Advances in Bioresearch
Adv. Biores., Special Issue (2) 2025: 55-60
©2024 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585. SPL2.25.5560

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A Critical review on nanotechnology in treatment of Thalassemia

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ABSTRACT
Nanotechnology has become a significant research focus due to the unique properties of nanomaterials. Managing iron ion intoxication is crucial in treating thalassemia and anemia linked to regular blood transfusions. Given the side effects of existing medications, safer alternatives are needed. In developing countries, Hematopoietic Stem Cell Transplantation (HSCT) is gaining attention as a promising cure for thalassemia, despite risks. Gene therapy, eliminating the need for a donor, poses risks like vector toxicity and tumor formation. CRISPR/Cas9 offers precise genome editing but has off-target effects and ethical concerns. Managing hemoglobinopathies, especially thalassemia, is challenging due to genetic variations and symptoms. Traditional methods alleviate patient burdens, but advancements in nanotechnology have improved diagnosis and treatment. Nanoparticles enhance targeted drug delivery, gene therapy, and gene editing, significantly improving conventional therapies. Utilizing these diagnostic and therapeutic strategies can lead to more efficient and cost-effective methods for detecting and treating thalassemia. Accurate diagnosis and treatment criteria may pave the way for nano-based personalized medicines, potentially transforming the theragnostic approach to thalassemia and other hemoglobinopathies. This approach also simplifies target design and reduces implementation time.

Keywords :*Thalassemia, HSCT,hemoglobinopathies, Nanoparticles ,targeted drug delivery.*

Received 18.10.2024 Revised 28.12.2024 Accepted 22.02.2025

How to cite this article:
Ashly Merin G, Ashok J M, Hemalatha K, Dawn V T. A Critical review on nanotechnology in treatment of Thalassemia. Adv. Biores. Special Issue [2] 2025. 55-60

INTRODUCTION

Nanotechnology has been utilized since 1974, encompassing various nanoformulations such as nanomaterials, nanomedicine, nanovaccines and nanotheranostics. These are favored for their small particle size enhancing reactivity, solubility and efficiency. Nanoparticles offer benefits such as biocompatibility, safety, ability to cross the blood-brain barrier and other physiological barriers and effective targeting of intracellular and multi-drug-resistant pathogens [1].This review explores nanotechnology in thalassemia treatment, an inherited blood disorder caused by mutations in α-like and β-like globin genes. Advances in clinical and genetic research have led to near eradication in some countries through mass testing. From HLA-matched donors collected allogeneic Hematopoietic Stem Cell (HSCT)transplantation exhibits more than 90% transfusion-independent survival for thalassemia patients [2]. This treatment is most effective, if is carried out at younger age. Factors like HLA matching, age and iron overload mainly influences the disease-free survival rate.

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and the Cas system (CRISPRCas) ha ve transformed gene therapy for inherited genetic disorders. This powerful duo enables the precise target ing of single base insertions or deletions (Indels) in genomic DNA [3]. In their 2021 study, Frangoul *et al*. demonstrated the effective use of CRISPR to modify hematopoietic stem cells. By suppressing the B globin gene, they managed to diminish defective beta globin chains and boost the reactivation of fetal hemoglobi n (G globin protein) [4].This approach aims to alleviate anemia and reduce blood dependency in thalassemia patients. The trial showed promising results, with no reported discrepancies so far. Thalassemia treatment can be prohibitively expensive, costing around $1.6 billion which is often unaffordable for patients' families and healthcare providers.

Pathophysiological Pathways

During fetal development, α-globin and γ-globin are produced. Within a year, γ-globin is replaced by β-globin forming adult hemoglobin. Thalassemia arises from an imbalance in α-globin and β-globin production leading to toxic accumulation of excess globin chains. This causes reactive oxygen species formation thereby damaging red blood cells and leading to hemolysis and abnormal erythroid maturation[5].In β-thalassemia, genetic defects reduce β-globin production causing α-globin accumulation. This leads to functional defects, ineffective erythropoiesis, hemolysis, severe anemia, frequent transfusions, organ defects and bone deformities. In female β-thalassemia patients, those with wild-type α-globin(αα/αα)showed higher iron levels. In contrast, those with α-globin deletions had normal iron levels, suggesting that inheriting a deleted α-globin gene can reduce serum iron levels.

Treatment available of Thalassemia

The main health challenge for thalassemia major patients is iron overload, resulting from multiple blood transfusions [6].

To tackle this issue, patients are given iron chelating drugs which helps to prevent the buildup of iron in organs and tissues. It's a necessary step to manage iron levels effectively and avoid complications.

In thalassemia major and intermedia patients,severe anemia results from two main issues: the inadequate production of normal hemoglobin and the buildup ofαglobin chains. This combination disrupts the norm al process of erythropoiesiswhich is a tough condition to manage. These patients require regular blood transfusions, with the frequency varying from person to person. Regular transfusions help increase life expectancy by preserving organ function and reducing symptoms such as lethargy, laziness and fatigue[7].

Hydroxyurea is an effective, affordable drug that increases hemoglobin levels by activating the γ-globin gene, boosting fetal hemoglobin (HbF) production. This reduces the need for frequent blood transfusions in some thalassemia patients. In this process, two α-globin chains combine with γ-globin chains to form HbF substituting for defective hemoglobin[8].

Hematopoietic Stem Cell Transplantation (HSCT) is a widely available and successful treatment for certain conditions. Gene therapy such as ZYNTEGLO®, employs recombinant genes to offer therapeutic benefits. With ZYNTEGLO®, hematopoietic stem cells are transduced with vectors that encode the β-globin gene. Even though it has received conditional authorization, it still necessitates a 15-years follow-up to assess its long-term efficacy and safety [9]. Gene editing is an advanced technique for treating genetic disorders by correcting mutations in specific DNA sequences and restoring them to their normal state. This 12 [10].

To produce substantial amounts of fully functional hemoglobin, a largequantity of corrected hematopoieti c stem cells (HSCs) is essential. CRISPR/Cas9 can be used as a therapeutic aid in this endeavor.

Nanoparticles in the treatment of Thalassemia

Nanoparticles have demonstrated significant promise in overcoming challenges associated with macromolecular drugs such as poor permeability through biological membranes, short biological half-life, large size, high molecular weight and structural instability. Organic nanoparticles encompass polymeric micelles, polymeric nanoparticles, dendrimers, polymer drug conjugates and liposomes[11].Inorganic nanoparticles like carbon nanotubes, carbon nanofibres, gold nanoparticles, quantum dots, magnetic nanoparticles, nanographene and metal-based nanoparticles are paving the way in gene therapy, cancer treatment, biomedical imaging, tissue scaffolds, implantable materials, biosensors and drug delivery systems.These innovation are particularly promising for the precise diagnosis and treatment of thalassem ia, bridging the gap between cutting-edge science and tangible healthcare improvements[12].

Nanobased approaches are becoming quite the trend in healthcare diagnostics and therapy. These tiny engineered materials (1-100 nm) have special properties -electrical, chemical, magnetic, optical biological-that make them ideal for non-invasive, highly sensitive, reliable and cost-effective diagnostic problems [13]. Nanotechnology and nanomedicine are definitely changing the game in biomedical science. New biosensors and therapeutic strategies are emerging, particularly for hemoglobinopathies. Nanotechnology can tweak physical and chemical properties to tackle drug-related challenges like poor solubility or bioavailability. By altering how drugs interact with the body, these nanosolutions can lead to more effective treatment. Nanparticles are definitely game changer for macromolecular drugs tackling issues like less permeability, short half-life, large size, high molecular weight and instability issues. This innovative approach is being explored for more accurate diagnosis and treatment of thalassemia and other genetic disorders[14].Various nanoparticles used for treatment included in Fig.2.

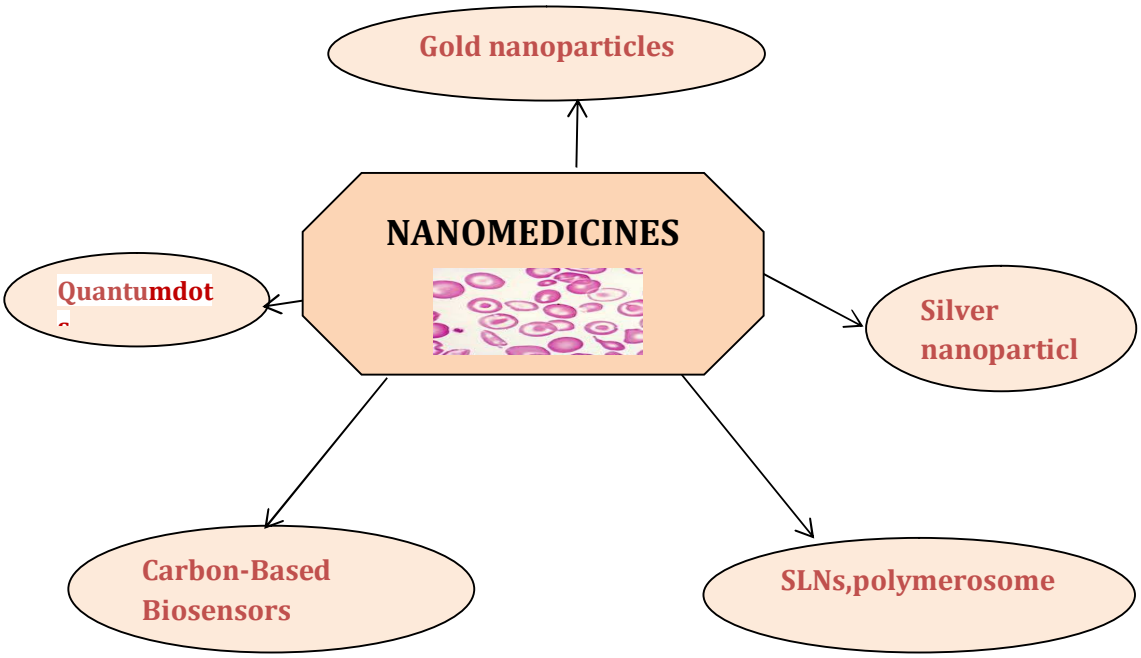

Fig.1: Nanomedicines used in the treatment of Thalassemia

Nanobiosensor for the Diagnosis of Thalassemia

Nanoparticles and nanosensors offers advanced, cost-efficient methods with high sensitivity for diagnosing thalassemia. This condition involves hemoglobin abnormalities causing anemia and oxidative stress which damages cells. Various methods to analyze hemoglobin includes colorimetric methods, fluorescence spectroscopy, specific gravity, Kurt electric resistance, spectrophotometry and electrochemical techniques. These approaches help in understanding and managing thalassemia more effectively[15].Among these, electrochemical analysis stands out as the most reliable method.

Electrochemical sensors have evolved with diverse levels of efficiency, significantly advancing diagnostics and treatments. Individuals with thalassemia experience anemia and other complications due to qualitative and quantitative defects in hemoglobin. This condition leads to lipid peroxidation and the formation of reactive oxygen species, causing oxidative stress that weakens antioxidant defenses and damages cells[16].Nanotechnology based diagnostics for thalassemia pinpoint mutations by targeting DNA involving optical nanocarriers with nanoechnology. Researchers have explored various nano structures -silver, gold, graphene, silica and quantum dots-for their diagnostic and therapeutic potential in hemoglobinopathies[17].

Quantum Dot-Based Diagnosis

Quantum dots (QDs)are tiny semiconductor nanocrystals with standout optical properties. A nano-diagnostic genotyping method uses QDs and magnetic nanoparticle-based probes to detect point mutations in the beta-globin gene. This non-PCR based technique boasts 85.45% sensitivity and 95.77% specificity for identifying thalassaemic mutations evidenced by shifts in fluorescence colour[18].

Researchers have designed a CdS/TiO2 nanocomposite-based molecularly imprinted photo-electrochemical sensor to detect hemoglobin using visible light. The sensor forms a heterojunction of CdS quantum dots with TiO2, increasing the photogenerated current through efficient charge transfer. Hemoglobin attachment decreases the photocurrent, enabling detection of conformational changes and disorders based on attachment patterns and fluorescence[19].

Gold nanoparticles, boasting unique chemical and physical traits, offer an exceptional platform for material and biological uses. Their operational prowess and colloidal stability make them invaluable in diagnostics and therapeutic applications [20]. Yi *et al* effectively used a combination of ligase detection reaction and PCR with a nanogold based universal array to pinpoint mutations in fetal DNA extracted from maternal plasma. They precisely identified the IVS2 654(C→T) mutation in the β-globin gene, a mutation in which results consistent with PCR/ reverse dot blot analyses of amniotic fluid cell DNA[21].

Silver Nanoparticles-Based Diagnosis:

Silver nanoparticles (Ag NPs) are utilized in biosensors for hemoglobin detection because of their stability and sensitivity. Ye *et al.* created molecularly imprinted polymers with Ag NPs/PbTiO3 electrodes for quantifying hemoglobin. Another study employed a quartz crystal microbalance with a silver electrode and biotinylated probe for speedy and patient friendly detection of α-thalassemia (SEA deletion)[22].

Carbon-Based Biosensors:

Electrochemical biosensors are effective in detecting hemoglobin in thalassemia. Xie *et al.* developed a Co3O4-doped carbon nanofiber composite with a carbon ionic liquid electrode to study hemoglobin's electrochemical behavior. Darabi *et al*. used a carbon paste-based electrode with ionic liquid and CdO-nanoparticle/rGO to measure vitamin C and deferoxamine in thalassemic patients, demonstrating high accuracy for both major and minor thalassemia[23,24].

Electrospun nanofibres with dye -intercalated DNA dendrimer probes(G3SG) enhance the fluorescent detection of nucleic acids, proteins and cancer cells. This technique can identify a 20 pM mutated beta-globin gene fragment causing thalassemia, thrombin and HeLa cells with high sensitivity. Additionally, NiTe nanorods based non-enzymatic sensors are used to measure hemoglobin levels in anemic pregnant women [25].

Nanotechnology for the Treatment of Complications of Thalassemia

Hemoglobinopathy-related problems, particularly anemia, must be treated in thalassemia patients. Frequent blood transfusions are typically used to achieve this, which results in secondary iron overload [26]. The liver's hepcidin–ferroportin combination maintains iron homeostasis. Hepcidin, a hormone produced in the liver, controls ferroportin, an iron export protein. In thalassemic patients, dysregulated iron metabolism suppresses hepcidin, significantly lowering the hepcidin-to-ferritin ratio. Iron overload from inadequate erythropoiesis, hepcidin suppression, and repeated transfusions damages key organs, ultimately leading to death [27].

Conventional iron chelation therapy, using FDA-approved drugs like deferoxamine, deferiprone, and deferasirox, reduces hazardous iron levels but can cause significant side effects on ocular, auditory, and renal pathways, especially at high doses [28].Nanoparticles offer various therapeutic benefits for treating hereditary and immunogenic illnesses [29].This rapidly developing field aims to overcome obstacles associated with traditional drug delivery methods. Researchers are exploring targeted drug delivery using nanoparticles for therapeutic purposes[30]. In both *in vitro* and *in vivo* studies, the effectiveness of deferoxamine, was compared with TLc-A, a new nanochelator developed using nanochelating technology[31].TLc-A showed superior efficacy in reducing iron overload in both iron intoxicated rats and the Caco2 cell line, emphasizing the effectiveness of nanochelating agents.A study on graphene oxide nanoparticles and the acquired protein corona during treatment regimens indicated that the effectiveness of nanochelating agents can vary among individuals[32].This suggests that personalized medicine, tailored to individual needs and immune responses, could be a beneficial approach.

Ali *et al*. investigated the hemolytic activity of cobalt ferrite (CoFe2O4) and nickel-zinc-iron oxide (Ni0.5Zn0.5Fe2O4) nanoparticles on human erythrocytes. They also studied the impact of these nanoparticles on albumin levels in the plasma of patients with β-thalassemia major. Interacting with these nanoparticles led to noticeable morphological variations in the erythrocytes [33].

In the treatment of thalassemia, Ni–Zn ferrite nanoparticles have shown greater efficacy compared to cobalt ferrite nanoparticles[34]. In a study with thalassaemic rats, silver nanoparticles developed with the tannin fraction from Myrtus communis extract (MC-AgNPs) effectively chelated iron, significantly reducing excess iron levels[35,36].

Ergün *et al*. developed Fe3+ imprinted beads embedded in cryogens, successfully chelating iron from the plasma of β-thalassemia patients. Capretto *et al*. encapsulated the DNA-binding drug mithramycin (PM-MTH) in polymeric micelles using microfluidic technology, resulting in better control, reproducibility, smaller size, reduced toxicity, and lower polydispersity. This drug may enhance γ-globin expression, increasing HbF levels and alleviating β-thalassemia symptoms [37]. Episomal vectors based on the scaffold/matrix attachment region (S/MAR) were created for episomal retention and β-globin replication, effectively producing γ-globin in hematopoietic stem cells. This technique was successfully tested in both mouse models and human hematopoietic stem cells [37].

CONCLUSION

Regular blood transfusions treat thalassemia but risk iron buildup. Hematopoietic Stem Cell Transplantation (HSCT)is gaining attention as a potential cure, despite risks. Gene therapy, removing the

need for a donor, faces challenges like vector toxicity and tumor formation. CRISPR/Cas9 promising for genetic diseases offers precise genome editing but has off-target effects and ethical concerns. Managing thalassemia is challenging due to genetic variations and symptoms. Traditional methods alleviate patient burdens, but advancements in nanotechnology have improved diagnosis and treatment. Nanoparticles aid in targeted drug delivery, gene therapy, and gene editing, achieving iron chelation, increased HbF levels, reduced hemolysis and gene correction. New cost-effective approaches are needed to reduce patient burdens. Leveraging advanced diagnostic and therapeutic strategies can lead to efficient, affordable methods for detecting and treating thalassemia. Nano-based personalized medicines are set to transform treatment approaches by making target design simpler and cutting down implementation times. Their precision and efficiency hold immense potential for individualized healthcare.

COMPETING INTERESTS Nil

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