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Mini Review

Silica Based Bio-hybrid Materials and their Relevance to Bionanotechnology

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Abstract

Bionanotechnology is an emerging interdisciplinary field which comprises of biotechnology and nanotechnology. In bionanotechnology, biological molecules are used in order to improve the applicability of nanomaterials. Synthesis of stable and functional bio-hybrid, biomimetic, self-assembled materials is important applications of bionanotechnology. Bio-hybrids are advanced materials which offer dual functionality and improved features. These materials have far fetching impact in the field of remediation, biosensor, biocatalysis and drug delivery. Herein, the focus is on the silica based bio-hybrid materials and their applications.

Keywords: Silica nanoparticles (Silica NPs); Bio-hybrids; Bionanotechnology; Immobilization; Biosensor; Drug Delivery; Remediation

Introduction

Biological materials like microorganism, enzymes (biocatalysts) etc., are valuable for various bioprocess applications. However, certain limitations associated with biomolecules like poor mechanical and chemical stability need to be addressed. Biotechnology is the discipline wherein the focus is on the application of biological components like organisms/their product/their part such as enzymes and Genetically Modified Organisms (GMOs) to produce products and processes which are useful to mankind. In other words, biotechnology creates wealth using biological molecules. Different methods of genetic engineering are used as tools for modification in the genetic material of the organism of interest in order to improve the required features in organism. Genetically Modified Organisms (GMOs) are further used for the production of various products like enzymes, antibiotics and vaccines etc. [1]. Biotechnology has enormous scope for use in health care, agriculture, food industry, environmental clean-up and biofuel.

Immobilization is another aspect in biotechnology used to improve the applicability of biological molecules. Immobilization is a simple and efficient method wherein a biological molecule is attached to a suitable support which provides a favourable micro-environment to the biomolecule and improves the various physico-chemical aspect of the immobilized biomolecule [2]. There are various methods of immobilization like adsorption, covalent attachment, gel entrapment, microencapsulation etc., (Figure 1). For efficient immobilization, suitable immobilizing supports with features like high surface area, biocompatibility, stability etc., are required. A wide range of supports varying from organic to inorganic supports have been explored for immobilization [3-10]. In recent years, nanoparticles have emerged as suitable immobilizing support. The unique quantum phenomenon at the nanoscale size is responsible for beneficial and unique features of nanomaterials. Scientists are in constant search for developing advanced materials through use of nanoparticles for various applications like drug delivery systems, biosensors with increased sensitivity, efficient and recoverable immobilizing supports and sorbents with high sorption capacity.

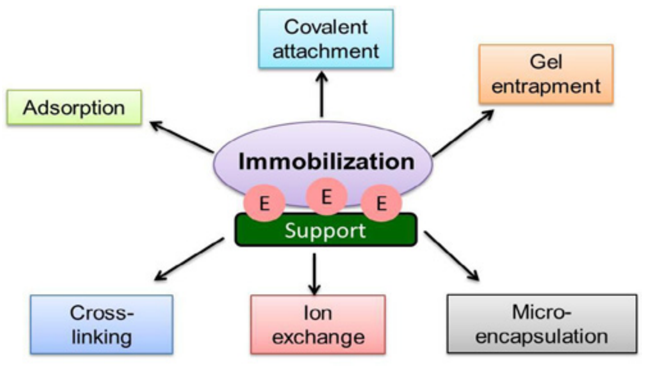

Figure 1: Immobilization and various methods of immobilization.

Silica nanoparticles (silica NPs)

Inorganic nanoparticles are well known immobilizing supports due to high stability, mechanical resistance and good sorption capacity. Previously, nanoparticles of aluminium, zirconium and titanium oxides have been explored as immobilizing support for different enzymes [11-13]. Due to its wide availability, easy functionalization and biocompatibility, silica nanoparticles are one of the most suitable inorganic supports for immobilization and various other applications [14]. Also, silica allows efficient bio-component attachment due to availability of several silanol groups on its surface and reduces diffusional limitations.

Colloidal silica NPs have been used as catalytic supports [15-17], biosensor supports [18-21], remediation [22-25] and drug carriers [26-29]. For various applications, commercially available colloidal silica is used because naturally available mineral silica is contaminated and is not suitable for application in scientific research and industrial applications [30]. Also, mineral silica offers less surface area (with a few exceptions) and exists in crystalline form which is damaging to health. Thus, for most of the applications chemically synthesized

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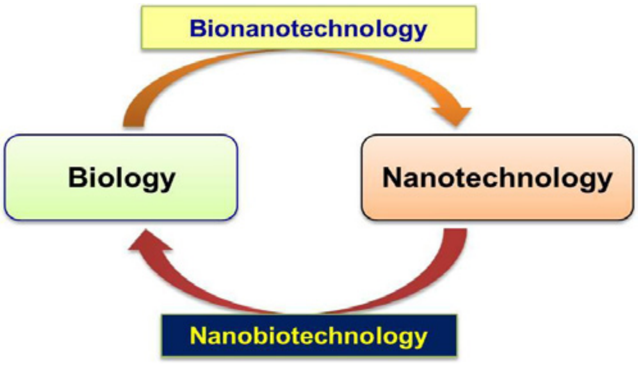

Figure 2: Relation between bionanotechnology and nanobiotechnology.

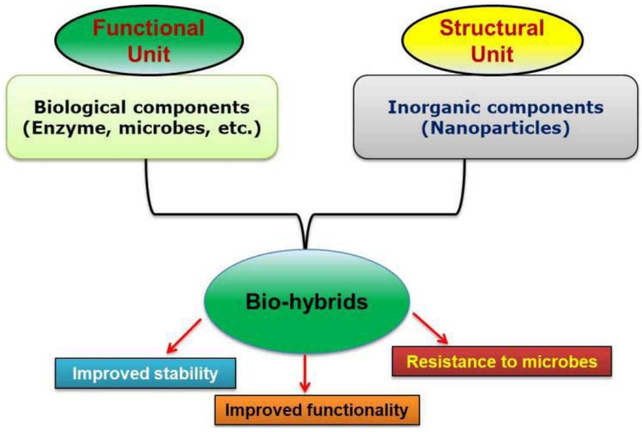

Figure 3: Two components of a bio-hybrid and its improved features.

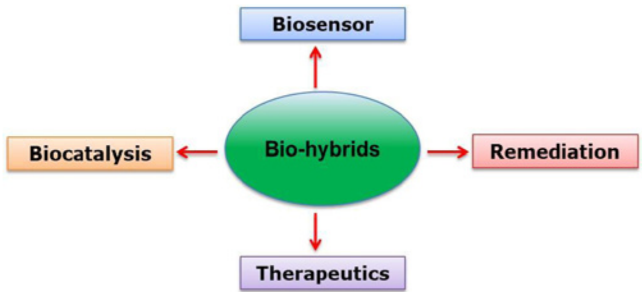

Figure 4: Applications of bio-hybrid materials.

colloidal silica is preferred.

Bionanotechnology

Bionantechnology is an emerging interdisciplinary field wherein a biological molecule is applied to improve the applicability of nanomaterials by synthesizing bio-hybrid, self-assembled and advanced materials. On the other hand, nanobiotechnology deals with application of the nanodevices/nanotechnology to understand a biological phenomenon or structure of a biological molecule [31]. In principle bionanotechnology and nanobiotechnology is a combination of biotechnology and nanotechnology (Figure 2). Bionanotechnology will help to understand the following aspect:

(a) The interaction between a biological and non-biological (nanoparticles) components

(b) Developing efficient drug delivery systems for targeted drug delivery

(c) To understand and study toxicology

(d) Developing highly sensitive and miniature screening systems

Important application of bionanotechnology is to help in the development of advanced materials like bio-hybrids, biomimetic, self-assembled materials in order to address the issues associated with conventional materials.

Silica based bio-hybrid materials

A hybrid material is a mixture of inorganic components and organic components, or both types of component [32]. The combination of silica nanoparticles (as inorganic component) with organic materials results in the synthesis of silica based hybrid materials [33]. Extensive work has been carried out on silica based hybrids and published wherein silica nanoparticles have emerged as suitable inorganic component [34-36]. The requirement for materials with advanced properties is continuously increasing. The efficiency of mesoporous silica for various applications is mainly due to their porous structure which allows molecules to diffuse into their large internal surface. However, need for functionalization, small pore size and limited accessibility to the internal surface creates are the bottlenecks which causes mass-transport issues. In this regard, by using a suitable method the bio-component of interest can be conjugated with nanomaterial and new 'bio-hybrid' materials can be developed. The conjugation between nanomaterial and bio-component will lead to new functional materials with improved features.

A number of advantages associated with silica, makes it a suitable inorganic component for the synthesis of bio-hybrid materials. From last decade "bio-hybrid" has gained attention wherein biological component acts as functional unit and inorganic component acts as structural unit and imparts dual functionality (Figure 3). Association of biological components with silica nanoparticles have opened a window of interest for synthesis and application bio-hybrids.

Applications of silica based hybrid/bio-hybrid materials

In present scenario, significant interest has been observed in the synthesis of silica nanoparticles based bio-hybrid materials with various sizes, shapes, morphologies and functional properties. These materials have gained popularity because of their advanced features and applied in various fields. Herein, the applications of silica nanoparticles in four major areas i.e. remediation, bioprocessing, biosensor and drug delivery are discussed (Figure 4).

In the field of remediation

In previous studies, silica based supports like mesoporous silica and nanosize silica have been prepared for the remediation of metal pollutants like uranium [22-25]. Hybrid mesoporous silica was applied for removal of heavy metal and in another study, functionalized mesoporous silica showed excellent binding affinity for mercury (II) [37,38]. Among different forms of silica, SBA-15 gained attention as sorbent for metal remediation [39] because it is hydrothermally more stable in comparison to MCM-41 [40]. To improve the performance of mesostructured silica, it was organically functionalized and used for the uptake of heavy metals [39,40].

Despite the several studies and motivating results, functionalization of mesoporous silica is still a big hurdle which is difficult to achieve in real environment. Thus, there is need to devise low cost methods to functionalize silica based support with improved features. To address the issue, we devised spray drying as a single step, efficient route to synthesize functionalized silica microstructures using microorganisms (*Streptococcus lactis* and *Saccharomyces cerevisiae*). Silica-*Streptococcus lactis* and silica-*Saccharomyces cerevisiae* microstructures were prepared and applied for sorption of uranium (VI) and mercury (II), respectively [41,42]. Spray dried doughnut silica-*Streptococcus lactis* microstructures showed significantly rapid uranium removal and maximum sorption capacity (q_{max}) was 169.5 mg/g [41]. In case of silica-*Saccharomyces cerevisiae* microstructures more than 98% of total mercury sorption was observed in 30min and q_{max} was 185.19 mg/g [42]. These studies suggest, the use of spray drying technique as a simple route for the functionalization of silica based microstructures using micro-organisms as functionalizing agent. It also helps to address the limitations associated with application of silica nanoparticles and micro-organism alone to be used for remediation. Also bio-hybrid has emerged as advanced material for the environment remediation.

In the field of biocatalysis

Among the available immobilizing supports in the field of enzyme technology, silica based supports stand out because of its beneficial features. Silica based materials are the best immobilizing support for immobilization of enzymes. Presence of functional groups on the surface of supporting material improves the enzyme-support binding and therefore reduces the chances of leakage of immobilized enzymes. Lei *et al.* have observed that immobilized enzymes within functionalized mesoporous silica showed improved activity compared to free enzymes [43]. Chong *et al.* have also proved that the activity of Penicillin G Acylase (PGA) immobilized on vinyl-functionalized mesoporous silica was higher than free PGA [44]. For efficient immobilization of enzymes and to maintain the activity and stability of immobilized enzyme, functionalized silica based supports are utilized as most suitable materials. However, limitations like substrate diffusion due to small pore size of mesoporous silica, its synthesis and multi-step functionalization processes are the bottleneck for its application as immobilizing support.

To overcome the above issues, dendtritic/fibrous bio-hybrid supports could be a possible solution. Our group has synthesized fibrous silica nanoparticles-*Ocimum basilicum* seeds bio-hybrid support and used it as immobilizing support for immobilization of invertase enzyme [10]. Swollen *O. basilicum* seeds show fibrous pellicular structure which was explored as template for assembly of silica nanoparticles. Incorporation of the nanoparticles results in an increase in the available surface area of the seeds and improvement in the physico-chemical properties. Another added advantage is that enzyme immobilized on bio-hybrids can be easily separated out and reused. Further, such type of fibrous bio-hybrids also have potential applications in other areas like remediation, carbon dioxide capturing, developing sensitive sensors, developing drug carrier and energy storage.

In the field of biosensor

Previously, enzymes immobilized on silica based supports have also been applied for developing biosensors [18-21]. Ponamoreva *et al.* have synthesized yeast-based self-organized hybrid for the application as biosensor [45]. Diana *et al.* have encapsulated genetically engineered *Moraxella* spp. cells using sol-gel technique for the detection of organophosphates pesticide [46].

Methyl Parathion (MP) is an insecticide used in agriculture [47]. However, it also causes harm to human health. For the detection of methyl parathion, microbial cells with Organophosphorous Hydrolase (OPH) enzyme which hydrolyzes MP into a coloured Product P-Nitrophenol (PNP), were immobilized on to a number of matrices and further explored as biosensor to detect MP [48-53]. Our group has observed that immobilized *Flavobacterium sp.* and *Sphingomonas sp.* could detect MP in the range of 1-20 ppm and storage stability in days [50-52]. However, the low sensitivity and poor storage stability in the previous studies motivated us to work for the improvement in the sensitivity (up to 0.1 ppm) as well as to improve the storage stability. To encounter the issue associated with previous methods, functionalized silica nanoparticles were associated with *Sphingomonas sp.* cells and immobilized on the 96 well microplate and associated directly with the optical transducer of microplate reader [14]. Immobilized bio-hybrid of functionalized silica nanoparticles-*Sphingomonas sp.* significantly improved the sensitivity and storage stability of the biosensor. There was improvement in linear detection range from 1 – 10 ppm to 0.1 – 1 ppm which is in the range of MRL. Also, the storage stability of developed was significantly enhanced ten times. This study showed that interaction of functionalized silica nanoparticles with cells has positive impact on developing bio-component and synthesized functional bio-hybrid was enough stable to improve the sensitivity as well as storage stability of biosensor for detection of methyl parathion pesticide.

In the field of developing drug carriers

Silica based materials are suitable candidates for efficient drug delivery systems because of their unique characteristics [54]. Several studies have been carried out wherein mesoporous silica nanoparticles were applied as drug carriers for cancer treatment [26-29]. Interestingly, the finding that cells can take up and internalize silica NPs without any cytotoxic effects has improved the practical applicability of applications of silica NPs as drug carriers [55].

For application of drug carriers in industry, there is need to produce the drug carriers in large scale under Good Manufacturing Practices (GMP) conditions with reproducibility and low cost. In view of this, we have synthesized silica nanoparticles-sodium alginate bio-hybrid as drug carrier for doxorubicin using spray drying [56]. Synthesized bio-hybrid drug carrier showed excellent drug loading efficiency. *In vitro* release study of entrapped doxorubicin in bio-hybrid showed a slow and pH-responsive release. The intracellular uptake study and *in vitro* cytotoxicity results showed that the presence of silica NP along with sodium alginate provided an opportunity to enhance the drug concentration and their cytotoxicity to cancer cells. Thus, spray drying has emerged as an efficient technique for synthesis of drug carriers with high drug loading efficiency and also suitable for cancer treatment.

Conclusion & Future Prospective

Bionanotechnology (a combination of biotechnology and nanotechnology) is helpful to develop advanced materials with various beneficial characteristics. This utilizes beneficial aspect of biological systems to improve the utility of a nanomaterial. The important applications of bionanotechnology include synthesis of stimuli responsive materials, self- assembled bio-hybrids, bio-mimetic biological assembly, drug delivery carriers and bioelectronics which supports biotechnological processes.

In future, bionanotechnology could be applied to develop sensitive nanodevices and also to develop efficient *in vitro* processes using bio-hybrids. Cancer bionanotechnology is also another growing area wherein one can address the limitations associated with conventional drug delivery systems by developing efficient and targeted drug delivery systems using bio-hybrids with less/no adverse effects to normal cells. In the field of biosensors, there is requirement to come up with efficient handy and easy to use instruments for detection of pesticides or other analytes using bio-hybrids.

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