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## Nanotechnology: A novel technique for aquaculture and fisheries development

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### Abstract

Nanotechnology is a extremely hopeful technology that spans many areas of science and technological applications. Fast advancements in nanotechnologies and nanosciences in recent years have started novel horizons for many consumer sectors and industrial that have been regarded as the hotbed of a new industrial revolution including agriculture, fisheries and allied sectors. Nanotechnology has a remarkable possible to transform agriculture and allied fields including fisheries and aquaculture. It can offer new techniques for aquaculture, fish biotechnology, fish genetics, aquatic health and fish reproduction etc. Nanotechnology tools like DNA nanovaccines, nanomaterials, nanosensors, smart drug delivery and Gene delivery etc. have the promising to solve many puzzles related to animal health, production, reproduction, prevention and treatment of diseases. It is sensible to assume that in the forthcoming years, nanotechnology research will reform the science and technology and will help boost livestock production. Nanotechnology applications in the fish processing industry can be used to detect bacteria in produce stronger flavors, colour quality, packaging, and safety by increasing the barrier assets. The review of nanotechnology and its applications in aquaculture and fisheries is discussed in this paper.

**Keywords:** Nanotechnology, gene delivery, dynamic light scattering (DLS), tagging and nano-barcoding

### Introduction

Nanotechnology (from the Latin *nanus*, meaning *dwarf*) is defined as the technique of using particles or structures whose size is measured in nanometers, and that bears an immense application potential in diverse areas such as physics, chemistry, and biology (Buzea *et al.*, 2007) [1]. The U.S. NNI has defined nanotechnology as “understanding and control of matter at dimensions of roughly 1 to 100nm where unique phenomena enable novel applications”. There are many variables to deliberate when working with nanomaterial and these include the type of material, their shape, surface, charge, coating, size, dispersion, agglomeration, aggregation, concentration, and matrix. By cautiously considering the experimental conditions, *in vitro* toxicity, bio-distribution, *blood-brain barrier* (BBB) deliveries, machine of cellular uptake and other related studies of nanoparticles can minimalize the usage of *in vivo* system to assess these. However, animal systems are really complicated with unique bio-distribution, clearance, immune response, and metabolism. Hence, the *in-vitro* study can only complement animal studies in considering Nano Delivery System (NDS). Though, adequate and expressive screening using cell culture technique will ensure a reduction in the number of animal usage significantly. Properly planned *in-vitro* technique is expected to serve as a good screening mechanism for NDS. This will likely reduce the cost, time and more importantly conserve animals. Nanotechnology exhibits a tremendous potential in agriculture and its allied fields like aquaculture and fisheries. Nanotechnology employs nanomaterials, nanosensors, DNA nanovaccines, Fluorescent biological labels in drug delivery, gene delivery, probing of DNA structure, bio-detection of pathogens, separation and purification of biological molecules and cells, antimicrobial activity, cell toxicity, antioxidant activity etc. Nano-structures have a miraculous ability to solve many complications related to animal health, reproduction, production, as well as in prevention and treatment of diseases, thus expanding their applications in aquaculture, fish biotechnology, fish genetics, fish reproduction and fish health management. Application of Nanotechnology in the fish processing industry helps in rapid detection of fish bacteria in packaging, for producing stronger flavors, enhancing color quality.

### Tissue cell culture

Animal cell culture is an essential branch of life science which deals with removal of cells, tissues or organs from animals and propagating them in an artificial environment.

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Primary cultures are derived directly by the excision of normal animal tissue and culturing them either as an explant culture or following dissociation into a single cell suspension by enzyme digestion. Initially, the cells of primary culture are heterogeneous but later become dominated by fibroblast. Primary tissue cultures involve five stages namely, preparation of donor fish, isolation and decontamination of tissue, dissection and or disaggregation, seeding into culture vessel and subculture.

### Importance of cell culture in nanotechnology

Cell culture is being used to carry out various nanotechnology researches in *in-vitro* condition. To study the uptake and delivery of nanoparticles across the blood-brain barrier, cell culture models serve as essential tools in investigating drug delivery to the brain without unnecessarily torturing animals, especially during the initial screening phase. Contrasting the animal models, cell culture technology has the benefit of rapid evaluation in nanoparticle uptake mechanism, toxicity potentials and other related molecular mechanism needed in achieving drug delivery. For imaging studies of a live cell, *in vitro* techniques are employed. This method observes living cells; images are developed from microscopes and other great content screening systems. The system gives a well view of cellular dynamics as it relates to any form of treatment and the researcher sees changes, as they are unfolding. For

studying Nanotoxicity, the cell culture is a valuable tool, and many such studies are in progress. However, the absence of an immune outcome, the endocrine system, blood proteins, and the general lack of complex interaction of the entire animal in most *in-vitro* system are certain drawbacks for potential transfer of cell culture-based results of nanoparticle studies to animals/humans. Tissue-specific difference functions of many cells and their physiological situation of the primary cell cultures in *in vitro* systems are lost, like the loss of bio-transformational enzymes in primary liver cell cultures (Worth *et al.*, 2002) [2]. This loss will hinder the usage of primary liver cells in assessing nanoparticle for metabolism and toxicity potentials.

### Synthesis of nanoparticle

There are various methods for synthesizing nanoparticle such as physical, chemical, biological and hybrid method (Fig.1). Materials compacted to the nanoscale can suddenly show very different properties compared to what they exhibit on a macroscale, enabling unique applications. At nanoscale level, the properties of the particles also change. For example, opaque substances convert into transparent (copper); inert materials become catalysts (platinum). These change in properties help in using the particles for various purposes. A material such as gold, which is chemically inert at normal scales, can assist as a potent chemical catalyst at nanoscales.

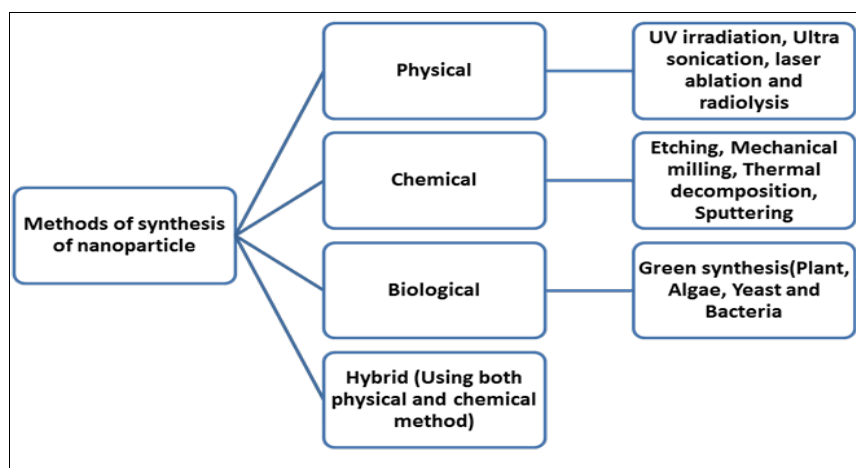


Fig 1: Different methods for synthesis of metal nanoparticles

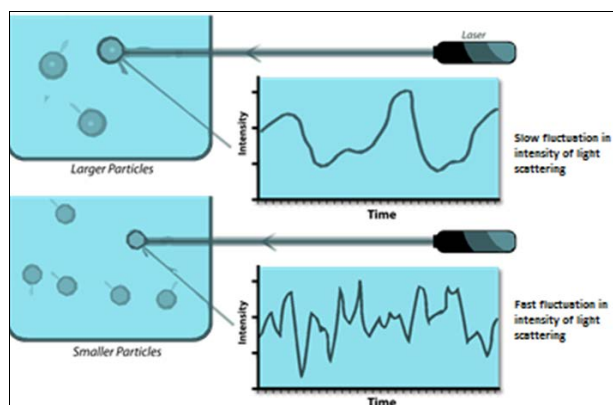
### Characterization of nanoparticle

After synthesis of a nanoparticle, various parameters need to be assessed which is called characterization. Various parameters that are measured for characterization are - Particle size & size distribution, Charge Determination, Surface Hydrophobicity, Chemical analysis of surface, Carrier drug interaction, Nanoparticle dispersion stability, Drug stability, etc. The techniques used for characterization of nanoparticles are - Dynamic light scattering (DLS) Electron microscopy (TEM, SEM), Atomic force microscopy (AFM), X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), Fourier transform infrared spectroscopy (FTIR), Ultraviolet-visible spectroscopy, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF), Nuclear magnetic resonance (NMR) and dual polarization interferometry.

### Dynamic light scattering

Size of nanoparticle is very important as it influences

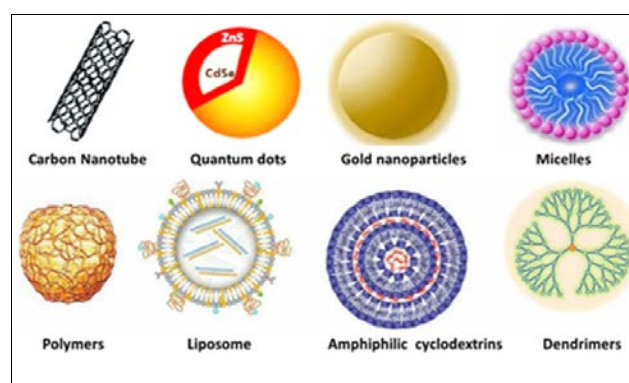
particle's material properties, such as reactivity, colloidal dispersion stability, dissolution velocity, and drug carrier system delivery efficiency. Dynamic light scattering (DLS) is a widely used method to calculate the diameter of different types of nanoparticles dispersed in a liquid medium. These particles can be organic and comprise polymers, proteins, carbohydrates, and surfactants or inorganic and comprise metals, such as silver, gold, and transition metal oxides. Hybrid particles with inorganic cores coated with organic molecules can also be characterized. In this technique, light is allowed to pass through a liquid suspension containing nanoparticles that have the ability to undergo random motion. When light passes through the suspension, it is scattered off the particles immersed in solvent. Since the random motion of these nanoparticles cause changes in the refractive index, and thus this random intensity variations produced by the particles can be evaluated (Fig 2).



**Fig 2:** Hypothetical dynamic light scattering (DLS) for large and small nanoparticles

### Types of nanomaterials

Nanomaterial can be classified as –Carbon based (fullerenes, nanotube), Metal based (quantum dots, nanogold, Nanosilver, metal oxides), Polymeric (dendrimers) and composites. (Fig 3). As nanomaterials are small in size; they have a much higher surface area to volume ratio than the conventional forms. They can be produced in one dimension (surface film), two dimensions (strand or fibers) or three dimensions (particles) and in different irregular and regular shape such as a sphere, rod tube wires, etc. Because of their unique electrical, catalytic, magnetic and thermal features, these materials have received much attention among researcher in any fields of biological science including Fisheries and Aquaculture.



**Fig 3:** Different types of Nanoparticles

### Application of nanotechnology in fisheries and aquaculture

Nanotechnology has tremendous potential to revolutionize the fisheries and aquaculture industry with new tools for rapid disease detection, improving the ability of fishes to absorb drugs rapidly like hormones and vaccines, as well as nutrients, etc. Although much more research and development potential is desirable for the use of nanotechnology in aquaculture, but, still there are numerous in fish health management, water treatment in aquaculture, animal breeding and Harvest and post-harvest technology. Some of the major upcoming applications of nanotechnology in aquaculture are briefly defined.

#### Fish health management

Nanotechnology holds the possibility for early detection and eradication of diseases. Immunoglobulin G- capped gold nanoparticles are used to image pathogenic organism like

*Staphylococcus aureus*, *S. pyogenes*, etc. IgG can specifically bind to the antigenic epitopes of the bacteria. Thus Ig G – capped gold nanoparticles are used to label the bacteria specifically. Using biodegradable nanoparticle carriers like chitosan and poly-lactide-co-glycolide acid (PLGA) with DNA vaccine, one may achieve high levels of protection to fishes and shellfishes not only against bacterial but also from individual viral diseases. Further mass vaccination of fishes can be done using Nano delivery system which has the potential to elicit an effective immune response in a fish population. The USDA is completing trials on a system for mass vaccination of fish using ultrasound. Nanocapsules containing short strands of DNA are supplemented to a fishpond where they are absorbed into the cells of the fishes. Ultrasound is then used to break the capsules, discharging the DNA and stimulating an immune response in the fishes. This technology has so far been verified in rainbow trouts by Clear Springs Foods (Idaho, US) - a significant aquaculture company that produces about one-third of all US farmed trouts. The nanoscale device can detect and treat infections long before symptoms are evident at a macro scale. Silver nanoparticles (AgNPs) have potent bactericidal activities against pathogenic gram positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and gram negative bacteria (*E.coli*, *Proteus vulgaris*), etc. Positively charged AgNPs can bind to the negatively charged bacterial cell walls (due to the presence of teichoic acids/ LPS) by electrostatic attraction, penetrate it and subsequently interact with the negatively charged cell membrane (due to the presence of carboxyl, phosphate and amino groups), causing damage to the cell membrane and leaking out the cell contents that eventually causes death of the pathogen. Moreover, once inside the cell, the AgNPs releases Ag<sup>+</sup> that can interact with biomolecules like DNA, enzymes, proteins, lipids leading to DNA damage, lipid peroxidation, inactivation of enzymes and proteins (Yun'an *et al.*, 2018) [3].

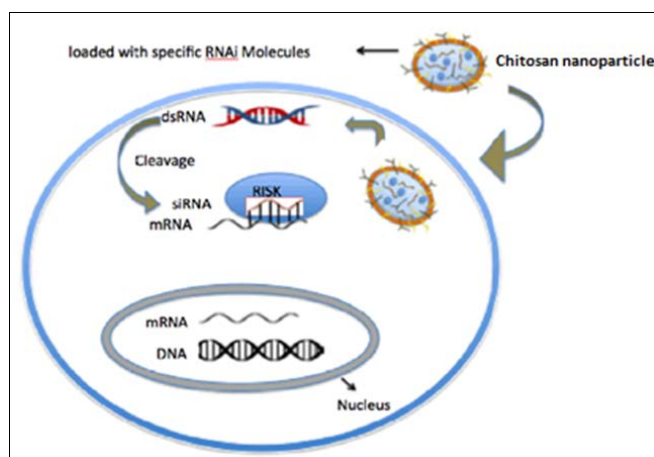
#### Nano-biosensors

Nanotechnology-based biosensors can be used in the aquaculture industry for microbial control. Researchers at NASA, USA have developed sensitized carbon nanotubes based biosensor that is capable of detecting a minute amount of microbes including bacteria, virus, parasite, and heavy metals from water and foods sources. Nano colloidal silver is one the most beneficial product of nanotechnology which can act as a catalyst and work on a wide spectrum of bacteria, fungi, parasites, and viruses by rendering an enzyme, which is used for their metabolism, inoperative (Selvaraj *et al.*, 2014) [4]. Unlike antibiotic resistant strains of bacteria, no such strains are known to develop by using colloidal silver. Silver nanoparticles are even able to kill methicillin resistant *Staphylococcus aureus* (Hoon *et al.*, 2005) [5]. Tracking nano sensors like "Smart fish" may be fitted with sensors and locators that relay data about their health and geographical location to a central computer. Such technology may be used to control reasoning cage systems or individual fish (ETC Group Report 2003) [6].

#### Gene delivery

Gene delivery characterizes a promising technology that aids in the eradication of genetic disorders by insertion of a gene into patient's cells instead of using drugs or surgery, for replacing/ inactivating a mutated gene or announcing a novel gene into the body that will fight out a disease. (Genetics

Home reference by NIH, US National Library of medicine). However, a serious barrier to successful gene therapy remains the formulation of an efficient and safe delivery vehicle. Non-viral delivery systems have been increasingly proposed as alternatives to viral vectors owing to their ability safety and stability to be formed in large quantities. Some methods employ DNA complexes covering lipid, protein, peptide or polymeric carriers, as well as ligands capable of targeting DNA complexes to cell-surface receptors on the target cells and ligands for directing the intracellular trafficking of DNA to the nucleus. Hopeful results were reported in the formation of complexes between chitosan and DNA. Although chitosan rises transformation efficacy the addition of appropriate ligands to the DNA—chitosan complex seems to achieve a more efficient gene delivery via receptor-mediated endocytosis. Furthermore, incubation of cells with chitosan demonstrated low cytotoxic activity. These results suggest that chitosan has similar efficacy without the associated toxicity of other artificial vectors and can, therefore, be an actual gene-delivery vehicle *in vivo*. Researchers have synthesized hybrid lipid-polymer nanoparticles for siRNA delivering. While, other up to date studies have shown the improved cancer treatments obtained with co delivery (Fig 4).



**Fig 4:** Process of RNAi delivery using Chitosan nanoparticles.

### Water filtration and remediation

Nanoparticle-based technologies are available today for the removal of contaminants from water. Nanomaterial in the form of stimulating materials like carbon or alumina, with additives like zeolite and iron-containing compounds can be employed in aquaculture for holding aerobic and anaerobic biofilm for the removal of ammonia, nitrites, and nitrate contaminants. Similarly, a nanoscale powder made from iron can be used as a useful tool for cleaning up contaminants such as trichloroethane, carbon tetrachloride, polychlorinated biphenyls and dioxins to simpler carbon compound which are less toxic thus concrete the way for a Nano-aquaculture. Nevada-based Altair Nanotechnologies makes a water cleaning product for swimming pools and fishponds called 'Nano Check.' It uses 40 nm particles of a lanthanum-based compound which absorbs phosphates from the water and prevents algae growth. Nano Check is currently undergoing large-scale testing in swimming pools.

### Breeding

Managing of breeding is a time consuming and a laborious problem for culture animals. One solution that is presently

being studied is a nanotube fixed under the skin to provide real-time measurement of changes in the level of estradiol in the blood. The nanotubes are used as a means of tracking oestrus in animals (O'Connell *et al.*, 2002) [7] because these tubes can bind and detect the estradiol antibody at the time of oestrus by near-infrared fluorescence. The signal from this sensor will be combined as a part of a central monitoring and control system to actuate breeding. In fisheries also, various hormones and steroids are being delivered using nanoparticle for enhancing gonadal growth and reproductive output.

### Tagging and Nano-barcoding

Radio frequency ID (Rfid) is a chip with a radio circuit integrating nanoscale component with an documentation code fixed in it. These tags can hold more data, scanned from a distance and embedded in the product to categorize any object anywhere automatically. These tags may be used as a tracking device as well as device to monitor the metabolism, swimming pattern and feeding behavior of fishes. A nano-barcode is a monitoring device consisting of metallic stripes containing nanoparticles where variations in the striping provide the method of encoding information. By incorporating the nanobarcoding, processing industry and exporters can monitor the source or track the delivery status of their aquaproduct until it reaches the market. Further, coupled with nanosensors and synthetic DNA tagged with colour coded probes, nano-barcode device could detect pathogens and monitor temperature change, leakage etc., thus improving the product quality (Rather *et al.*, 2011) [8].

### Control of bio-fouling agents

Bio-fouling is one of the major problems in fisheries management. The growth of bio-fouling organisms such as bacteria (as biofilm), algae (diatoms and seaweed) and invertebrates animals (barnacles, mussels) in a man-made structure in aquaculture environment imposes a costly and hazardous threat causing economic damage in various ways. Coating or painting through the incorporation of metals oxide nanoparticle ( $\text{Cu}_2\text{O}$ ,  $\text{ZnO}$ ,  $\text{SiO}_2$ ) can control bio-fouling agents as these coating bound to metal nanoparticles develops an efficient anti-fouling surface and improve the performance of antifouling paints.

### Enhancement of fish growth

Fish production can be enhanced by increasing the growth rate of fishes using nanotechnology in fish farming. Nanotechnology may also increase the absorption of nutrients. Scientists from the Russian Academy of Sciences had reported that young carp and sturgeon exhibited a faster growth rate (30 and 24 percent, respectively) when they were fed nanoparticles of iron (ETC Group Report 2003).

### Conclusion

Nanoparticles exhibit a wide array of roles in agriculture and its allied fields. Application of Nanotechnology in aquaculture and fisheries has brought advances in diagnosis & treatment of genetic disorders in fishes, detection and eradication of pathogens even while they are in minute densities, rapid detection of heavy metals in water and food, improving the ability of fishes to absorb drugs and nutrients thus magnifying production, filtration and remediation of contaminated water, enhanced breeding, controlling bio-fouling agents and many more applications are yet to be explored. Nanotechnology can not only bring a revolution in aquaculture and fisheries but

also in other sectors like horticulture, animal husbandry, dairy and food industry, soil conservation and several others.

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