A REVIEW ON SAFER MEANS OF NANOPIERCLE SYNTHESIS BY EXPLORING THE PROLIFIC MARINE ECOSYSTEM AS A NEW THRUST AREA IN NANOPHARMACEUTICS

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ABSTRACT

Nanotechnology has many implications in our day today life with its core being the synthesizing of nanoparticles. The synthesis of nanoparticles is significant because of its application in the biomedical field. The biological means of synthesizing nanoparticles are more advantageous than the physical and chemical methods, as it involves simple methodology that are non-toxic and obtained at a minimum cost. The biological synthesis implies the use of plants, plant parts and different microorganisms. Marine life has always been a unique source of bioactive compounds with tremendous impact in the field of medicine and pharmacetics. The marine ecosystem has captured a major attention in recent years, as they contain valuable resources that are yet to be explored much for the beneficial aspects of human life. This review focuses on the biological synthesis of nanoparticles from the huge diversified marine ecosystem, including, variety of species like bacteria, algae, fungi, yeast and sponges.

Keywords: Nanoparticles, Biological synthesis, Marine, Nanotechnology, Bioreduction

INTRODUCTION

In this era of nanotechnology (Greek word ‘nanos’ - meaning ‘dwarf’), research on nanomaterials have become the subject of interest for researchers and academia due to its technology, rapid improvements and innovations in various aspects of everyday lives. Nanotechnology is able to control and manipulate matter at the atomic scale. Nanotechnology can be defined according to the National Cancer Institute as “technology development at the atomic, molecular, or macromolecular range of approximately 1–100 nm to create and use structures, and devices, and systems that have novel properties” [1]. Nanotechnology is broadly classified into three major categories as i) wet nanotechnology, which comprises of living biological systems ii) “dry” nanotechnology, which includes engineered objects at the nano-scale level [2] and iii) computational nanotechnology, which involves modeling of nano-scale structures [3].

What are nanomaterials? Nanomaterials are materials that consist of or contain nanoparticles with improved physical and chemical properties such as lower weight with higher strength. These can be categorized as (i) one dimensional nanomaterials having one dimension less than 100 nm (thin films or surface coatings), (ii) two dimensional nanomaterials (nanowires, carbon nanotubes, inorganic nanotubes, biopolymers, nanoribbons), (iii) three dimensional nanomaterials [4] (fullerenes [5], dendrimers [6]), and (iv) zero dimensional nanomaterials (quantum dots) [7].

The dimension factor is considered as one of the most important aspects in nanotechnology. Because, at the nanoscale, new properties emerge due to size confinement, surface effects and quantum phenomena [8] which are quite different from the properties of bulk materials. To be clear, materials at the nanoscale have a larger surface area to volume ratio as compared to bulk materials that show a major impact on the mechanical response [9-11]. On the other hand, the quantum effect also plays a dominant role at the nanoscale affecting their optical, electrical and magnetic properties. These new properties are utilized in many novel applications. Nanotechnology produces materials that range between 1 and 100 nm [12]. A nm is, therefore, defined as one billionth of a metre, mathematically given as 10\(^{-9}\)m. An interesting fact is that a human hair measures approximately 80,000 nm in width. We can then imagine the uniqueness of the nanoscale materials.

Nanoparticles are considered have all three external dimensions at the nanoscale. Nanoparticles are present in the following ways: (a) naturally occurring (anthropogenic) (lipoproteins, virus, ferritin [13] and particles from volcanic ash are some of the examples) and (b) engineered nanoparticles. Engineered nanoparticles are obtained either commercially or by experimental procedures in the research laboratory. Fullerenes, carbon nanotubes, quantum dots, metal and metal oxide nanoparticles are the few examples of engineered nanoparticles [14]. Nanoparticles can be categorized into different types based on various factors such as: (1) size (for example, nanoclusters), (2) shape, (3) metallic, (4) semiconductor nanoparticles, (5) quantum dots, (6) semi-solid and soft nanoparticles.

Nanotechnology finds its application in diverse areas. The unique properties of carbon nanotubes make it ideal for electronic applications [16]. Several nanomaterials are developed to provide food safety [17], good taste, health benefits [18] and as packaging material [19]. Nanotechnology plays an important role in improving an eco-friendly environment by reducing pollution due to industrial emissions [20] and also helps in minimizing contamination of groundwater [21]. The use of silicon nanowires helps in conversion of solar to electric energy to obtain an efficient cost effective solar cells [22]. The Zn/ZnO nanoparticles coated on the surface of cotton fabrics emitted 82% of excessive infrared intensity [23]. Nanotechnology, in the field of medicine offers many exciting possibilities. Use of nanorobots in treating human disease is one of the major developments of modern medicine [24]. Nanomedicine has found wide applications in drug delivery [25], gene delivery, molecular diagnostics [26], fluorescent biological labels [27], cardiovascular [28] and cancer imaging purposes [29], detecting anti-microbial activity [30], detection of protein analytes [31], purification of biomolecules and cells [32], and many others. Nanowire nanosensors are used in detecting wide range of chemical and biological species and this is exhibited in in-vivo diagnostics [33]. The nanomaterials play an important role in the field of medicine because of their size that is similar to most of the biological molecules and structures. Due to this property, they are used in both in-vivo and in-vitro biomedical applications.

Regarding the synthesis, there are different methods employed which can be broadly classified as follows: (1) wet synthesis, (2) dry synthesis and (3) milling. In both, wet and dry method of synthesis, nanoparticles are produced by a bottom-up approach in which the nanoparticles are formed first and then assembled into the final material using chemical, physical and biological procedures. Examples of bottom-up approach include chemical synthesis, self-assembly and molecular fabrication. The advantage of bottom-up approach is that the nanoparticles are obtained with lesser defects and the chemical composition is homogenous in nature. Wet
synthesis includes sol-gel and precipitation methods. Whereas, milling follows a top-down approach that involves breaking down of larger particles mechanically, i.e., mechanism and structures are miniaturized to a nm scale. An important example of this kind is the lithographic technique (such as photolithography, ion beam lithography, X-ray lithography) [34,35]. However, a major drawback of this approach is the imperfection of the surface structure that can have a significant impact on the properties of the nanoparticles. Dry synthesis encompasses combustion, furnace and plasma synthesis of nanoparticles. Attrition and pyrolysis are the commonly used procedures for the physical methods. In attrition, initially the macro or micro scale particles are ground in a ball mill or a planetary ball mill, or by other size reducing mechanism. The resulting particles are air classified to recover nanoparticles. In pyrolysis, an organic precursor (liquid or gas) is forced through an orifice at high pressure and burned. The resulting solid is air classified to recover oxide nanoparticles from by-product gases. Pyrolysis often results in aggregates and agglomerates rather than singleton primary particles. However, it seems that the productivity rate of the nanoparticles synthesized by these methods is very low, and, on the other hand, the expense of energy and pressure is much higher [36]. A wet chemical method involves the synthesis of metallic nanoparticles by using reducing agents such as sodium citrate [37], NaBH₄ [38], N₂H₄ H₂ gas [39], commonly available sugars such as glucose, fructose and sucrose [40] or by reduction of metallic salts in dry ethanol [41]. The metal colloids can be obtained from air-saturated aqueous solutions of polyethylene glycol (PEG) [42]. The mesityl derivatives are also used in synthesizing metallic nanoparticles [43]. Usually, the chemical methods are cost-effective for large-scale synthesis. However, there are quite a few drawbacks like contamination from precursor chemicals, use of toxic solvents, and generation of hazardous by-products. The advantages of biological synthesis over conventional methods are shown in Fig. 1. Hence, there is an increasing demand in the recent years to develop an eco-friendly procedure with low-cost and non-toxic method for the synthesis of nanoparticles.

A new approach to these problems is addressed by utilizing the huge variety of biological resources from nature. Over a decade, different varieties of microorganisms and plants are used for production of low-cost, energy-efficient, and nontoxic nanoparticles. This is called the ‘green-synthesis’ or ‘green-chemistry’ procedures [44,45]. *Avena sativa* [46], *Azadirachta indica* [47], *Aloe vera* [48], *Tamarindus indica* leaf extract [49], *Cinnamomum camphora* [50] are some of the plant sources which synthesizes nanoparticles. There are many reports of different microorganisms like bacteria, fungi, actinomycetes, yeast, and virus being used in the synthesis of nanoparticles. Few examples of nanoparticle synthesis by various micro-organisms include *Aspergillus fumigatus* [51] (Ag nanoparticle), *Candida glabrata* [52] (CdS nanoparticle), *Fusarium oxysporum* (silver [53], gold [54], zirconia [55], cadmium sulphide [56], silica and titanium particle [57], and CdSe quantum dots [58]), *Pseudomonas aeruginosa* [59] (Au nanoparticle), and many others.

The biologically synthesized nanoparticles are multi-functional with diverse biomedical applications. They are widely used in therapeutics [60], drug delivery systems [61], tissue regeneration [62], separation techniques and sensors [63]. They are potentially used in nano-medicine [63]-[65]. The applications orienting biologically synthesized nanoparticles are shown in Fig. 2.

The synthesis of nanoparticles from a huge diversity of marine resources has become the recent and most innovative area of research. Many biologically active compounds have been isolated and screened for pharmacological activity from various marine sources. These include marine algae, fungi, vertebrates, invertebrates and other marine microorganisms from which potentially active chemicals with interesting anti-inflammatory, antiviral, antibacterial, antifungal, antimalarial and anticancer properties which have been studied [66].
In this review, we have discussed in detail about various reports on the synthesis of nanoparticles from variety of marine sources. Predominantly, silver, gold and cadmium sulphide were the only nanoparticles synthesized so far using marine resources. The tabulation in Table 1 represents different marine sources used for nano-synthesis. As this finds a new approach on the synthesis of nanoparticle, extensive research is being carried out with different methodologies in this area.

**Fig. 2: Diverse applications of biologically synthesized nanoparticles**

**Table 1: Synthesis of metallic nanoparticles from marine sources**

<table>
<thead>
<tr>
<th>Marine source</th>
<th>Nano-particle synthesized</th>
<th>Size &amp; shape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sargassum wightii (algae)</td>
<td>Gold</td>
<td>8-12 nm Mostly thin planar structures, some are spherical</td>
</tr>
<tr>
<td>Yarrowia lipolytica NCIM 3589 (yeast)</td>
<td>Gold</td>
<td>Particle size varied with varying concentration. Mostly spherical nanoparticles, some hexagonal or triangular nanoplates</td>
</tr>
<tr>
<td>Fucus vesiculosus (algae)</td>
<td>Gold</td>
<td>Both size and shape varied according to different initial pH values</td>
</tr>
<tr>
<td>Acanthella elongata (sponge)</td>
<td>Gold</td>
<td>7-20 nm Spherical</td>
</tr>
<tr>
<td>Penicillium fellutanum (fungi)</td>
<td>Silver</td>
<td>5-25 nm Spherical</td>
</tr>
<tr>
<td>Brevi-bacterium casei MSA19 (sponge-associated)</td>
<td>Silver</td>
<td>Uniform and stable</td>
</tr>
<tr>
<td>Pichia capsulata (yeast)</td>
<td>Silver</td>
<td>5-25 nm Spherical</td>
</tr>
<tr>
<td>Rhodopseudomonas palustris</td>
<td>Cadmium sulphide</td>
<td>8.01 ± 0.25nm, Cubic crystalline structure</td>
</tr>
</tbody>
</table>

**Biosynthesis of gold nanoparticles**

Due to unique physical and chemical properties of gold nanoparticles, they are extensively used in many applications [67]. They find their application in photonics, electronics [68], electromigration and chromatographic techniques [69], catalysis [70] and biomedicine. The biomedical applications may include identification of protein-protein interaction [71], diagnostic assays, thermal ablation drug and gene delivery [72]. Silicon nanowire embedded with gold nanoparticle is a good example of application of these nanoparticles in biomedicine for DNA detection [73]. The gold nanoparticles can be synthesized either by physical or chemical method. The green synthesis of these nanoparticles is of recent interest because of its multiple advantages.

The bioreduction of auric chloride using a marine alga, *Sargassum wightii* yielded uniform gold nanoparticles. The reports of TEM images clearly showed that the nanoparticles had an average size of 11 nm and there was an inclination forming at thin planar structures than the spherical structures. There was no evidence of the capping agent. The authors believe the coating to be a bioorganic component of *S. wightii* and the polysaccharide content to stabilize. But an interesting fact is that, not all the particles of the TEM images are in physical contact, but were separated from each other by uniform interparticles distance. An important and effective benefit obtained by this method is that the nanoparticles synthesized were stable in solution. Thus it makes it more potent compared to other biological methods which are currently in use. The extracellular polysaccharide confers the stability and this stable nature may inturn lead to easy bioprocessing of these gold nanoparticles [74]. In a certain literature, it was suggested that sulphated polysaccharides derived from marine seaweed, *Sargassum wightii* had a wide application in inhibiting free radicals. These polysaccharides were found efficient in preventing the dysfunction associated with Cyclosporine A induced toxicity, thereby playing a major role as a therapeutic agent [75,76]. The usage of *Sargassum wightii* has been extended for the synthesis of silver, Pt and Pd nanoparticles by the same researchers and they reported similar kind of reduction mechanism.

It was demonstrated that by varying the concentrations of HAuCl₄ and cell numbers of the tropical marine yeast isolate *Yarrowia*...
lipolytica NCIM 3589; different varieties of gold nanoparticles were produced with vivid colour reactions. This method offers extracellular synthesis of nanoparticles obtained by a simple procedure. The gold nanoparticles produced in a cell-associated manner when incubated in cold synthesized custom designed pattern of gold nanoparticles. Regarding the shape of nanoparticles obtained, about 86% were spherical and the remaining 14% obtained were hexagonal or triangular nanoparticles. It was also reported that the carboxyl, hydroxyl and amino groups associated with the cells were involved in the synthesis of nanoparticle after the interaction of the yeast cells with gold. After the reduction of HAuCl₄ to Au⁺, a capping agent helped in the stabilization of their structure. It was found from the results, that with intermediate number of cells and low concentrations of gold salt, nanoparticles were synthesized. With higher concentrations of gold salts, the excess gold was deposited on specific sites to form nanoparticles because the capping agent was in limiting concentration. It is also suggested that the proteins biomolecules could have been present which could be the possible reason for the insufficient reduction of HAuCl₄ and also capping of nanoparticles at a higher gold concentration. Studies are on-going on the identification of these biomolecules. It was also confirmed from the studies that the average mean of the nanoparticle size increased with an increasing concentration of the gold salt while the cell numbers remained constant. On the other hand, the nanoparticle size decreased with increasing cell numbers and the concentration of the gold salt being constant. Thus, the particle size varied with varying concentrations of gold salt and cell biomass [77].

In a certain study, dead biomass of brown alga Fucus vesiculosus was used. The brown algae are generally considered as the well known biomass for biosorption which in turn serves as cheap and best method for metal recovery at low concentrations. The recovery of gold has an important value in the market because of its high cost. It was done in two stages, first was the induction period with not much change observed. Moreover, in the second stage, hydroxyl groups present in the polysaccharides of algal cell wall were involved in the gold bioreduction and also in the nanoparticle formation. Au (III) was recovered as the metallic gold nanoparticle. These algae were also effective in bioreduction of Au (III) to Au (0). The metallic gold was obtained as microprecipitates on the surface of the algal biomass and as colloidal form of nanoparticles was detected from the solution. The size and shape of the nanoparticles varied with different initial values of pH. For example, at a pH of 7, uniform and smaller spherical nanoparticles were obtained compared to those with a pH of 4, which varied with different sizes and shapes. It was also suggested that sulphhydril groups, a highly reactive functional group with its chemical behavior similar to hydroxyl groups and also fucoxanthin, an algal pigment rich in hydroxyl groups could have been involved in the bioreduction of HAuCl₄. Thus it could have been acting as capping agents preventing the aggregation of nanoparticles in solution, and thereby playing an important role in their extracellular synthesis in a larger level making its purification process easier and also in the shaping of the nanoparticle [78].

The gold nanoparticles were also biosynthesized using a marine sponge Acanthella elongata at an extracellular level. The sponge extract when added to 10⁻⁴M HAuCl₄ aqueous solution at 45°C, it was changed to pinkish ruby red color solution and 95% of the bioreduction of AuCl⁺ ions occurred within 4 h of continuous stirring and yielded uniform gold nanoparticles. By high-resolution transmission electron micrographs (HR-TEM), it was reported that the nanoparticles obtained were monodispersed, spherical in shape with size ranging from 7 to 20 nm, and about 25% being 15 nm in diameter. The XRD analysis revealed that the nanoparticles obtained were crystalline in nature. The secondary metabolites obtained from the extract of marine sponge Acanthella elongata possibly acted as the capping agent and thereby helped in the prevention of agglomeration of the particles formed and stabilized them. It was also detected that the water-soluble manganese present in the extract were involved in the reduction of gold ions. It was also reported that highly stable gold nanoparticles were obtained by biotransformation using various species of marine sponges [79].

Biosynthesis of silver nanoparticles

The silver nanoparticles are used widely in technology, medicine and consumer products, but there are limited data from the marine sources. Among the nanoparticles, silver nanoparticles have been widely used in bio-labeling, antimicrobial agents, catalysts [80], and sensors [81]. Due to its unique optical [82], electrical [83] and magnetic properties, they mainly find their application in optics, electronics and catalysis.

A report showed that, by using the marine fungal strain of Penicillium fellutanum, it was possible to obtain silver nanoparticles at a faster rate by extra-cellular means. Silver nanoparticles were synthesized within 10 min of silver ions being exposed to the culture filtrate and proved that the increase in colour intensity of culture filtrate was due to increasing number of nanoparticles formed by reduction of silver ions. The particles obtained had a good monodispersity. The maximum synthesis of these nanoparticles occurred at a pH - 6.0, temp - 5 °C, 24 h of incubation time, silver nitrate concentration of 1 mM and 0.3% NaCl. According to TEM micrographs, most of the silver nanoparticles obtained were spherical in shape with size ranging from 5 to 25 nm. In this study, it is believed that the enzyme protein of nitrate reductase secreted out of the fungal biomass would have involved in the reduction of the silver ions nanoparticles that is detected by the presence of a single protein band of 70 kDa obtained from the culture filtrate [84].

The usage of biosurfactant has now emerged as a green alternative for stabilizing the nanoparticles and used in enhancing the silver nanoparticle synthesis. The biosurfactants are mainly made up of sugar and fatty acid moieties. In this study, a glycolipid biosurfactant was derived from the sponge-associated marine Brevisbacterium casei MS19 under solid-state fermentation. In this study of biosurfactant-mediated synthesis, the silver nanoparticles obtained were found to be uniform and stable for a period of about 2 months. The biosurfactant prevents the formation of aggregates and thus helps in the production and stabilization of nanoparticles [85].

In a certain study, twelve different species of marine yeasts were isolated from the coastal mangrove sediment for the in-vitro synthesis of silver nanoparticles. The nanoparticles were synthesized extracellularly by the marine yeast, Pichia capsulata which exhibited the highest activity as compared to other species screened. The synthesis of silver nanoparticles was observed by a change in the colour intensity from medium to brown under specific conditions favorable for synthesis. These conditions (pH - 6, incubation time - 24 h, substrate concentration - 1.5 mM AgNO₃, temperature - 5 °C, 0.3% salinity) favored maximum synthesis of silver nanoparticles. The particles obtained were mostly spherical in shape and the size ranged from 5-25 nm. Pichia capsulata followed by Saccharomyces cerevisiae, Debaryomyces Hansenii, Schizosaccharomyces pombe and Debaryomyces Hansenii are some of the species that synthesized nanoparticle at insignificant concentrations. The other marine yeasts isolated comparatively showed lower activity of the silver nanoparticle production [86].

The bioreduction of AgNO₃ to silver nanoparticles using seaweeds like Ulva lactuca and Gelidilla s.p. has also been reported. Seaweeds constitutes of various phytochemicals like carbohydrates, alkaloids, steroids, phenols, saponins and flavonoids which aids in the reduction and stabilization of nanoparticles. The biogenic silver nanoparticles synthesized using green seaweed, Ulva lactuca, exhibited good antibacterial activity against clinical pathogens like Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis, Staphylococcus aureus and Pseudomonas aeruginosa [87]. The silver nanoparticles produced by bioreduction of aqueous Ag+ using seaweed extract from Gelidilla sp. demonstrated a considerable cytotoxicity against Hep-2 cell lines. The result showed that Hep2 cells proliferation were significantly inhibited by AgNPs with an IC50 value of 31.25 µg/ml of the concentration [88].

Biosynthesis of cadmium sulfide nanoparticles

Cadmium sulfide nanoparticle finds its use for various purposes such as an oligonucleotide label for detecting nanopalle terminator gene sequences [89], electrochemical DNA detection of
phosphinothricin acetyltransferase [90], and as matrices and co-
mattices for peptides and large proteins analysis respectively in
MALDI-TOF-MS [91].

When cadmium sulfate solution was incubated with a
photosynthetic bacterium Rhodopseudomonas palustris, it was
 demonstrated that the biomass changed to yellow color from 48 h
 onwards, which indicates the formation of cadmium sulfide
 nanoparticles. In the stationary phase of the R. palustris cells, the
 CdS nanoparticles obtained exhibited monodispersity and was
 spherical in shape. The average mean of the particle size was
 found to be 8.01 ± 0.25 nm. Reports of the electron diffraction pattern
 further confirm the presence of face-centered cubic (fcc) crystalline
 structure of CdS. The colloidal form of cadmium sulfide nanoparticles
 remained very stable even after 2 months without any
 aggregation. The results from FTR spectrum suggests the presence
 of biological molecules which could have involved in both
 stabilization and formation of nanoparticles. From the study, it was
 also suggested that the cysteine desulhydrase (C-S lyase), an
 intracellular enzyme present in the cytoplasm of R. palustris could be
 responsible for the formation of nanocrystal, and the protein
 secreted by the bacteria helped in the stabilization of cadmium
 sulfide nanoparticles. The nanocrystals formed had an average size
 of about 4–6 nm. The R. palustris was also able to efficiently
 transport cadmium sulfide nanoparticles out of the cell [92].

SUMMARY

The biological synthesis of nanoparticles fulfills the need for a safe
and environment friendly method of synthesis. One of the major
concerns despite the beneficial aspects of nanotechnology is now
 focused on the physico-chemical properties of nanoparticles which
in turn may cause unknown effects on the biological ecosystem. This
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