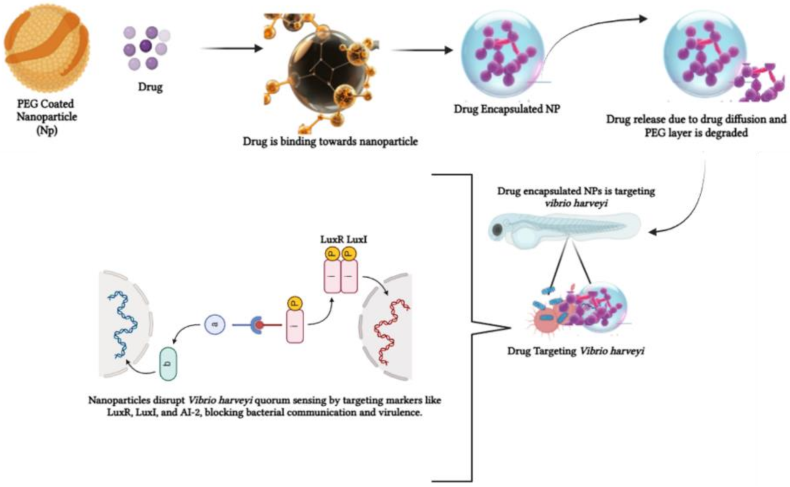

Figure 2. PEG-Coated Nanoparticles (NPs) Aimed Against *Vibrio harveyi*

Potential Impact

Implications for Aquaculture Disease Management

The application of biodegradable nanoformulations for recombinant protein delivery in aquaculture has the potential to transform disease management by providing more efficient, targeted, and sustained therapies. In contrast to traditional antibiotics that necessitate frequent administration and promote antibiotic resistance, nanoformulations provide regulated, sustained release of therapeutic agents, preserving effective medication concentrations for extended durations. This diminishes the necessity for recurrent treatments, thereby alleviating stress, expenses, and pharmaceutical usage. Nanoencapsulation enhances bioavailability and safeguards the recombinant protein, enabling reduced dosages without sacrificing efficacy, which may decrease treatment burdens while promoting fish health and production.

Reduction in Antibiotic Use and Environmental Impact

When used in aquaculture, combining antibiotics with recombinant protein-based nanoformulations can greatly reduce the need for antibiotics and the environmental risks that come with them. Overuse of antibiotics results in residue accumulation in aquatic habitats, fostering antibiotic-resistant bacteria, and jeopardizing both aquaculture sustainability and human health. Conversely, recombinant proteins are biodegradable, mitigating infections or augmenting fish immune responses without residual toxicity. Nanoencapsulation guarantees precise, sustained release, thereby reducing environmental effects. This transition promotes biodiversity, safeguards microbial ecosystems, and aids in the global combat against antimicrobial resistance (AMR).

Economic and Environmental Sustainability

Nanoformulated recombinant protein therapy is used in aquaculture. It has big economic benefits because it lowers the death rate of fish caused by diseases, especially infections like *Vibrio harveyi*, which can kill up to 70% of fish and cost billions of dollars. These nanoformulations facilitate more efficient, focused therapies, diminishing the necessity for frequent medication administration and personnel expenses by ensuring sustained drug release. Furthermore, they alleviate the financial strain of antibiotic resistance and correspond with the increasing demand for sustainable, environmentally friendly aquaculture operations, thereby improving market competitiveness and facilitating access to premium markets via certifications and eco-labels.

Potential for Broader Application

Nanoformulated recombinant protein therapies show promise for more than just fighting *Vibrio harveyi* in fish. They could also help treat many other bacterial diseases in aquaculture species. In shrimp aquaculture, these therapies may aid in the fight against the White Spot Syndrome Virus (WSSV), while in mollusc aquaculture, they have the potential to alleviate diseases like Perkinsus marinus. This system can be customized for many species, including tilapia, catfish, salmon, and trout, improving disease management. With the global expansion of aquaculture, these sustainable, environmentally friendly therapies may significantly enhance fish health, mitigate environmental effects, and foster sustainable aquaculture practices.

Suggested Improvements and Future Directions

Optimization of Nanoparticle Composition

Biodegradable polymers like PLGA, chitosan, and alginate can help medicines work better by making nanoparticles for recombinant protein treatment in aquaculture better and more advanced. Hybrid materials that integrate synthetic and natural polymers may enhance drug release regulation, and biocompatibility and diminish toxicity. Stimulus-responsive polymers, such as pH- or temperature-sensitive compounds, can be designed to release slowly in the digestive system or when an organism's temperature changes, like when salmon migrate. Surface functionalization with targeting ligands, such as mannose or lectins, may augment tissue-specific delivery, thereby enhancing bioavailability and therapeutic efficacy, especially against infections like *Vibrio harveyi*. Additional nanoparticle research is needed for aquaculture efficacy.

Multifunctional Nanoparticles

The development of multifunctional nanoparticles for the co-delivery of medicinal agents, such as recombinant proteins, antibiotics, or vaccinations, presents significant potential for enhancing disease therapy in both human and veterinary medicine. In aquaculture, these nanoparticles may simultaneously target infections and augment the immune system, thereby improving therapeutic efficacy. Liposomal nanoparticles can provide antibiotics to diminish bacterial load and recombinant proteins to confer long-term immune protection. This method might also help with vaccinations, maybe making fish healthier against bacterial, viral, and parasitic diseases, similar to how nanoparticle-based vaccines have helped people (Fig. 3).

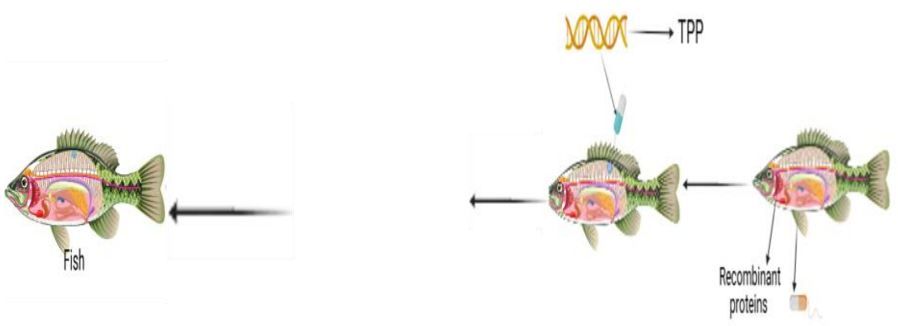

Figure 3. Process for Delivering Recombinant Proteins to Fish Using PLGA-Based Nanoparticles and TPP

Customizing Nanoformulations for Fish Species

Different fish have different physiological, morphological, and immunological traits, making nanoformulations for aquaculture need to be species-specific. Nanoparticles work differently for different species because of differences in how they absorb, break down, and get rid of drugs. Other factors that affect their effectiveness are digestive resistance and environmental conditions (like saltiness and temperature). Pharmacokinetic and biodistribution analyses enhance pharmacological efficacy and safety optimization. Moreover, variations in immune systems, including more robust innate responses in certain species, need the customization of nanoparticles to improve therapeutic efficacy. By comprehending these species-specific characteristics, researchers can develop more efficient, focused therapies for various aquaculture systems.

Safety and Regulatory Considerations

As nanotechnology progresses in aquaculture, evaluating the safety of nanoparticle-based therapies for fish and the aquatic ecosystem is essential. Although biodegradable polymers such as PLGA and chitosan are typically considered benign, further long-term studies are necessary to elucidate their impacts, especially concerning nanotoxicity and bioaccumulation. Toxicity assessments must account for effects on fish health, including organ damage and immunological suppression, as well as consequences on aquatic microbes and non-target species. Collaboration with regulatory agencies such as the FDA and EMA is crucial for setting safety standards and alleviating apprehensions over bioaccumulation and environmental effects. This will enable the ethical commercialization and use of nanoparticles in aquaculture, guaranteeing appropriate dosing and application. Improving biodegradable nanoformulations for recombinant protein delivery can help fish stay healthy and live longer. However, more research needs to be done on safety, regulations, and formulations that are specific to each species.

Conclusion

Biodegradable nanoformulated drug delivery devices integrated with recombinant protein therapies provide a revolutionary approach to disease control in aquaculture. These advancements provide precise, long-lasting treatments that reduce the need for frequent administration and minimize environmental impact. By augmenting treatment efficacy and diminishing dependence on antibiotics, they tackle the escalating problem of antibiotic resistance, enhancing both fish health and industrial sustainability. Through additional research and regulatory cooperation, these new technologies could transform aquaculture, guaranteeing its sustainability and profitability in the long term.

Future Outlook

With the advancement of nanoformulation technology, its wider applicability in aquaculture and other fields has become increasingly promising. Enhancing nanoparticle composition, optimizing targeted distribution, and guaranteeing long-term safety are essentials for the technology's future. Multifunctional nanoparticles capable of co-delivering pharmaceuticals, vaccines, and immune modulators have the potential to transform disease control in aquaculture, terrestrial agriculture, and human healthcare.

Biodegradable nanoformulations provide a sustainable solution for aquaculture, diminishing dependence on antibiotics and improving disease management. This breakthrough facilitates eco-friendly methods and applies to other agricultural sectors, advancing the sustainable future of global aquaculture.

Conflict of Interest

There was no conflict of interest to declare.

Acknowledgement

We sincerely thank the Department of Biochemistry, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India for their contribution to this manuscript.

References

[1]. Khan, M. A., Shadan Khan, K., 2011, *Aquaculture as a food production system: A review. Early Pregnancy*. ;3: 291–302.

[2]. Ahmed, N., Azra, M. N., 2022,10.1007/s40572-022-00364-6

[3]. Afewerki, S., Osmundsen, T., Olsen, M. S., Størkersen, K., Misund, A., Thorvaldsen, T., 2023, Innovation policy in the Norwegian aquaculture industry: Reshaping aquaculture production innovation networks. *Mar Policy*. ;152: 105624. doi:10.1016/j.marpol.2023.105624

[4]. Assefa, A., Abunna, F., 2018, Maintenance of fish health in aquaculture: Review of epidemiological approaches for prevention and control of infectious disease of fish. *Vet Med Int.* ;2018: 5432497. doi:10.1155/2018/5432497

[5]. Ina-Salwany, M. Y., Al-Saari, N., Mohamad, A., Mursidi. F. A., Mohd-Aris, A., Amal, M. N. A., et al. ., 2019, Vibriosis in fish: A review on disease development and prevention. *J Aquat Anim Health.;*31: 3–22. doi:10.1002/aah.10045

[6]. Kah Sem, N. A. D., Abd Gani, S., Chong, C. M., Natrah, I., Shamsi, S., 2023, Management and mitigation of vibriosis in aquaculture: Nanoparticles as promising alternatives. *Int J Mol Sci.* ;24. doi:10.3390/ijms241612542

[7]. Habboush, Y., Guzman N., 2024. Antibiotic resistance. StatPearls. Treasure Island (FL): *StatPearls Publishing;* Available: https://www.ncbi.nlm.nih.gov/books/NBK513277/

[8]. Mondal, H., Thomas, J., 2022, A review on the recent advances and application of vaccines against fish pathogens in aquaculture. *Aquac Int. ;*30: 1971–2000. doi:10.1007/s10499-022-00884-w

[9]. Kumar, A., Middha, S. K., Menon, S. V., Paital, B., Gokarn, S., Nelli, M., et al.., 2024, Current Challenges of Vaccination in Fish Health Management. *Animals.;*14: 2692. doi:10.3390/ani14182692

[10]. Bøgwald, J., Dalmo, R. A., 2019, Review on immersion vaccines for fish: An update Microorganisms. ;7: 627. doi:10.3390/microorganisms7120627

[11]. Ma, J., Bruce, T. J., Jones, E. M., Cain, K. D., 2019, A review of fish vaccine development strategies: Conventional methods and modern biotechnological approaches. Microorganisms. ;7: 569. doi:10.3390/microorganisms7110569

[12]. Abram QH, Dixon B, Katzenback BA.., 2017 Impacts of low temperature on the teleost immune system. Biology (Basel). ;6. doi:10.3390/biology6040039

[13]. Mugwanya, M., Dawood, M. A. O., Kimera, F., Sewilam, H., 2022, Anthropogenic temperature fluctuations and their effect on aquaculture: A comprehensive review. Aquac Fish. ;7: 223–243. doi:10.1016/j.aaf.2021.12.005

[14]. Petrovic, S., Bita, B., Barbinta-Patrascu, M. E., 2024, Nanoformulations in pharmaceutical and biomedical applications: Green perspectives. *Int J Mol Sci.;*25: 5842. doi:10.3390/ijms25115842

[15]. Yeh, Y. C., Huang, T. H., Yang, S. C., Chen, C. C., Fang, J. Y., 2020, Nano-based drug delivery or targeting to eradicate bacteria for infection mitigation: A review of recent advances. *Front Chem.* 8: 286. doi:10.3389/fchem.2020.00286

[16]. Youssef, F. S., El-Banna, H. A., Elzorba, H. Y., Galal, A. M., 2019, Application of some nanoparticles in the field of veterinary medicine. *Int J Vet Sci Med.* 7: 78–93. doi:10.1080/23144599.2019.1691379

[17]. Cheng, X., Xie, Q., Sun, Y., 2023, Advances in nanomaterial-based targeted drug delivery systems. *Front Bioeng Biotechnol.* ;11: 1177151. doi:10.3389/fbioe.2023.1177151

[18]. Gupta, V., Sengupta, M., Prakash, J., Tripathy, B. C., 2017, Production of Recombinant Pharmaceutical Proteins. Basic and Applied Aspects of Biotechnology. Singapore: S*pringer Singapore;* . pp. 77–101. doi:10.1007/978-981-10-0875-7_4

[19]. Tripathi, N. K., Shrivastava, A., 2019, Recent developments in bioprocessing of recombinant proteins: Expression hosts and process development. *Front Bioeng Biotechnol.*;7: 420. doi:10.3389/fbioe.2019.00420

[20]. Gupta, S. K., Shukla, P., 2017, Sophisticated cloning, fermentation, and purification technologies for an enhanced therapeutic protein production: A review. *Front Pharmacol.* 8: 419. doi:10.3389/fphar.2017.00419

[21]. Lee, P. T., Yamamoto, F. Y., Low, C. F., Loh, J. Y., Chong, C. M., 2021, Gut immune system and the implications of oral-administered immunoprophylaxis in finfish aquaculture. *Front Immunol.* ;12: 773193. doi:10.3389/fimmu.2021.773193

[22]. Mokhtar, D., Zaccone, G., Alesci, A., Kuciel, M., Hussein, M., Sayed, R., 2023, Main components of fish immunity: An overview of the fish immune system. G. ;8: 93. doi:10.3390/fishes8020093

[23]. Nguyen, H. T., Nguyen, T. T. T., Chen, Y. C., Vu-Khac, H., Wang, P. C., Chen, S. C., 2018, Enhanced immune responses and effectiveness of refined outer membrane protein vaccines against Vibrio harveyi in orange-spotted grouper (Epinephelus coioides). *J Fish Dis.* ;41: 1349–1358. doi:10.1111/jfd.12828

[24]. Semple, S. L., Dixon, B., 2020, Salmonid antibacterial immunity: An aquaculture perspective. *Biology (Basel).* ;9: 331. doi:10.3390/biology9100331

[25]. Mba, I. E., Nweze, E. I., 2022, Antimicrobial peptides therapy: An emerging alternative for treating drug-resistant bacteria. *Yale J Biol Med.*;95: 445–463. Available: https://www.ncbi.nlm.nih.gov/pubmed/36568838

[26]. Imran, M., Jha, S. K., Hasan, N., Insaf, A., Shrestha, J., Shrestha, J., et al., 2022, Overcoming multidrug resistance of antibiotics via nanodelivery systems. *Pharmaceutics;*14: 586. doi:10.3390/pharmaceutics14030586

[27]. Makabenta, J. M. V., Nabawy, A., Li, C. H., Schmidt-Malan, S., Patel, R., Rotello, V. M., 2021, Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. *Nat Rev Microbiol.* ;19: 23–36. doi:10.1038/s41579-020-0420-1

[28]. Araujo, G. S., Silva, J. W. A., da, Cotas, J., Pereira, L., 2022, Fish farming techniques: Current situation and trends. *J Mar Sci Eng. ;*10: 1598. doi:10.3390/jmse10111598

[29]. Mohd Yazid, S. H., Mohd Daud, H., Azmai, M. N. A., Mohamad, N., Mohd Nor, N., 2021, Estimating the economic loss due to vibriosis in net-cage cultured Asian seabass (Lates calcarifer): Evidence from the east coast of Peninsular Malaysia. *Front Vet Sci.* ;8: 644009. doi:10.3389/fvets.2021.644009

[30]. Hegde, A., Kabra, S., Basawa, R. M., Khile, D. A., Abbu, R. U. F., Thomas, N. A., et al, 2023, Bacterial diseases in marine fish species: current trends and future prospects in disease management. *World J Microbiol Biotechnol.* ;39: 317. doi:10.1007/s11274-023-03755-5

[31]. Gao, W., Chen, Y., Zhang, Y., Zhang, Q., Zhang, L., 2018, Nanoparticle-based local antimicrobial drug delivery. *Adv Drug Deliv Rev.* ;127: 46–57. doi:10.1016/j.addr.2017.09.015

[32]. Fajardo, C., Martinez-Rodriguez, G., Blasco, J., Mancera, J. M., Thomas, B., De Donato, M., 2022, Nanotechnology in aquaculture: Applications, perspectives and regulatory challenges. Aquac Fish. ;7: 185–200. doi:10.1016/j.aaf.2021.12.006

[33]. Yang, F., Cabe, M., Nowak, H. A., Langert, K. A., 2022, Chitosan/poly(lactic-co-glycolic) acid nanoparticle formulations with finely-tuned size distributions for enhanced mucoadhesion. Pharmaceutics. ;14: 95. doi:10.3390/pharmaceutics14010095