Materials Science in Semiconductor Processing 16 (2013) 1842–1848

Contents lists available at SciVerse ScienceDirect

Materials Science in Semiconductor Processing

journal homepage: www.elsevier.com/locate/mssp

Synthesis, characterization and in vitro cytotoxicity study of calcium ferrite nanoparticles

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ARTICLE INFO

Available online 2 August 2013

Keywords:
Calcium ferrite nanoparticles
Magnetic nanoparticles
Superparamagnetic
Biocompatible
Cytotoxicity

ABSTRACT

In the present paper, calcium ferrite nanoparticles have been synthesized by the sol–gel method. The orthorhombic structure of calcium ferrite nanoparticles has been revealed by X-ray diffractometry. The morphology and size (5–10 nm) of the synthesized nanoparticles have been observed by scanning electron microscopy and transmission electron microscopy, respectively. Fourier transform infrared spectroscopy and thermogravimetric analysis have been studied, in order to ensure absence of impurities. The magnetic analysis has been studied by vibrating sample magnetometer, where the superparamagnetic behavior with saturation magnetization of 36.4 emu/g was observed. In vitro cytotoxicity test on T cell lines (Jurkat cells) using MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a tetrazole) assay revealed the biocompatibility of the synthesized calcium ferrite nanoparticles at particle concentration below 250 μg/ml.

1. Introduction

Magnetic nanoparticles have been extensively scrutinized in many inter-disciplinary areas such as materials science and bio-medicine, because of their non-toxic nature, distinct surface and magnetic properties [1–3]. For biomedical applications, the particles must be nano-sized, superparamagnetic and biocompatible. Magnetic nanoparticles (size ranging from a few nanometers up to tens of nanometers) have dimensions smaller than or comparable to those of a cell (10–100 μm), a virus (20–450 nm), a protein (5–50 nm) or a gene (2 nm wide and 10–100 nm long) [3]. This enables them to penetrate smaller capillaries and get close to any biological entity or target site [1–3]. The other characteristic required is that the nanoparticles must be superparamagnetic. Superparamagnetic materials are those which become magnetized in the presence of magnetic field and lose their magnetism as soon as the magnetic field is removed. Generally, a localized magnetic field gradient is used for attracting and retaining the nanoparticles to a selected site until the completion of the therapy, subsequently followed by their removal [4,5]. The advantage of using superparamagnetic nanoparticles for this application is that as the magnetic field is applied, the triggering response for its planned action begins and it stops as soon as the magnetic field is removed. This enables effective control on their planned action and time of exposure. In addition, compared to other metallic magnetic materials, superparamagnetic nanoparticles show superior chemical stability and biocompatibility [4]. Along with these features, biocompatibility is an important issue which needs to be taken well into care. In context to all the above-mentioned requirements, Fe_3O_4 [6–8], $NiFe_2O_4$ [4,6,9], $MnFe_2O_4$ [10,11], $CoFe_2O_4$ [5,12,13], $ZnFe_2O_4$ [6] nanoparticles have been extensively investigated by the researchers worldwide. However, excluding Fe_3O_4, the high inherent toxicity of Ni, Mn, Co, Zn metals raise apprehensions on their efficacy for biomedical applications, even though the ferrites of these metals exhibit superior magnetic property [14]. Ferrites of calcium are expected to be more biocompatible

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http://dx.doi.org/10.1016/j.mssp.2013.07.016

since calcium is inherently non-toxic. Instead of causing any harm to the body, it is expected to be safely metabolized by the body.

To determine toxicity of magnetic nanoparticles, many cell viability assays such as Lactate dehydrogenase (LDH) [22], Cell counting Kit-8 (CCK8) [23], 3-(4,5-dimethylthiazol-2yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium, inner salt (MTS) [22,24], Sulforhodamine B (SRB) [25], 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-dipheny ltetrazolium bromide, a tetrazole (MTT) [9,10,26–28] have been used, which measure the effect of nanoparticles' exposure on the cells. Among them, MTT assay is considered the "gold standard" for cytotoxicity [29] which is recommended for fast and accurate determination of cytotoxic effects [30,31].

In the bulk form, calcium and iron based compounds have been explored for optical memory devices, steel making industry (as deoxidizer, desulfuration, and dephosphorization) [15], as pigment [16] and as absorbent of hydrogen sulfide (H_2S) [17]. Their magnetic properties in the bulk form have also been investigated [18–20]. We have earlier studied the formation of calcium ferrite compound at different calcination temperatures in the nano-regime [21]. To the best of our knowledge, no report is available regarding its in vitro cytotoxicity using MTT assay. In the present work, we have studied the structural, thermal, magnetic, and morphological properties of calcium ferrite nanoparticles. Also, its in vitro cytotoxicity analysis using MTT assay on Jurkat cells (T-cell lines) has been performed, in order to ascertain its application in the biomedical field.

2. Materials and methods

2.1. Materials

All the chemicals used for synthesis were of analytical grade. Calcium nitrate ($Ca(NO_3)_2 \cdot 4H_2O$), ferric nitrate ($Fe(NO_3)_3 \cdot 9H_2O$), and citric acid ($C_6H_8O_7 \cdot H_2O$), were purchased from Loba Chemie, India and ethylene glycol was purchased from sdfine chemicals, India and were used without any further purifications.

2.2. Synthesis of $CaFe_2O_4$ nanoparticles

The synthesis of calcium ferrite nanoparticles was done by the conventional sol–gel method [26]. 1 M solution of calcium nitrate and 2 M solution of ferric nitrate were mixed. To this mixture, citric acid solution and ethylene glycol were added. The solution was constantly magnetically stirred and heated at 80–90 °C. During heating, the solution first turned into a viscous brown gel and later a dried gel was formed. The dried gel continued to burn in a self propagating combustion manner until the whole gel completely converted to a brown-coloured powder. This indicated the completion of auto-ignition process. The powder was thoroughly washed with ethanol and distilled water. It was dried overnight in vacuum oven at 60 °C. To obtain thorough crystallinity and to remove the impurities, the powder was calcined at 550 °C for 3 h, yielding calcium ferrite nanoparticles as the final product.

Fig. 1. Calcium ferrite nanoparticles dispersed in distilled water and their accumulation near handheld magnet.

Fig. 1 shows calcium ferrite nanoparticles dispersed in distilled water (by sonicating for 15 min) and the second picture shows the accumulation of nanoparticles near the handheld magnet. Once the particles have been collected close to the magnet, they can be easily dragged by moving the magnet, suggesting that the nanoparticles can be moved and influenced by an applied external magnetic field.

3. Characterizations

The morphology, composition and structure of synthesized calcium ferrite nanoparticles were studied by scanning electron microscope (SEM; JSM, 6510 LV, JEOL, U.S.A) where the dried sample was first coated with gold using JEOL, JFC sputter coater, transmission electron microscope (TEM; Hitachi (H-7500)), energy-dispersive X-ray analysis (EDAX, Oxford) and X-ray diffraction (XRD; X'PERT PRO Panalytical, MRD ML), respectively. To study the bond formations and confirm the absence of impurities and unreacted precursors, the FT-IR spectrum was recorded on Perkin Elmer Spectrum BX (II) spectrophotometer. The thermal analysis of the as-synthesized calcium ferrite nanoparticles was done with differential thermal analyzer (DTA; Pyris 1 TGA, Perkin Elmer). It was performed in order to determine the calcination temperature for obtaining pure and crystalline nanoparticles. The thermal analysis was carried out for the uncalcined nanoparticles whereas rest of the characterizations were carried out for calcined nanoparticles. The superparamagnetic properties and saturation magnetization of the synthesized calcium ferrite nanoparticles were determined using vibrating sample magnetometer (VSM; Princeton Applied Research Model 151/155).

The biocompatibility and toxicity of nanoparticles is an important issue before applying them for in vitro or in vivo applications [10,32]. The viability of T cell lines (Jurkat cells) incubated with synthesized calcium ferrite nanoparticles at different concentrations was investigated by the widely used and recommended 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a tetrazole (MTT) colorimetric assay [22–24]. Cells were cultured on tissue