Synthesis of functionalized rare earth metal as nanotherapeutics towards cancer diagnostics plethora

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Abstract

By amalgamating nanoscience and nanotechnology, the exploration of nanomaterials opens up new possibilities for a wide range of biomedical applications. The use of multifunctional nanoparticles for medication delivery has grown significantly in the field of cancer therapy. This proposed effort involves the manufacture of folic acid (FA) functionalized rare earth metal oxide for use as nanotherapeutics in the treatment and detection of cancer. Scanning electron and optical micrographs were used to track the cytomorphological architecture of the cancer cells. The data suggest that therapy inhibits the migration of malignant cells. Studies on cellular uptake also showed the cellular internalization signifying the diagnostics insight towards cancer chemotherapeutics area.

Keywords: Nanoparticle, functionalized nanoceria, cytomorphology, cancer, chemotherapeutics, cell migration.

INTRODUCTION

Due to its numerous distinguishing characteristics, nanostructured cerium oxide has recently attained unprecedented fame. The therapeutic effectiveness in a variety of application areas, such as antioxidant therapy, neuroprotection, radioprotection, and ocular protection, has been the subject of remarkable research [1–5].

Nanoceria are being developed due to strong antioxidant therapy, inflammatory response, good catalytic capacity, and reactivity with reactive oxygen species (ROS). Trivalent (Ce3+) and tetravalent (Ce4+) are promoted to protect cells against a variety of ROS by their simple cyclic oxidation states [6]. When there is an excessive release of ROS at the intracellular level, catalase and super oxide dismutase partially provide a shielding effect. Oxidase enzymes regulate intracellular ROS generation, which creates oxidative stress and ultimately damages cells and tissues. Traditional chemotherapy drugs have limitations when it comes to specifically targeting cancer cells. Targeted nanomedicine initiatives can address this by taking a path to deliver cancer therapies to tumour sites of interest without affecting healthy ones [7-9].

Due to their strong propensity for binding to folate receptors (FR), also known as folate, folic acid (FA), also known as folate, is extensively used as a targeted ligand [10]. In contrast to the relatively restricted expression of FRs in healthy cells, FRs are recognized as significant therapeutic tumour indicators overexpressed on the surface of roughly 40% of cancer cells, including lung, breast, kidney, ovarian, endometrial, and renal cancer. FA has a wide range of beneficial qualities, such as non-immunogenicity, small molecular size, high stability, and affordability [11]. These modifying characteristics cause simple cellular internalization within cancer cells after they penetrate the cell membrane. In this study, the nanotherapeutics efficiency of functionalized nanoceria using a good synergistic approach was evaluated via cellular uptake and cytomorphological investigation studies in in-vitro system.

A biosensor is a tiny analytical device that employs a transducer and recognition element to detect and measure a specific

molecular entity. New biosensor assembly, immobilisation methods, and applications of cutting-edge materials that can make these methods practical have been the focus of numerous studies. For the swift, inert, stable, low-cost detection of a target molecule, a trustworthy biosensor must provide this platform. Food analysis, bioprocess monitoring, environmental monitoring, clinical diagnosis and prognosis, and bioassays are just a few of the many potential applications for biosensors. Since glucose sensing has a wide range of clinical and experimental applications, the creation of speedy, precise, and reasonably priced biosensors is an exciting research topic in multiple labs throughout the globe. One of the few sensing items that has so far seen commercial success is glucose biosensors. In order to detect glucose, glucose biosensors either stimulate its conversion to other measurably electroactive molecules or do so directly. Both enzyme-based and enzyme-independent techniques have up till now been applied to the measurement of glucose.

Many materials have been used as immobilisers in enzyme-based biosensors, including polymers, porous alumina, clay, phospholipid bilayer, and zeolite. The objective of current research is to improve the stability, sensitivity, and detection precision of biosensors by incorporating nanomaterials into their design. Over the past ten years, the introduction of cutting-edge nanomaterials like nanotubes (NTs), quantum dots, nanosilica, nanofibers, nanoparticles (NPs), and nanorods has created interesting new opportunities for biosensors. Due to their several important inherent advantages over traditional materials, nanomaterials are well suited for inclusion into new minidevices, microdevices, and nanodevices. These features include large surface-to-volume ratios, very small sizes, distinctive behaviour, high chemical reactivity, and adaptable qualities. Due to their special characteristics, nanoscale materials are appealing candidates for biotechnology and bioanalytical chemistry.

In the biological sciences, metal nanoparticles (MNPs) have a variety of applications. MNPs have lately become widely used in glucose biosensors, which has increased detection signal by increasing surface area and assisting in the flow of electrons from enzyme to electrode. The most current advancements in MNP-based enzyme-based and enzyme-independent glucose biosensors are discussed in this article. Diabetes mellitus is one of the main causes of mortality and disability globally because it significantly increases the risk of heart disease, kidney failure, and blindness. 200 million people worldwide have diabetes mellitus. Regular physiological blood glucose measurement is necessary to avert diabetic situations since it confirms the effectiveness of the treatment. Therefore, the development of extremely sensitive, trustworthy, economical glucose sensors with exceptional selectivity has long caught the attention of the medical and food industries.

This is due to the high demand for sensitive and reliable glucose monitoring in biological and clinical aspects. The limitations of enzyme-based glucose measurement are still numerous. As examples, consider difficult enzyme immobilisation, crucial operational factors including the optimal temperature and pH, chemical instability, and high cost. The creation of nonenzymatic electrodes has received a lot of attention as a solution to these problems. Numerous researchers have thoroughly investigated the development of nonenzymatic glucose sensors. The range of applications that these electrodes can be utilised for is significantly constrained by their flaws, such as poor selectivity, excessive cost, or chloride ion poisoning. Therefore, it is still urgently need to develop a nonenzymatic glucose sensor that is low-cost, highly selective, fast, and accurate. Recent developments in nanotechnology have made it possible to create a variety of new materials and technologies with desirable characteristics. Applications for electrochemical sensors and biosensors are served by these items. In essence, it is possible to influence the fundamental properties of materials by creating nanostructures, even without changing the chemical composition of the materials. This has caused a fascinating universe of low dimensional systems to arise in the field of nanotechnology, as well as the current trends in the production of practical nanostructured arrays.

MATERIALS & METHODS:

In a nutshell, Cerium oxide nanoparticles (CeO2) were made by hydrolyzing the precursor ceric ammonium nitrate (NH4)2Ce(NO3)6 (99.999% pure) in basic media using NH4OH [12]. The precipitate was then neutralized with vigorous washing, which was followed by vacuum drying. Aminopropyl triethoxy silane (APTMS) was used as a linker agent during the surface functionalization of CeO2 with FA. Using 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide coupling chemistry, conjugation was accomplished [13]. The human The human mammary cancer cell line, MDAMB-231, was procured from the National Centre for Cell Science in Pune, India, and cultivated in Dulbecco's Modified Eagle's Medium (DMEM) with 10% foetal bovine before being incubated at 37°C with 5% CO2 [14].

The potential efficacy and therapeutic modality assessment was validated by the cytomorphological architecture of MDA-MB231 examined using scanning electron micrographs. Furthermore, fluorescence microscopy using the acridine orange (AO)/EtBr staining procedure was used to assess the results of the apoptosis experiment in breast cancer cell lines (MCF7).

Investigation of FA-CeO2 induced cell death efficacy

To monitor the chemotherapeutics efficacy, we have steered the research analysis toward cellular morphology investigation using SEM micrographs to support the conclusions.

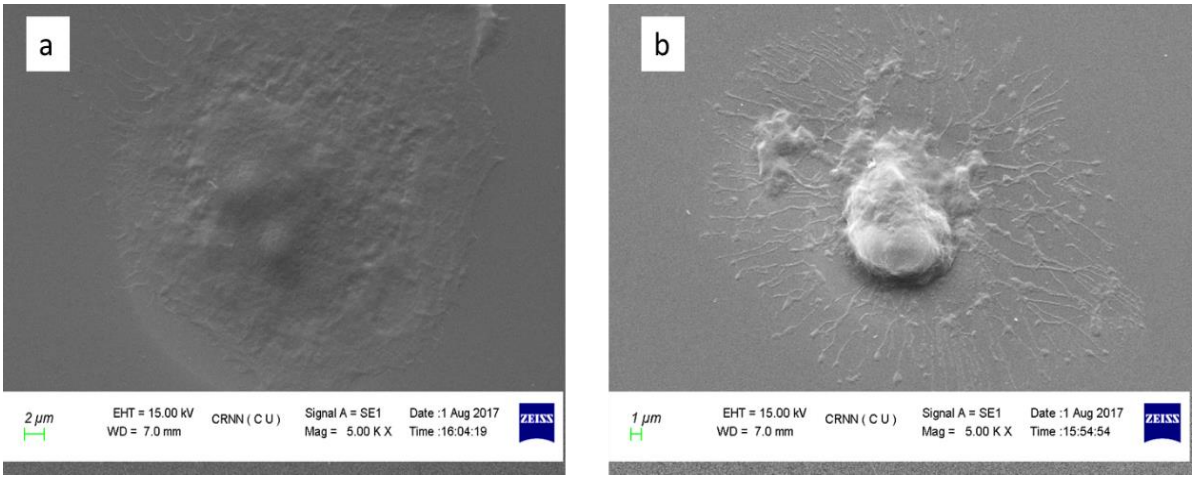

Fig.1 SEM micrographs representing the alteration of cellular morphology a) control & b) upon treatment with nanoparticles at 24 h

Functionalized nanoceria when exposed to cancer cells a substantial amount of membrane blebbing was noticed, which is one of the characteristics markers of apoptotic cells wherein the cytoskeleton of the cell disassembles during apoptosis (programmed cell death), which causes the membrane to protrude outward followed by bulging and becoming apoptotic blebs. Fig1a exhibits an intact cellular architecture in the control group as compared to that cellular apoptosis which ultimately led to cell death in MDA-MB231 cells (Fig1b) [15-17].

Further scrutinization of apoptosis scheme was carried out in human breast cancer cells (MCF7) through fluorescence micrographs utilizing the staining procedure [18].

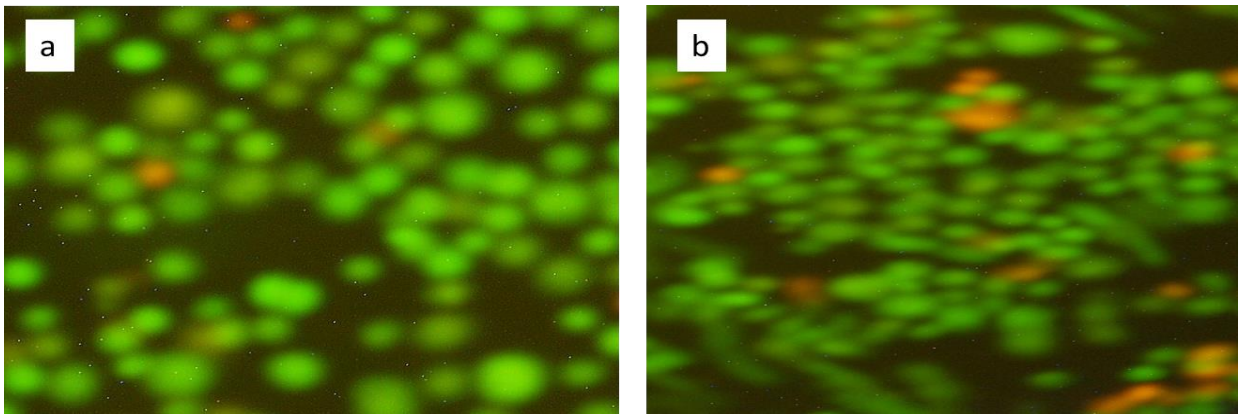

Fig.2 Fluorescence micrographs of MCF7 cells stained with AO/EtBr treated with a) control b)50 µg/ml of FA-CeO2 nanoparticles

Cells were discovered to be in the late stages of apoptosis, as depicted in Figure 2, while the population of dead cells with compromised membrane integrity and DNA fragmentation increased after treatment with multifunctional nanoparticles. From the aforementioned results, it is evident that majority of the cells-stained orange following treatment with FA-CeO2 (50 mg/ml), a characteristic of apoptotic cells undergoing programme cell death mechanisms. [19-20].

Furthermore, autophagy is a self-degrading process, wherein dysfunctional cellular components are impaired by lysosomes or vacuoles and recycled [21]. Thus, autophagy is well documented as a survival mechanism, even though its deregulation has been correlated with non-apoptotic cell death. Growing evidence reveals that autophagy caters pivotal role in the pathogenesis of a several of human diseases, including neurodegeneration, cancer, infection and immunity, aging, myopathies, and liver and heart diseases. Taking account of these significant portrayal of autophagy in human diseases [22]. In this aspect autophagy regulators offers an advanced strategic approach towards therapeutics. Engineered nanoparticles such as nanoceria has acquired immense potential as autophagy regulators [23]. Several mechanistic insights have been identified wherein nanoceria may induce oxidative stress ultimately modulating autophagy. Besides that, ROS generation stimulates and activates several cellular pathways thereby instigating autophagy mechanism [24].

CONCLUSION:

In summary we have elucidated the facile synthesis of folic acid conjugated nanoceria and fundamentally explored its anti-cancer efficacy towards breast cancer cell line. Further the cytomorphological micrographs validated the above findings illustrating the inhibition of cellular protrusion aiding disrupt cellular morphology. The late apoptosis pathway was also substantiated using AO/EtBr staining method. Henceforth, it is imperative that these functionalized nanoceria spectacle promising applications in plethora of therapeutic and diagnosis area. Therefore, this functionalized nano-ceria may act as novel cell death regulators and illustrate its immense potential applicability in the treatment of various diseases by impeding pathogenesis and arresting cellular pathways.

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