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REVIEW PAPER

Magnetic nanobeads: Synthesis and application in biomedicine

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ABSTRACT

Nanobiotechnology appears to be an emerging science which leads to new developments in the field of medicine. Importance of the magnetic nanomaterials in biomedical science cannot be overlooked. The most commonly used chemical methods to synthesize drugable magnetic nanobeads are co-precipitation, thermal decomposition and microemulsion. However monodispersion, selection of an appropriate coating material for *in vivo* application, stability and unique physical properties like size, shape and composition of nanobeads remain unsettled challenge. The use of hazardous reagents during chemical synthesis is another impediment for *in vivo* application of the magnetic nanobeads. The current minireview put forth the pros and cons of chemical and biological synthesis of magnetic nanobeads. We critically focus on chemical and biological methods of synthesis of the magnetic nanobeads along with their biomedical applications and subsequently suggest a suitable synthetic approach for potential biocompatible nanobeads. Biogenic synthesis is proposed to be the best option which generates biocompatible nanobeads. Reducing enzymes present in plants, plant materials or microbes reduce precursor inorganic salts to nano sized materials. These nanomaterials exhibit biomolecules on their surface. The use of biologically synthesized magnetic nanobeads in diagnostics and therapeutics would be safe for human and ecosystem.

Keywords: *Bio-synthesis, Biomedical application, Chemical synthesis, Magnetic nanobeads, Nanobiotechnology*

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INTRODUCTION

Nanobeads have been widely used in biomedical research for two decades. It is not only the unique surface properties of nanobeads but also the nano-scaled size which make them fabulous. Various biological and chemical methods have been used to synthesize the nanobeads [1].Magnetic and non-magnetic nanobeads have been extensively investigated regarding their usage in biomedical devices and techniques like magnetic resonance imaging (MRI) [2], magnetic cell sorting [3], magnetic cell separation systems [4], protein purification [5] and targeted drug delivery [6]. These varied technological applications of nanobeads have instructed their significance and safety in the fields of biology and medicine. Metallic nanobeads depict magnetic nature. Frequently used metallic materials for the synthesis of magnetic nanobeads are magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) which are superparamagnetic below 20 nm in size [7]. Around 30-100 nm sized nanobeads behave as paramagnetic materials [8]. Various size controlling protocols have been tested previously [9]. Metals like iron (Fe), cobalt (Co) and nickel (Ni) have also been used to synthesize magnetic nanobeads for various applications in biomedical research.

Despite vast usage of magnetic nanobeads, various limitations have also been noticed. One of the major limitations is the aggregation of magnetic nanobeads

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which hinders their unique physical properties at nano scale [10, 11]. However, it can be resolved using additives during synthesis of magnetic nanobeads. Additives form a coat and give a single particle suspension (monodispersion) which keeps the nano scale intact [12, 13]. Different coating materials like phospho-ethylene glycol (PEG), starch or silica are being used in synthesis of monodispersed particles [14].Magnetic nanobeads can be conjugated with various distinct structures including iron oxides (Fe_3O_4 and Fe_2O_3) [15, 16], un-adulterated metals (Fe and Co) [17, 18], spinel-sort ferromagnets ($MgFe_2O_4$, $MnFe_2O_4$, $CoFe_2O_4$) [19, 20] and combinations ($CoPt_3$ and FePt) [21, 22]. In recent decades, research has been focused on conjugated magnetic nanobeads.

Various formulations of drug—conjugated [23], antibodyconjugated [4] and nucleic acidconjugated magnetic nanobeads [24] are used in biomedical research. In summary, entire research (chemical and biological synthesis) related to nanobeads is focused on modifications to control the size, shape, stability, and monodisperseion of magnetic nanobead. Several methods including co-precipitation, thermal decomposition or reduction, micelle synthesis, hydrothermal synthesis and laser pyrolysis techniques are directed to synthesize high-quality magnetic nanobeads. This minireview focuses on chemical and biological methods of synthesis of magnetic nanobeads and their biomedical applications. We present representative XRD micrographs of nanobeads of magnetite (iron oxide), strontium (Sr), and Ni synthesized by chemical or biological methods. The critical analysis of these methods helps the readers to choose a better approach to synthesize magnetic nanobeads for biomedical application.

Chemical Synthesis

Co-precipitation

Co-precipitation is a simple technique where ferric and ferrous ions are mixed at 1:2 molar ratio in basic solution. Co-precipitation is used to synthesize nanobeads like iron oxide (Fe_3O_4 or γ-Fe_2O_3) from an aqueous solution of salt of different concentrations at variable temperatures (at or above 25 °C).

The size, shape and structure depend on the type and concentration of salt used at specified pH and temperature [25]. Keeping these factors in focus, the protocol for synthesis of magnetic nanobeads can be optimized for biomedical application.

The magnetic index for magnetite nanobeads synthesized by co-precipitation is mostly found to be 30-50 $emug^{-1}$. Magnetite nanobeads are not exclusively stable.

They are effectively oxidized to maghemite or broken down in an acidic medium [26, 27]. Since maghemite is a ferrimagnetic, it does not oxidize [28].

Maghemite nanobeads are stable in alkaline or acidic medium [29]. Iron oxide nanobeads synthesized by co-precipitation method from $Fe(NO_3)_3$ showed characteristic XRD pattern corresponding to their Miller indices as shown in Fig. 1a.

The oxidation of magnetite into maghemite can be ruled out by providing non-oxidizing environment after the synthesis of nanobeads. The aggregation of magnetite nanobeads, however, remains an enigma due to their magnetic properties and tendency to attract and combine. This limitation leads to false positive calculation of the size of nanobeads and also affects the downstream bio-medical application. Nanobeads synthesized by co-precipitation show the tendency to aggregate, which causes the loss of monodispersion character and results in polydispersion. However, generation monodispersion phase of nanobeads is essential for its biomedical applications [30]. Kinetics of co-precipitation reactions are very fast. It does not allow controlling the size and distribution of nanobeads in the medium. Various efforts to control the size and the monodispersion phase of nanobeads are made by using different coating molecules. For instance, 1% polyvinlyalcohol (PVA) is used to monodisperse the magnetite nanobeads of smaller than 10 nm size. Nonetheless, while using PVA containing 0.1% carboxyl as a settling agent, magnetite nanobeads precipitate as chain-like bunches [31].

Thermal decomposition

The materials having semi conduction potential (e.g. Sr) can be converted into nanobeads in their native solid state. Different optimized protocols (hot-injection or conventional reaction) for thermal decomposition have been developed [14, 15]. The size and shape of nanobeads can be controlled by exposure at different temperatures for different time periods. Monodispersion phase can be achieved by coating of nanobeads with surfactant molecules [16, 17]. For the zero valent magnetic meterials [iron (0) pentacarbonyles], thermal decomposition yields

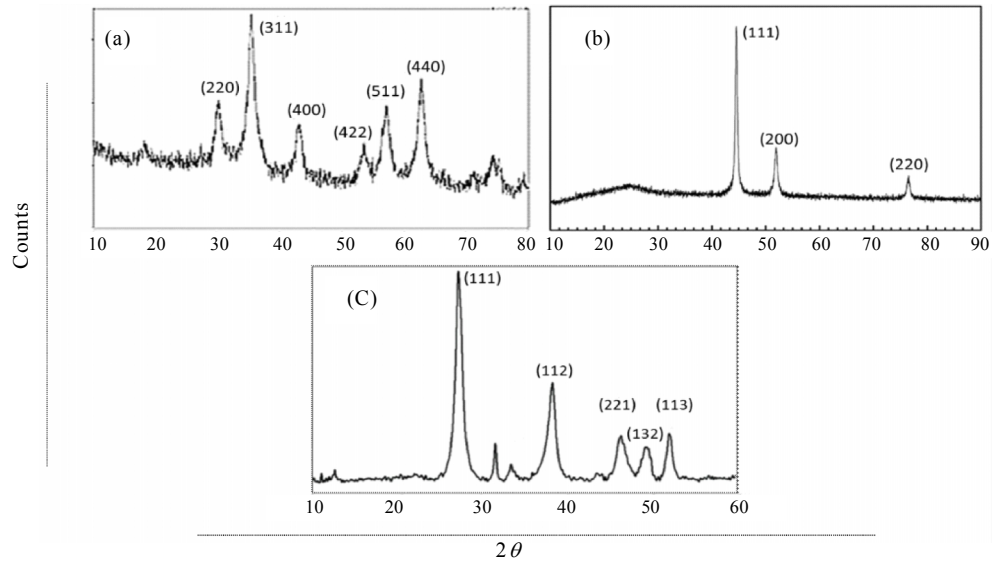

Fig. 1. XRD micrograph of nanobeads. Chemically synthesized nanobeads of (a) iron oxide, (b) strontium and biologically synthesized nanobeads of (c) nickel exhibit characteristic peaks which are corresponding to their Miller indices

metallic nanobeads. By using thermal decomposition, nanobeads can be synthesized in two steps. Organic salt (containing carbonyl group) is decomposed at high temperature into constituents followed by synthesis of nanobeads. For instance iron pentacarbonyl can be decomposed into a mixture of octyl-ether and oleic acid at 100 °C in the presence of trimethylamine oxide (an oxidant) which yields nanobeads (Fe_2O_3) of around 13 nm size [32]. Monodispersion phase can be achieved by mixing starch or PEG while preparation.

Synthesis of amorphous cobalt nanobeads by thermal decomposition of carbonyl salt has been achieved. Ishikawa, Yang and colleagues reported the combination of cobalt nanodiscs by thermal decomposition of cobalt carbonyl [33, 34]. Cornell and colleagues described the aggregation of cobalt [35, 36] and Ni nanorods [37] by thermal decomposition of non-carbonyl organometallic structures.

However thermal decomposition has some discrepancies. Temperature used to decompose a salt is high enough to denature it instead of its conversion to nano sized particles. It may lead to loss of resources and information. Pre-treatment of precursor salt like $Sr(NO_3)_2$) with a base (NaOH) makes it decomposable at reduced temperature upto 200°C. Otherwise, $Sr(NO_3)_2$ decomposes at 600-700°C.

A representative XRD micrograph of Sr nanobeads synthesized by thermal decomposition of Sr $(NO_3)_2$ is shown in Fig. 1b.

Microemulsion

Microemulsion is thermodynamically stable isotropic mixing of two immiscible fluids, where the micro domains of fluids are balanced by an interfacial film of surfactant molecule [38]. In water-in-oil microemulsions, the aqueous phase is scattered as micro droplets (1–50 nm in diameter) coated by a monolayer of hydrophilic surfactant molecules. Hydrophilic surfactant interacts with aqueous phase and makes it a continuous phase while oil droplets are emulsified and *vice versa*. The extent of micelle formation is dictated by molar ratio of water to surfactant [39]. By mixing two indistinguishable water-in-oil microemulsions where one contains the metallic salt and other contains a ruductant, the micro droplets are perpetually broken down into micelles [40]. Random collision among the micelles results in exchange of reductant and metallic ions leading to the formation of metallic nucleus. Recovery of nanobeads from the microemulsion is a big challenge. Separation of continuous phase from emulsified micro droplets is another problem of microemulsion. Addition of polar organic compounds for example ketone or ethanol to

the microemulsions helps in separating the phases. Ultracentrifugation can also be used to recover nanobeads. In this sense, a microemulsion acts as a nano-reactor for the synthesis of nanobead.

Magnetic nanobeads have also been synthesized through microemulsion technique. The size and shape of nanobeads can be controlled by adjusting molar ratio of water to surfactant or reductant to precursor salt. Yields of nanobeads are dependent on kinetics of the micelles. Collision of micelles dictates the exchange rate of reductant to precursor salt between the micelles. Higher the exchange rate, higher will be the yield and *vice versa*.

Bioreduction

Despite chemical synthesis, biological materials have also been used to synthesize nanobeads. Reducing enzymes present in biological materials are used to convert a salt to corresponding metals or its oxides. The major objective of biological synthesis is to make the nanobeads safe and biocompatible for *in vivo* applications. Biological synthesis corresponds to green nanobiotechnology which is safe for human and ecology [41, 42].

Plant Materials

Physical, chemical and biological methods are employed now a days to produce nanobeads [42]. Different physiochemical methods, for instance, laser ablation [43], photochemical method [44], chemical reduction [45] and c-radiation [46] are used to synthesize nanobeads [47, 48]. However these methods exhibit limitations. Physical methods require continuous maintenance of high temperature, pressure, energy [49], expensive equipments and high vacuum technology [50, 51]. Chemical Methods include chemicals like starting materials, reactants and solvents which are mostly toxic and have serious concerns to environment and biosphere [52-54]. In addition, capping agent is needed to prevent agglomeration of the particles due to high surface reactivity [50, 51]. Production of toxic by-products is another drawback of chemical methods [55]. Stability and safety of nanobeads synthesized by chemical methods in biological systems is a point of vital interest. Hence, for the safe application of nanobeads particularly in medicine, an alternate biocompatible method is required [53, 56]. Green synthesis or biological method provides an attractive alternate strategy, particularly for medicine. Biological synthesis is eco-friendly [55, 57, 58], cost-effective and does not require high temperature, pressure, energy or toxic chemicals that may produce adverse effect in living system [50, 51]. Biological method produces large amount of nanobeads which are contaminant—free having well-defined size and shape [59]. [60]. A representative XRD pattern of Ni nanobeads synthesized by biological method, using peal of *Punica granatum,* is shown in Fig. 1c.Nanobeads are synthesized by a variety of biological methods. Biological materials like micro-organisms [61, 62] , whole plant, plant tissues and fruits, plant extracts and marine algae [63-65] are used to reduce the metallic salts [49, 55, 63, 65-76]. For the last three decades, plants or plant extracts have preferably been used to produce nanobeads for various biological or biomedical applications. Safety and simplicity of biogenic methods have increased its reliability and compliance [69, 70, 74, 77-86]. Use of microorganisms as a source of reducing enzymes to produce nanobeads is pretty expensive. To handle microorganisms in the lab is another issue in terms of biosafety and economy. Plants and their extracts are hazardous free, reliable, easy to handle and produce large biomass [53, 62, 63, 67] as compared to microorganisms [78, 79, 81, 84, 85]. Plant extracts exhibit dual properties during synthesis of nanobeads. It conducts reduction of precursor metallic salt into corresponding metals or metallic oxide as well as stabilization of nanobeads. Medium of extraction like methanol, ethanol, phenol or water affects the properties of nanobeads [84]. Methanolic, ethanolic, phenolic or aqueous extracts possess variable quantities of reducing agents which affect the downstream physical properties of nanobeads [87]. Aqueous plant extract is mixed with aqueous solution of metallic salt at room temperature for a typical plant extract mediated bioreduction. The reaction generally completed within few minutes [88]. Recovery of the nanobeads is also easy. Nanobeads synthesized by a biogenic method are naturally stabilized by the organic compounds present in the extract. These organic compounds make a protective coating on the surface of nanobeads, hence, they do not need further functionalization or stabilization. Despite plant extracts, biological waste materials like pomegranate peel, green tea leaves and grass leaves possess a significant quantity of reducing agents.

They are used to reduce metallic salts into corresponding metals or metallic oxides. Biogenic methods would also be suggested as a part of waste management strategy.

Microbes

Despite plant materials, microorganisms like *Geobacter metallireducens, Magnetospirillum gryphiswaldense, Bacillus subtilis and Actinobacter sp.* are involved in bioreduction of precursor salts into nano sized materials. Most of the microbial enzymes work in anaerobic environment to synthesize nanobeads. However, *Actinobacter sp.* is reported to produce stable superparamagnetic maghemite nanobeads in aerobic condition. Despite the ease of green production, biological methods warrant further investigation to understand underlying mechanisms of reduction and size control.

Applications in Biomedicine

Biocompatibility, long retention time in the blood, safe excretion and low toxicity make the nanobeads suitable for biomedical application. Magnetic nanobeads have extensively been used *in vitro* or *in vivo* as a drug career in the fields of biotechnology and biomedicine. Magnetic separation is a basic technique used to purify a homogenous population of biological cells or molecules from a heterogeneous mixtures. Nano scaled magnetite or maghemite particles coated with a polymer shell and conjugated with dopamine are being used to purify proteins [89]. Dopamine coordinatively changes the surface chemistry of magnetic nanobeads to octahedral geometry. Dopamine, as a bidentate enediol ligand, binds tightly to the surface of unsaturated magnetic nanobeads. [90]. Magnetic nanobeads are perfect molecular transporters for efficient separation [91]. Tan and colleagues have integrated a geno-magnetic nano-capturer (GMNC) for the collection, separation, and detection of minute quantities of nucleic acids having point mutations [92]. GMNC was prepared with a magnetic core, coated with silica to ensure biocompatibility, and avidin-biotin particle as a linker to conjugate a molecular reference for a sample. It is established that GMNC is efficient in separating the trace amounts of mutated DNA or mRNA. Magnetic activated cell sorting (MACS) and fluorescence activated cell sorting (FACS) are based on the antibodyconjugated superparamagnetic nanobeads. These techniques are gold standards of cell separation. Superparamagnetic nanobeads conjugated with monoclonal antibodies are used to isolate specific type of cells from a heterogeneous mixture. For stem cell therapy, MACS is used to isolate hematopoietic stem cells from human umbilical cord blood or bone marrow. Superparamagnetic nanobeads are coated with anti-CD34 or anti-CD133 antibodies, which bind to the cognate molecule on the surface of target cell and capture it through its magnetization in an external magnetic. Another fascinating use of magnetic nanobeads, as a drug carrier (nanomedicine), have made it possible to achieve targeted drug delivery at diseased tissue [93]. Magnetic nanomedicine is guided under the influence of external magnetic field to the target site. The required therapeutic level of drug can be achieved through the targeted concentration of nanomedicine. Application of nanomedicine to treat variety of diseases seems safe and reliable. Undesirable effects, reported with conventional drug delivery systems, can be minimized by optimizing the dosage and strengthening external magnetic field. Despite the successful usage of nanomedicine *in vivo*, more clinical trials with huge number of subjects are needed to justify its significance. Magnetic nanobeads are used in diagnostics and therapeutics. Polymer coated magnetic nanobeads (core-shell type) are used in hyperthermia and MRI. To treat the tumors, provision of high temperature matters a lot to kill the target cells. Hyperthermia, a supplementary treatment to chemotherapy, radiotherapy or surgery, is achieved through magnetic nanobeads at the target site [94, 95]. Tumor cells are temperature sensitive. Tumors are exposed to magnetic liquid (ferrofluids) and strong external magnetic field is applied which generates high temperature and destroys the cells. The amount of heat generated depends on the size and shape of magnetic nanobeads. Methotrexate-coated nanobeads are used as a chemotherapeutic agent against tumor cells which overexpress folate receptors on their surface. Controlled drug delivery through nanobeads is currently in juvenile stage of human trials. Underlying mechanisms of cytotoxicity, safe excretion and immunological profile of nanobeads need to be investigated.

CONCLUSION

Although the chemical method is quick and produces a substantial amount of magnetic

nanobeads, however, hazardous reagents used in chemical synthesis make them non-drugable. Biological synthesis of magnetic nanobeads makes them biocompatible, drugable, safe and eco-friendly. Despite all fascinating biomedical applications of magnetic nanobeads, the challenges remain to be addressed in clinical applications. Multifunctional, biocompatible, eco-friendly and stable magnetic nanobeads would be the focus in basic research and clinical biomedicine.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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