Implementations in Biomedical Therapies by using "Bacterial Magnetosomes" as Drug Loader

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Abstract: ***The Magnetotactic Bacteria have potential to navigate along magnetic field line due to the presence of magnetosomes. The magnetosomes having unique feature and make them applicable for the nanotechnology and biomedical therapies. In this article, we describe the mass cultivation of magnetosomes. The biomineralization of magnetosomes is a process within a specific compartment called crystals, which are shown to be excellent magnetic-nanocarrier for antibodies, enzymes, ligands, nucleic acid and chemotherapeutic drugs thus, further part of the article focuses towards the strategies for loading drug onto the bacterial magnetosomes (BM's). Finally, we described the potential of BM's in various biomedical applications.***

Keywords: Magnetotactic Bacteria, Magnetosomes, Mass Cultivation, Purification, Drug Loading

1. Introduction

The biomineralization of magnetosomes is an intriguing exploration for the biological synthesis of nanoparticles which perform with in the membrane MTB (Magnetotactic Bacteria) and serve as navigational device for spatial orientation in marine and fresh water habitats by interaction with the earth's magnetic field [1]. Modified techniques now can easily produce and isolate the magnetosomes. These particles have exceptional properties which represent a new class of magnetic nanoparticles. The magnetosomes provide numerous attractive possibilities to modify the medical treatments, and nanotechnologies. Bacterial magnetic nanoparticles have been suggested for a number of in-vitro applications such as magnetic separation and procedures for labeling and immobilization of various bimoleculas. The magnetosomes has been using for numerous purification procedures such as mRNA and DNA extraction from biological samples such as tissues, blood and bacterial cells. For instance, the efficiency of DNA recovery with dendrimer-modified magnetosome particles was 6-fold higher with bacterial particles than with artificial Magnetic particles [2]. The DNA extraction automation procedure which is based on dendrimer- modified particles has been recently reported [2, 3]. Oligo(dT) modified magnetosomes facilitates the mRNA isolation [4]. Magnetosomes similarly modify with Oligonucleotides have been employed in an automated. Magnetic microarray for the deletion of different Cyanobacterial DNA with genes specific probes [5].

Immobilization of protein, peptides and enzymes on magnetic particles, facilitates another set of biotechnological applications which allows selective separation and reuse of immobilized enzyme form a reaction Mixture [6]. Likewise, the immobilization of immunoglobulin has received great attention and inspired the development of diverse application relied on chemical cross-linking of the antibody with the MM [7]. And it also provide genetic modification of magnetosomes membrane proteins (MMP) to generate protein fusions of a MMP and an immunoglobulin binding proteins such as the staphylococcal protein A or Streptococcal protein G [8,9] . Along with all the above approaches, an additional approach is potentially superior to chemical cross-linking as the antibody is oriented more accurately, although it remain to be show that the polypeptides used for the genetic fusion are infect native constituents of the MM. Automated immunoassays uses the antibody-magnetosome conjugates to detect environmental pollution, hormones and toxic- substances [8,10]. The most successful use of antibody modified magnetosomes is the specific separation of target cells from human blood [10]. Streptovidin modified magnetosomes were used for discrimination of single nucleotide polymorphism. The streptovidin- modified particles were coupled to biotinylated oligonucleotides to facilitate magnetic separation of DNA hybrids. The magnetic particles can be used for the purification and immobilization of bio-molecules and also for their detection. The highly sensitive detection and quantification of stresptovidin immobilized on glass slides was done by magnetic force microscopy where the magnetosomes conjugated with biotin [11]. Chain of magnetosomes is one of the most complex and highly ordered structures within a MTB and it has been suggested that biomimetic approaches could be used for the fabrication of self-assembling magnetic nanostructures inspired by magnetosomes chain, such as magnetic nanotubes and nanowires as building blocks in magnetic devise. It has been recently shown by Banerjee and co-workers that assembly of magnetic nanotubes is possible due to incorporation of isolated bacterial magnetosomes into peptide nanotubes [12]. In short, the application of Magnetic nanoparticles provides impressive demonstration towards bio and nanotechnological potentials. But still numerous fundamental questions have remained unsolved thus far; have prevented an application of magnetosomes at technical scale. Thus, this article concluded the production and applications of bacterial magnetosomes particles from the isolated strain (unknown) for their use in a number of applications.

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2. Mass Cultivation and Purification of Magnetosomes

For the production application of magnetosome particles, the large scale production of magnetic bacteria and magnetosomes were required and leads to the construction of BMs loaded drugs for medical application. For the biochemical and biophysical characterization of magnetosomes, huge amount of magnetosomes are required. MTB are fastidious organism and difficult to grow [13, 14]. Therefore, we seek to establish optimum conditions for the mass cultivation of the micro-aerophilic MTB in flask and fermented. The first step was the optimization of the medium to increase cell yield, magnetism, resulting in a defined medium in which high yield of cells and magnetosomes can be obtained at least costs. The MTB, isolated from “Ketham” lake located in Agra, UP, INDA; identified as it produces magnetite only under microaerobic condition whereas higher oxygen concentration act as growth inhibitor and repress magnetite formation. However, we observed and increased mutability within the Magnetosome Island if cells were grown at higher oxygen levels leading to the irreversible loss of the capability to form magnetosomes [13]. For the initial growth experiments, the MTB cells were cultured in flasks under microaerobic gas mixture containing 99% nitrogen and 1% oxygen [15]. These conditions are limited only of short duration for growth experiments and large-scale cell production because the oxygen supply during the incubation period. In fact, the cellular respiration decreases the oxygen partial pressure and due to this, the metabolic activity shifts from microaerobic to anaerobic growth and reduction in growth rate. To overcome the problem, a protocol for mass cultivation of MTB was established, which allows the continuous maintenance of low O_2 concentration [15]. A Dual vessel “BIOSTAT” Bioreactor was used for the microaerobic cultivation of MTB under appropriate oxygen condition .This was done by associating high oxygen amplifier and assembly equipment for gas supply. The regulation of low oxygen partial pressure was control via separate and independent gassing with nitrogen and air .The flow meter used to controls the nitrogen and the air supply was regulated by thermal massflow controller and pulsed solenoid control valve. The flowmeter was installed with pulsed solenoid gas valve [16]. The actual partial pressure within the medium facilitates the switching between oxygen and nitrogen gassing. The oxygen partial pressures above 0.5% switch the nitrogen into the medium at constant rate and the pressure below 99.5%. The aeration rate was regulated via the mass flow controller and the pulsed solenoid value. This oxystat fermented has been used to determine optimal oxygen partial pressure to produce and cultivate MTB Isolated from “ketham” lake. The magnetosomes synthesis within the MTB through above discussed procedure was purified by a proper technique which was separated by ultra-centrifugaration into a 55% [W/w] sucrose cushion after disruption by french press and centrifugation of magnetosomes to separate it from the crude extracts through magnetic separation columns [17].This procedure results in suspensions of purified magnetosome particles with intact enveloping membrane structure. The mild detergents do not affect the isolated magnetosomes; that is the isolated magnetosomes are relatively stable in mild detergents. But strong detergents like “like 1% SDS” Solubalise the MM at high temperature (95°C) and results into the agglomeration of membrane free magnetite particles [17, 18].

3. Strategies and types of Drug loading onto BM’s

The modification of BMs are very easily with diverse bioactive molecules due to the abundance of primary amino groups on the surface of magnetosomes membrane and chimeric proteins displayed specifically on the surface of genetically engineered isolated magnetosomes [19]. Most probably, the two most popular strategies are employed to construct the drug loaded BM’s they are (i) Direct drug loading onto BM’s with Dual Functional Linkers. (ii) And indirect drug loading onto BM’s after modification of BM’s and/or Drugs. The chemotherapeutic drugs which contain one or more then amino group per drug molecules are prepared through Direct drug loading onto BM’s Doxorubin, Apiubicin, Daunorabicin , and Pirarubicin contain one amino group per molecule while Betomycin and Peplomycin contain Multiamino group per molecule BMs can be linked to these drugs by the homobifunctional cross-linking agents such as aliphatic binary aldehyde, disocyanates, di-isothiocyanates, di(succinmido) aliphatic esters and there derivatives[20]. Drugs with carboxyls or phosphate groups also can be linked to the amino group of BM’s by using EDC (1Ethyl-3-[3-dinmethyyllam–inopropyl] carbodiimide).

Indirect Drug loading onto BM’s involves the loading of drugs without amino or carboxyl groups cannot be directly linked to BMs through the previously maintained methods. Such type of drugs are loaded to BM’s by three ways through attaching an amino or carboxyl group to the drugs by modification of the drugs, modifying the BMs so that they can be linked with bi-functional reagents. Drugs with sulphydryl or disulfide bond can be linked to BM’s with modified SPSP (N- sucinimidyl-3-[2pyridyldithio] Propionate and then reduced with DTT [Di- thiothetol] [7, 21]. Drugs with primary amino groups can also be loaded onto BM’s with this indirect method. For other drugs without amino group, sulphydryl or disulfide bonds, drug loading onto BM’s can be achieved by introducing one of these residues into drug and then using the strategies mentioned above. There were some another method to load drugs onto BM’s. One of them is by linking BM’s with macromolecules which are loaded with numerous small drug molecules [22]. Such macromolecules include poly-Glu, poly-bys, poly-Asp, polyethylene glycol and dextran. Poly-L-glutamic acid (PLGA) is a polymer of amino acids which contains multicarboxy group and only one single amino group, the single amino group of PLGA can be masked with a thiol group first to form PLGA – 3-[2-pyridyldithiol] propionyl (PLGA- PDP). With the help of EDC, PLGA-PDP react with small drug molecules carrying amino groups such as doxorubicin (DOX) and form PDP-PLGA (DOX)n which is next react with the BM’s modified with SPDP and DTT and PLGA- (DOX)n can be loaded onto BM’s [19]. The drug loading onto BM’s were also possible without additional chemical reaction because BM’s had a negatively charged surface could be modified with cationic silane such

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as isothiouronium chloride, 3-aminopropyltriethoxysilane. The BM's with modification shows cationic surface and may absorb nucleic acid drug [3,23].There are four major groups of drugs that are able to be loaded onto BM's they are – (i) Protein drugs (ii)Nucleic Acid Drugs; (iii)Radioactive Isotopes (iv) Chemotherapeutic Drugs.

Protein drugs are of all shape and sizes; recombinant human proteins such as Insulin, Growth Hormones and Erythropoietin; Monoclonal Antibodies such as Remicade, Rituxan and Erbitux; viral or bacterial proteins used as vaccines to elicit a specific immune response [24]. These protein drugs have some drawback as it fail to deliver in one or more target areas because they are digested or disrupted very rarely during the process of crossing biological barriers such as organs, cells and intracellular compartments. Therapeutic anticancer antibodies suffer from poor curative affect against solid tumors. To solve the above problem related to protein drugs can be done by loading antibodies onto BM's and maintained in the solved tumors with magnets. This was first done by T.Matsunaga in 1987. He with the coworkers successfully immobilized glucose Oxidase and immobilized on BM's was increased to 40 times. Glucose Oxidase and urease coupled with BM's retained their activities when they were again and again five times [6]. Nucleic acid such as DNA and RNA act as drugs through different mechanisms such as binding with the synthesized proteins and hybridizing to a messenger RNA that leads to translation altering or inducing degradation of target RNA. This process facilitate as the drugs for gene expression and regulation. BM's were reported for DNA and RNA extraction and gene delivery and detection [3, 4, and 19]. BM's have a negative charged surface and a membrane that contains 25% phosphatidylethanolamina and can absorb much lower nucleic acid directly. Matsunaga's group modified BM's with cationic silanes such as N-isothiouronium chloride, 3- Aminopropyltriethoxysilane, and 3-[2-(2-aminoethyl)-ethylarino]propyltrimethoxysilane. The DNA binding efficiency of the modified BM's increases with the number of amino groups presented. On the silane compound and was 14 fold higher than that of untreated BM's [3]. They have developed much better methods of direct formation of a cascading hyperbranched polyamindoamina-dendrimer onto the surface of amino silane modified BM's [3]. Radiotherapy can be used to treat cancer, using radiation to destroy or weaken particular targeted cells, such as X- rays, Y- rays, particle beams, protons or neutrons [25, 26]. Radioactive isotopes such as 99mTc, 131T, 123T and 111In can be linked to BM's with suitable chelates, radioactive labeled molecules such as nucleic acid and proteins and by including the radioactive isotopes in culture medium during BM's formation. These BM's shows advantages in internal radiation or brachytherapy of soild tumors due to their targeted delivery. In addition of this, the majority of chemotherapeutic drugs can be divided into alkylating agents, antimetabolites, anthrocyclines, plant alkaloids, topoisomerase inhibitor and other anti-tumor agents [27]. All these drugs affect cell division or DNA synthesis mostly chemotherapeutic drugs suffer from the inability to accumulate selectively at the site of action. BM's membrane contains an abundance of primary amino group which can be modified and /or linked with chemotherapeutic drugs by various strategies. *Sun.et.al.* provide an effective method of loading doxorubicin (DOX) onto BM's with Glutaradehyde and explored the clinical potential of magnetosome as drug carriers in target therapy of caner [28]. The nanoparticles with super-paramagnetic property are used as magneto-pharmaceuticals for diagnostic purpose. They serve as contrast agents in MRI [Magnetic resonance imaging] to outline the contrast between normal and diseased tissue or to indicate the status of an organ. Magnetosomes based ferrofluids facilitate the Magnetic Resonance Tomography contrast agents, which can be deleted at very low concentration in clinically employed MR tomography. *Herborn.et. al.* shows that magnetosomes are an expedient alternative to synthetic ferrofluids [29]. Another promising application for magnetosomes might be the treatment methods for hyperthermia in which the heating of tissues is controlled to promote cell necrosis in tumors [30]. From previous studies by Herget and co workers found the exceptionally high specific powers losses, which substantially overtake the result obtained with artificial particles in respect to biomedical applications such as hyperthermia and the rmablation [31].

4. Conclusion and Discussion

Magnetosomes formation in MTB provides a novel magnetic a nanoparticles that generated by a mineralization process with control over the morphology, size intracellular location of magnetic minerals and chemical composition. In summary this research article is contributed to a greatly improved knowledge about the production, loading of drugs onto BM's and its application. Here we highlighted most of the recent knowledge about the magnetosome with respect to biomedical treatments. Beside the establishment of techniques for the improved handling and cultivation of MTB in laboratory and strategies of Drug loading onto BM's and types of drugs employed for treatment by utilizing Magnetic-nanoparticles. BM's are shown to be excellent magnetic nano-carrier for antibodies, enzymes, ligands, nucleic acid and chemotherapeutic drugs. It has been reported that functionalized magnetosomes could be used for DNA/ RNA recovery, ELESA, cell sorting, target therapy of cancer, gene delivery and as a contrast agent in MRI and cellular markers for gene expression. These findings indicated that it will be very easy to develop multifunctional magnetosomes for clinical applications. For instance, pre-modification of magnetosomes with anticancer drugs can be linked with radioactive isotope labeled antibodies and can recognize Carcino-embryonic antigens. The multifunctional magnetosome can simultaneously be used as molecular probes for tumor detection with MRI and as targeting drug carries for tumor chemotherapy and radio-immunotherapy combined with magnetic hyperthermia. Likewise, the biochemical composition of MM may be altered in vivo by genetic engineering. A highly attractive and promising approach will be the design of magnetosomes with functionalized surface. This can be achieved for instance by generation of chimeric protein that display on the surface of isolated magnetosomes, or by the magnetosomes and bio-molecule conjugation based on chemical composition. BMs have great potentials for the preclinical and clinical applications.

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References

[1] Bazylinski D.A and Frankel R.B. Nature Review.Microbiol.2, pp 217, 2004.
[2] Yoza B, Arakaki A, Matsunga T.J Biotechnol, 101, pp219, 2003.
[3] Yoza B, Arakaki A, Maruyama K, Takeyama H and Matsunga T. Journal of Bioscience and Bioengineering, 95, pp21, 2003.
[4] Soda K, Kudo S, Sakaguchi T, Nakamura N and Matsunga T. Biotechnol. Tech, 7, pp688, 1993.
[5] Nakamura N, Matsunga T, Okochi M and Takeyahia N.Biotechnol, 75, pp400, 2004.
[6] Matsunaga T and Kamiya S .Applied Microbiology and Biotechnology .26. pp328.1987.
[7] Nakamura N and Matsunaga T.Analyt. Chem. Act281, pp585, 1993.
[8] Yoshino T, Takahashi M, Takeyama H, Okamura Y, Kato F and Matsunaga T. Applied and Environmental Microbiology, 70, pp2880, 2004.
[9] Tanaka T, Takeda H, Ueki F, Obata K,Tajima H, Takeyama H, Goda Y, Fujimoto S and Matsunaga T. Journal Of Biotechnology, 108, pp153, 2004.
[10] Kuhara M, Takeyama H, Tanaka T and Matsunaga T. Analytical Chemistry, 76, pp6207, 2004.
[11] Amemiya Y, Tanaka T, Yoza B and Matsunaga T.Biotechnol , 120, pp308, 2005.
[12] Banrejee IJ ,Yu L, Shimam, Yoshino T, Takeyama H, Matsunaga T and Matsui H. Advance Mater, 17, pp1128, 2005.
[13] Ullrich S, Kube M, Schubbe S, Reinhaedt R and Schuler D .Bioteriol, 187, pp7176, 2005.
[14] Schultheiss D, Kube M, and Schuler D. Applied Environmental Microbial, 70, pp3624, 2004.
[15] Heyen U and Schuler D. Applied Microbial and Biotechnology, 61, pp536, 2003.
[16] Lang C and Schuler D. Microbiol and Bionanotechnology: Biological Self- Assembly System and Biopolymer-Based nanostructures Rehm B editor Wymondliam Narfolls UK Horizon Scientific press 107, 2006.
[17] Grunberg K Muller EC, Otto A ,Reszka R, Linder D, Kube M,Reinhardt R and Schuler D. Applied Environmenteal Microbiology, 70, pp1040, 2004.
[18] Grunberg K, Wawer C, Tebo BM and Scholar D. Applied Environ.Microbiol, 67, pp4573, 2001.
[19] Matsunga T, Sato R, Kamiyya S, Tanaka T and Takeyama H. Journal of Magnetism and Magnetic material, 194, pp126, 1999.
[20] Harmanson G. Bioco. Tech. Academic Press, New York, USA, 2_{nd}edition, 2008.
[21] Nakamura N, Burgess JC, Yagiuda K ,Kudo.S, Sakaguchi T and Matsungat T. Analytical Chemistry, 55, pp2036, 1993.
[22] Yoza B, Masumoto M and Matsunga T .Journal of Biotechnology, 94, pp217, 2002.
[23] Tamilonson T.M. Nature Biotechnology, 77, pp914, 2004.
[24] Boswell C.A and Brechbiel M.W. Nuclear Medicine and Biology, 34, pp757, 2009.
[25] Anil Kumar A.V.S, Kumar P.G and Shankar S. Indian Journal Of ....4.2, pp51, 2009.
[26] Minev .B.R. Biological Therapy .Springer. First Edition, 2011.
[27] Sun J.B, Duan J.H, Dai SL. Biotechnology and Bioengineering, 101, pp1320, 2008.
[28] Hartung A, Trost R, Lisy R, Hilger I ,Kaiser Wa and Reichenbach J.R. Scientific and Clinical Application of Magnetic carrier Krems, Australia, 2006.
[29] Hilger I, Hergt R and Kaiser Wa. Investigative Radiology, 35, pp170, 2000.
[30] Hergt R, Hiergt R, Zeisberger M, Schuler D, Heyen U, Hilger I and Kaiser Wa. Magnetism Magnetic Materials, 293, pp70, 2005.

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