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Short Communication

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Preparation and Characterizaion of Plumbagin Loaded Fibrin Functionalized Iron Oxide, Graphene Oxide Nano Composite

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ABSTRACT

Nano Tecnology employs knowledge from the fields of Physics, Chemistry, Biology, Material Science, Health Science and Engineering. Nano Composite made up of Fibrin, Graphene Oxide and Iron Oxide. Nano Particals are synthesized by a variety of methods depending upon the particles and its final application. The methods can be classified as physical, chemical or biological. Physical techniques (mainly top-down techniques) include attribution or mechanical milling, thermal and vapour gas decomposition (Castro and Mitchell, 2009) and laser ablation synthesis in solution (LASIS) (Amendola and Meneghetti, 2009). Chemical synthesis involves bottom-up techniques. In Micro-emulsion/micelle based techniques the introduction of microwave and ultrasound irradiation into a reaction system has been employed. Bovine blood was hygienically collected from a municipal slaughter house at Chennai, India. It was mechanically stirred with glass rod to isolate the fibrin, which was in crude form. Crude fibrin was washed thoroughly with cold distilled water and treated with 0.5 M Sodium Acetate and 30% Hydrogen Peroxide. About 5 mg of iron oxide was dissolved in 10 ml distilled water and sonicated for 30 minutes. 5 mg of graphene oxide was dissolved in 10 ml distilled water and sonicated for 30 minutes. Both the sonicated samples were mixed together and kept for stirring for 2 h. Then 5 ml of fibrin solution was added to the above sonicated solution.. A black color residue with precipitate particle was obtained.

Keywords: Nano composite; Fibrin; Iron oxide; Graphene oxide; Plumbagin

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INTRODUCTION

The word “Nano” is derived from Greek word Dwarf, means “A Billionth”. A Nanometer is billionth of a meter, which is 250 millionth of an inch, about 1/80,000 of the diameter of a human hair or 10 times of the diameter of hydrogen atom. The term ‘Nanotechnology’ was coined by Prof. Norio Taniguchi, Tokyo Science University in 1974 to describe the accurate manufacture of materials with nanometers tolerances and was unknowingly appropriated by Drexler in his 1986 book ‘Engines of creation: The Coming Era of Nanotechnology employs

knowledge from the fields of physics, chemistry, biology, materials science, health sciences, and engineering. It has immense applications in almost all the fields of science and human life [1,2]. An article summarized the relationship between the color of stained glass and the size/shape of the nanoparticles (The New York Times, 2005).

Fibrin is a naturally occurring protein generated during the blood coagulation cascade and is broadly used in numerous surgical procedures for haemostasis and wound healing applications [3-6]. Fibrin serves as a perfect substrate for cell attachment, proliferation, extracellular matrix formation in wound healing and tissue engineering. It is widely used in the form of sponge, glue and micro beads for various biomedical applications. It exhibits good biocompatibility and biodegradability. Fibrin also acts as an effective carrier of anticancer drugs for the treatment of retinoblastoma [7]. Fibrin nanoparticles (FNPs) have excellent biocompatibility, immunocompatibility, haemocompatibilty and biodegradable properties.

Graphene is a single-atom thick, two-dimensional sheet of hexagonally arranged carbon atoms packed into a honey-comb lattice, and is of great interest in the fields of materials science, physics, and chemistry. Due its unique physical, chemical, and biocompatibility properties, graphene has been used in various applications, including bio sensing, diagnosis, antibacterial development, antiviral material development, and cancer targeting. Due to its unique physical, chemical, and biocompatibility properties, graphene has been used in various applications including diagnosing [6] and cancer targeting. Graphene oxide (GO) is a lamellar flexible material with a large number of functional groups such as epoxy (C–O–C), hydroxyl (OH) and carboxyl (COOH) groups on both basal planes and edges [5]. Graphene oxide exists as a two-dimensional Nano sheet comprised of sp2 hybridized carbon atoms.

The magnetic iron oxide nanoparticle (IONP) based system promises to have many bio-medical applications such as tumours targeting drug delivery carriers, (MRI) contrast agents and hyperthermia treatment. Inorganic nanoparticles such as gold, silica, iron oxide, quantum dots, and calcium phosphate are the most significant materials in the field of biology and medicine. Among these, iron oxide nanoparticles (IONPs) have gained a major attention owing to their ease of synthesis and biocompatible nature. Due to their analogous dimension with biological compounds and distinctive physical traits, IONPs are widely investigated for various applications in tissue-specific drug delivery, cell separation and (MRI). IONPs are predominantly promising as contrast enhancement agents for MRI and also serve as ideal platform for drug delivery.

Plumabagin (PLB) (2-methyl-5-hydroxy-1,4-naphthoquinone) is a yellow crystalline phytoconstituent that is widely present in the roots of the plants belonging to the family Boraginaceae, Droseracea, Ancestrocladaceae, Juglandaceae and Plumbaginacea. PLB isolated from the roots of Plumbago indica has been used in the Indian traditional medicine (Ayurveda) for more than 2000 years as an anti-bacterial, anti-inflammatory, anti leishmanial, anti-fungal, anti-hyperglycemic and anticancer agent. Plumbagin is also used as an ingredient in a number of traditional drug formulations for treating disorders of the liver and cancers of the uterus, breast, lungs and oral cavity, in addition to haemorrhoids. The studies of on nude mice is very encouraging that PLB at a dose of 2 mgkg-1 day-1 decreased the tumours volume significantly and did not exhibit significant toxicity on the normal tissues. PLB was also found to have a chemo-preventive activity against cancer in experimental rat model.

EXPERIMENTAL SECTION

Preparation of Fibrin

Bovine blood was hygienically collected from a municipal slaughter house at Chennai, India. It was mechanically stirred with glass rod to isolate the fibrin, which was in crude form. Crude fibrin was washed thoroughly with cold distilled water and treated with 0.5 M Sodium Acetate and 30% Hydrogen Peroxide. It was yet again washed with cold distilled water, pulverized using a blender and stored at -20◦C until used.

Preparation of Fibrin Solution

Fibrin nanoparticles were prepared by dissolving 250 mg of fibrin in 50 ml of 0.5% NaOH and kept for stirring using magnetic stirrer for overnight which eventually lead to the formation of fibrin nanoparticles. Then, the obtained fibrin solution is filtered using Whatman filter paper.

Synthesis of Iron Oxide

1 g of iron sand was dissolved in 50 ml of concentrated HCl and kept for stirring process using magnetic stirrer for 5 h. The solution was filtered using Whatman filter paper. The filtered iron sand was kept for stirring using magnetic stirrer. During this process, Ammonia solution was added drop by drop to maintain the pH of 9. Once the pH 9 was attained, it was subjected to heating process to 60◦C and dried powder of iron oxide was obtained.

Preparation of Fibrin, Iron Oxide, Graphene Oxide Nanocomposites

About 5 mg of iron oxide was dissolved in 10 ml distilled water and sonicated for 30 minutes. 5 mg of graphene oxide was dissolved in 10 ml distilled water and sonicated for 30 minutes. Both the sonicated samples were mixed together and kept for stirring for 2 h. Then 5 ml of fibrin solution was added to the above sonicated solution. The pH was adjusted to 6-7 using 1% HCl. A black color residue with precipitate particle was obtained. Then the precipitated particles were separated using magnets and transferred into eppendorf and centrifuged. After centrifugation, the obtained pellets were lyophilized.

Preparation of Plumbagin Stock

2 mg of Plumbagin was weighed and dissolved in 2 ml of DMSO solution.

Preparation of Plumbain Loaded Fibrin Functionalized Iron Oxide, Graphene Oxide Nanocomposites

30 mg of lyophilized sample, 0.5 ml of Plumbagin stock was added. To this, 20 ml of distilled water was added and mixed well. The solution is kept for overnight stirring using magnetic stirrer. The stirred sample was centrifuged for 15 minutes. The supernatant was discarded and the pellets were collected and lyophilized.

RESULT AND DISCUSSION

Vibrating Sample Magnetometer (VSM)

The magnetic property of the prepared PLB loaded nanocomposites was measured using Vibrating Sample Magnetometer (VSM). Magnetic hysteresis loop of the composite was S-like curve and the saturation magnetisation of PLB loaded nanocomposites were 27.2 emu/g which would be sufficient for MRI studies as supported by the literature report. The magnetic intensity of the final compound was lower than that of bulk iron oxide nanoparticles (62.4 emu/g) due to the presence of GO as well as due to the presence of fibrin and drug incorporated iron oxide nanostructures. The results suggested that the nano bio composites exhibit ferromagnetic behaviour and this

magnetic property is required for the soft magnetic applications. These nanoparticles can also be tried as MRI contrast agents to enhance the cellular imaging of cancer lesions both for in vitro and in vivo applications (Figure 1).

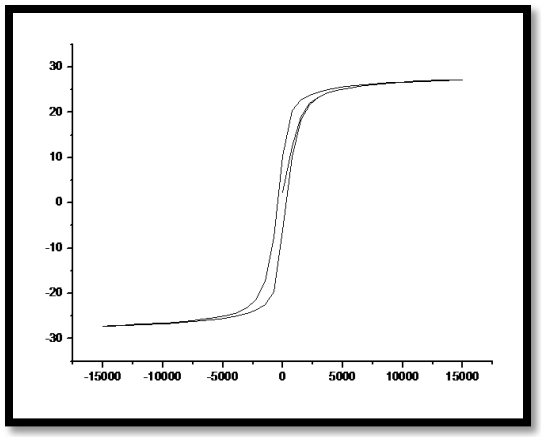

Figure 1. PLB loaded nanocomposites

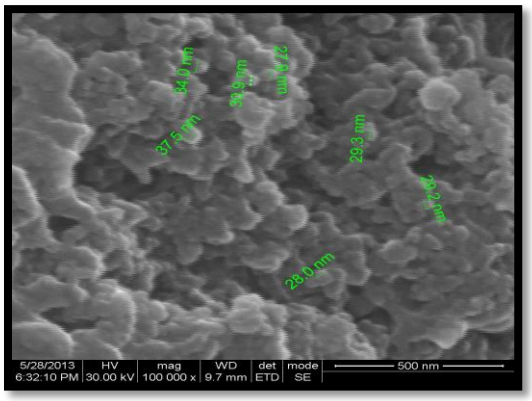

Figure 2. SEM image of Plumbagin loaded nanocomposites

Scanning Electron Microscope (SEM)

The Plumbagin loaded nanocomposites were observed through JEOL Cold Field Emission Scanning Electron Microscope (SEM) operated at 3 kV. They were found to be spherical in shape and the size ranges from 27.9-37.5 nm (Figure 2).

Haemocompatibility

In vitro haemolysis is considered to be a simple method to evaluate the compatibility of nanomaterials towards RBCs. Complete lysis of RBCs has been noticed on incubation with water (Positive control). The supernatant obtained was found to be red in colour resulting in high absorbance at 577 nm (absorbance maxima of haemoglobin). Incubation of different concentration of (F+IO+GO) nanocomposites, Plumbagin loaded nanocomposites for a period of 4 h did not result in haemolysis as observed visually. The supernatant obtained after centrifugation of the treated RBCs was clear. Quantitation of the lysed haemoglobin was performed. The absorbance measurement of haemoglobin at 577 nm showed very low haemolysis. The percentage haemolysis in the presence of (F+IO+GO) nanocomposites was 0.8 at 25 µg/ml which increased to 2.2 at 100 µg/ml, Plumbagin loaded

nanocomposites showed less than 5% in the presence of 25 µg/ml and 100 µg/ml indicating that the prepared nanocomposites were haemocompatible (Table 1).

Table 1. Haemolysis assay result

Nanocomposites	Concentration µg/ml	% haemolysis (mean ± S.D)
(F+IO+GO)	25	0. 76 ± 0.14
Nanocomposites	50	0.82 ± 0.54
	100	2.2 ± 2.1
Plumbagin loaded nanocomposites	25	0.4 ± 0.28
	50	1.2 ± 0.07
	100	3.1 ± 0.58

Alamar Blue Assay

Viability of nanocomposites with PBMCs was investigated using Alamar blue assay.Treatment of PMBCs with lowest concentrations of nanocomposites does not decrease the viability of significantly up to a treatment period of 72 h. On treatment with concentration of 100 µg/ml of (F+IO+GO) nanocomposites, the viability values were 86, 83 and 76 at 24, 48 and 72 h respectively. The corresponding values were 85, 80 and 79 on treatment with 100 µg/ml of Plumbagin nanocomposites. When treated with 150 µg/ml of the two nanocomposites, decrease in viability around 70% at 24 h, 60% at 48 h and 50% at 72 h were observed. This result indicated that nanocomposites up to concentrations of 100 µg/ml are not toxic to the cells while the treatment with 150 µg/ml of nanocomposites is toxic. Hence for further studies, nanocomposites up to a concentration of 100 µg/ml could be used.

CONCLUSION

This paper demonstrated a safe and promising use of fibrin nanoparticles (FNP) for biomedical applications. The stable, haemocompatible nanocomposites (F+IO+GO) synthesized in novel way using PLB. Plumbagin loaded nanocomposites was 176.2 nm and the surface charge was -8.65 mV which was found by DLS analysis. Their high specific surface energy was proved by SEM analysis. The nano bio composites exhibited ferromagnetic behaviour through VSM, which is required for the soft magnetic applications. The significance of the present work evaluated all the important parameter related to its positive in vivo applications which includes the exposure of normal human blood cells (RBCs and PBMCs) to the PLB loaded fibrin functionalized Iron oxide, Graphene oxide nanocomposites showed only a slight hemolytic activity at 0.7-3.1% at 25-100 µg/mL of Plumbagin loaded nanocomposites with an acceptable range. The low concentration (25%) of Plumbagin loaded nanocomposites would be used for in vivo applications since it showed less viability and less haemolytic effect. These analyses demonstrated that Plumbagin loaded nanocomposites did not induce significant toxicity in vivo and thus may be used as potential vehicles for drug delivery applications.

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