



Interfacing of dextran coated ferrite nanomaterials with cellular system and delayed hypersensitivity on Guinea pigs



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ABSTRACT

The study focused on the interfacing of dextran coated ferrite nanomaterials (DFNM) with the cellular system and delayed hypersensitivity on Guinea pigs. *In vitro* study investigated the cytotoxic potential of DFNM on L929 cells, effect on antioxidant enzymes and Lipid peroxides (LPO) production on rat brain homogenates. DFNM was also repeatedly exposed topically to Guinea pigs for the evidence of skin sensitization and toxicity at the molecular level. Biochemical and hematological parameters were estimated. Liver and brain of Guinea pigs were homogenized and evaluated for the induction of LPO, glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx), superoxide dismutase (SOD) and 8-hydroxyl-2-deoxyguanosine (8-OHdG). The results of the study demonstrated that there was no significant alternation in the level of antioxidant defense enzymes, LPO, hematological, biochemical or oxidative stress related DNA damage. Hence, it can be concluded that the synthesized DFNM was non-skin irritant or non-toxic at the molecular level under the laboratory conditions.

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1. Introduction

The major toxicological issue associated with the manufactured nanomaterials is that some of them are redox active that can transport across cell membranes and interact with sub-cellular organelles. Delivery of nanomaterials through skin has potential application in drug delivery because of its large surface area and is challenging because skin acts as a barrier from external environment. Nanoparticles due to their small size can translocate from these portals of entry into circulation [1,2].

Tan et al. [3] reported that TiO₂ (10–50 nm) could penetrate through the skin and reaches stratum corneum and even to the dermis following repeated application. Magnetic nanoparticles are being used as contrast agents in magnetic resonance imaging, tissue repairing, hyperthermia, drug delivery, etc. These particles were made of iron derivatives, have potential application in skin delivery and were found to accumulate in stratum corneum [4]. The molecular mechanisms of toxicity of nanoparticles are still underway. Experimental evidence has shown that nanoparticles induce DNA damage and apoptosis through ROS generation and

oxidative stress [5,6]. Due to the high reactivity of ROS, most cellular components are likely to be the target of oxidative damage ultimately results in cellular dysfunction and injury [7]. Cell confronts and combat against the free radicals generated with the aid of antioxidant defense system, which includes both low-molecular-weight free radical scavengers, such as glutathione (GSH), as well as antioxidant enzymes, like superoxide dismutase (SOD), Glutathione reductase (GR) and glutathione peroxidase (GPX) [8,9]. Increase in ROS generation often results in oxidative stress that damages cellular components and alter their functions. Free radicals have an affinity to DNA bases leading to their modifications. Of these, 8-OHdG with a hydroxyl group at the eight position of guanine is formed easily and abundantly by oxidative stress. This biomarker is sensitive and proportionate to the degree of oxidative stress [7,10].

Sensitization or delayed type hypersensitivity assays on Guinea pigs (closed patch assay) is the most popular assay for evaluating the dermal allergenicity and molecular toxicity [11,12]. This *in vivo* method is to assess the skin sensitization potential of a compound used in cosmetics, topical drugs, latex gloves, etc. Very little published information is available on the skin allergenicity or dermal absorption of these particles. Hence the present study is to investigate the *in vitro* toxicity, delayed hypersensitivity reaction and reactive oxygen species generated molecular toxicity of an in-house synthesized dextran coated ferrite nanomaterials topically exposed to Guinea pigs.

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2. Experimental

2.1. Chemicals and reagents

Dextran coated ferrite nanoparticle (DFNM) [SCTIMST, India], thiobarbituric acid (TBA), reduced glutathione (GSH), oxidized glutathione (GSSG), dithio-bis-2-nitrobenzoic acid (DTNB), RNase, bromophenol blue, ethidium bromide, hydrogen peroxide (H₂O₂) (all from Sigma, USA), disodium hydrogen phosphate (Na₂HPO₄), sodium dihydrogen phosphate (NaH₂PO₄), ethylene diamine tetra acetic acid (EDTA), agarose, physiological saline, Genelute mammalian genomic DNA miniprep kit (Sigma Aldrich, USA) and high sensitive 8-OHdG check ELISA kit (Japan Institute for the Control of Aging, Fukuroi, Japan and Genox Corp., Baltimore, MD). All the chemicals and reagents used were of analytical grade.

2.2. Equipments

Rotor stator homogenizer (Polytron, PT 3100, USA), freezer mill (Labconco, Germany), micro-plate reader (Asys Expert plus, Austria), refrigerated centrifuge (Eppendorf, USA), UV visible spectrophotometer (UV-1601, Shimadzu, Japan).

2.3. Experimental animals

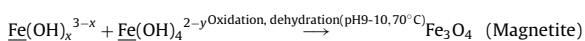
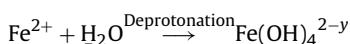
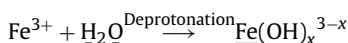
Albino rats (200–250 g) and Guinea pigs (350–500 g) were used for the *in vitro* and *in vivo* study respectively. The animals were procured from the Division of Laboratory Animal Sciences, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala, India.

2.4. Animal husbandry and welfare

All animals were handled humanely, without making pain or distress and with due care for their welfare. The care and management of the animals complied with the regulations of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Govt. of India. All the animal experiments were carried out after prior approval from Institutional Animal Ethics Committee and in accordance with approved institutional protocol. Animals were maintained in a controlled environment with a temperature 22 ± 3 °C, humidity of 30–70% and a light/dark cycle of 12 h. The animals were provided with commercially available feed and aqua guard filtered fresh drinking water, *ad libitum*.

2.5. Dextran coated ferrite nanomaterial (DFNM) synthesis and characterization

DFNMs were prepared using the co-precipitation method. Briefly, the stoichiometric mixtures of FeCl₃ and FeCl₂·4H₂O (Fe³⁺/Fe²⁺ = 2:1) were heated at 70 °C in N₂ atmosphere. Ferrite nanoparticles were precipitated by the addition of 3 M NaOH drop wise, for 1 h followed by heating with stirring for about another 1 h. The precipitate formed was then washed with deionized water to get uniformly dispersed spherical magnetite particles. All reactions were carried out in N₂ atmosphere to prevent oxidation of magnetite to maghemite.



2.6. Coating of dextran on ferrite nanomaterial (DFNM)

Surface coating of ferrite nanoparticles with dextran was done by stirring the ferrite nanomaterial in a solution of dextran of appropriate concentration, overnight (at 37 °C). The precipitate was then washed and lyophilized to get dextran coated nanomaterials (DFNM) [13].

2.7. Characterization of DFNMs

The synthesized DFNM were physico-chemically characterized by the following techniques:

1. Hydrodynamic size profile of dextran coated iron oxide particles by dynamic light scattering (Malvern Instruments, Malvern, UK).
2. Transmission electron microscope for particle size analysis (TEM-H-600).
3. X-ray diffraction technique for phase purity analysis (Siemens D5005).
4. Thermo gravimetric analysis for quantifying the dextran in dextran coated iron oxide (simultaneous TGA-DTA, TA Instruments Inc.).
5. Fourier transform infrared spectral analysis which implies the effectiveness of coating (Nicolet Impact 410 FT-IR spectroscopy).

2.8. Cytotoxicity studies

The *in vitro* cytotoxicity assay using direct contact method was performed with the synthesized DFNM by MTT assay [14]. To determine cytotoxicity, L929 cells were plated at a density of ~20,000 cells/well in a 96-well micro plate. Different concentrations (10, 25, 50, 100 and 200 µg/mL) of DFNM were added onto the wells. After incubation of cells with DFNM at 37 ± 1 °C for 24 ± 1 h, cell culture was examined microscopically for morphological defects and cytotoxicity was evaluated by MTT assay. Phenol served as a positive control and untreated cells served as negative control. 20 µL of MTT dye solution (5 mg/mL in phosphate buffer pH 7.4) was added to each well. After 4 h of incubation, the MTT was removed and formazan crystals were solubilized with 200 µL of DMSO. The absorbance was read on a microplate reader (ELx 808 iu ultra microplate reader, Bio-Tek instruments, USA) at 540 nm. The relative cell viability (%) related to negative control was calculated.

2.9. Delayed hypersensitivity

The delayed hypersensitivity assay is performed as per ISO 10993-10: 2010 (E) Biological Evaluation of medical devices-Part 10: Test for irritation and skin sensitization. Clause: 7.6: Closed patch test [15]. Fifteen healthy adult Guinea pigs (10 animals for test and 5 animals for control) were chosen for the assay. During the induction phase, the clipped area on the upper back skin was swabbed using 70% alcohol and air dried. A concentration of 80 mg/animal of DFNM was made into a paste with physiological saline and applied topically on the clipped upper back region of ten animals. A patch of four ply gauze (wet in physiological saline) alone was applied to other five animals. This procedure was repeated thrice a week for three weeks. The challenge phase was fourteen days after the last application (induction period). During this stage, all the test and control animals were challenged with the DFNM. Hairs were removed from the flank area (untested area) and swabbed with 70% alcohol and DFNM was applied as done in the induction phase. Following this the skin sensitization potential was evaluated at 24, 48 and 72 h.

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