Supplementary Information

Core-shell nanostructured Fe₃O₄ - Poly(ST-co-VBC) grafted PPI dendrimers stabilized with AuNPs/PdNPs for efficient nuclease activity

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2.1 MATERIALS USED

Monomers such as Styrene (Alfa Aeser), Divinylbenzene (Sigma Aldrich) and Vinyl benzyl chloride (Sigma Aldrich) were used with prior purification. Other chemicals like ferrous sulphate heptahydrate (SRL), ferric chloride hexahydrate (Loba Chemie), aqueous ammonia (SRL), oleic acid (Merck), gelatin (BDH), poly(vinylalcohol) (Fluka), boric acid, sodium nitrite, sodium hydroxide, chloroauric acid (Sigma Aldrich), Pottassium tetrachloropalladate (Sigma Aldrich), benzoyl peroxide (Alfa Aeser), polyethylene glycol-Molecular weight-4000 (SRL), Poly(propyleneimine)-G(2) and G(3) dendrimer (Symo Chem, Netherland), 3-amino benzoic acid, dicyclohexyl carbodiimide (Alfa Aeser), dimethylaminopyridine (Alfa Aeser), potassium hydroxide (SRL), hydrogen peroxide (Alfa Aeser) and sodium borohydride (SRL), solvents like methanol, ethanol, acetone, diethylether, dimethyl sulphoxide, dichloroethane and distilled water were used as such without any further purification. Agarose gel electrophoresis kit with buffers was used as received for DNA cleavage studies.

2.2 INSTRUMENTS USED

The FT-IR spectra were recorded on Bruker Tensor-27 FT-IR spectrophotometer. The sampling was done making use of KBr pellets for background calibrations. All the measurements were carried out in the range 400 - 4000 cm⁻¹ at 25 ºC. The UV-Vis spectra were measured on Techomp 8500 instrument with software. The measurements were carried out in the wavelength range of 200-800 nm. The surface morphology study was performed using JEOL JSM-6360 scanning electron microscope (SEM). The samples were spread on the surface of double sided adhesive tape, one side of which was already adhered to surface of a circular copper disc pivoted by a rod. JEOL JSM-6360 auto fine-coating ion sputter was used for the gold coating. The crystallographic structure of core-shell magnetic nanoparticle catalysts were obtained by X-ray diffraction on desktop X-ray diffractometer using
a Cu kα monochromatized radiation source (λ=1.54045) operating at 30KV and 15 mA and scanned at the rate of 1-10⁰ min⁻¹ over the range of 5⁰-80⁰. The magnetic susceptibility values were recorded for prepared magnetic nanoparticles using Lakeshore, USA, Model 7404 at maximum magnetic field -2.17 T (0.64” air gap) with dynamic moment range :1 x 10⁻⁷ emu at 10⁻³ emu moment and Field Accuracy -1%. The thermogravimetric analysis too was performed quantitatively by taking the same weight of the core-shell magnetic nanoparticles in a SDT Q600 V20.5 Build 15 instrument at a heating rate of 10⁰ C/min from 20 to 700⁰ C under nitrogen atmosphere. XPS measurement was made in a DAR400-XM1000 (OMICRON Nanotechnologies, Germany) equipped with dual Al/Mg anodes as the x-ray source. An Mg anode was used to obtain the survey and elemental spectra. Pass energy of 50 eV was used to obtain the survey spectrum and 20 eV pass energy was used for elemental spectra. The Raman spectra for core-shelled magnetic nanoparticles were acquired quantitatively by taking equal amount of each catalyst and performed in a Witec Confocal Raman instrument (CRM200) with Ar ion laser (514.5 nm). The ultrasonication of Fe₃O₄ and various functionalized Fe₃O₄ was done by Cole Parmer sonication bath at 40 KeV. The DNA cleavage activity of the metal complex was studied by using agarose gel electrophoresis. Supercoiled plasmid pBR322 DNA was used for the study. Electrophoresis was undertaken for 1h at 50 V in Tris-acetate-EDTA (TAE) buffer. The gel was stained with ethidium bromide (EB) for 5 min after electrophoresis, and then photographed under UV light. The proportion of DNA in each fraction was quantitatively estimated from the intensity of each band with the Alpha Inno tech Gel documentation system (Alpha Imager 2200).
2.3 SYNTHESIS OF Fe$_3$O$_4$NPs

4.45 g of FeSO$_4$.7H$_2$O and 7.785 g of FeCl$_3$.6H$_2$O were dissolved individually into a 10 ml deionized water in two different beakers and then the corresponding solutions were mixed together and stirred for 3 min and then both the solutions were transferred to a 250 ml three-necked round bottomed flask equipped with a Teflon blade mechanical stirrer and a nitrogen inlet. Then the solution was heated to 60$^0$C to which 40 ml of 25% (w/w) NH$_3$.H$_2$O was added drop wise until the pH of the solution reached to 10-11. After addition of this base, the orange coloured solution turned dark brown, followed by the formation of black precipitate, which indicates the formation of iron oxide in the system (Fe$^{2+}$+ Fe$^{3+}$ + 8OH). Then the resulting solution was heated at 80$^0$C for 1hr. The obtained precipitate was isolated from the solvent by magnetic decantation and then repeatedly washed with deionized water until the washed solution shows neutral pH and then washed with ethanol. The resulting Fe$_3$O$_4$NPs were dried at room temperature under vaccum for 12 hours.

Mechanism for DNA Cleavage

The general mechanism is a ferrous ion acts as a catalyst in the presence of hydrogen peroxide. The hydrogen peroxide is broken down into a hydroxide ion and a hydroxyl free radical. It has been shown that ·OH radicals induce DNA damage via hydrogen abstraction from the sugar moiety hence forming sugar radicals \(^1\). Schraufstatter and coworkers were the first to show that exposure of H$_2$O$_2$ to P388 D1 cells resulted in an increase in 8-hydroxyguanine (8-OH-Gua) \(^2\). Later, Floyd et al. was able to prove that the major product formed by ·OH DNA damage was 8-OH-Gua suggesting that ·OH radicals are involved in the attack of purine bases \(^3\). Under aerobic conditions, DNA react with oxygen to produce O$_2^-$ (E = ~0.9 V vs. NHE) \(^4\). However, several works in literature have shown that O$_2^-$ radicals cannot induce DNA damage.
These $O_2^-$ can react with $H^+$ to generate $H_2O_2$ which in turn can react with more $O_2^-$ to generate more ${\cdot}OH$ via Harber-Weiss reactions. The redox potential for $\cdot$OH radicals is $E = \sim 2.4$ V vs. NHE. Since the redox potential for $\cdot$OH is high, $\cdot$OH can oxidize DNA, resulting in DNA damage. In summary, these results indicate that the cleavage reaction involves hydroxyl radicals, that is, a Fenton type reaction may lead to the formation of these oxygen active species which finally cleave the DNA. Therefore, from the earlier studies it is established that although different methods are available to perform DNA cleavage studies, the DNA cleavage performed with OH radical is proved to be a better and benign method than the other techniques.

3. RESULTS AND DISCUSSIONS

![Figure S1 FT–IR spectra](image)

Figure S1 FT–IR spectra of (a) Fe$_3$O$_4$-Poly(ST-DVB-VBC)-PPI-G(2)-PdNPs, (b) Fe$_3$O$_4$-Poly(ST-DVB-VBC)-PPI-G(3)-PdNPs, (c) Fe$_3$O$_4$-Poly(ST-DVB-VBC)-PPI-G(2)-AuNPs and (d) Fe$_3$O$_4$-Poly(ST-DVB-VBC)-PPI-G(3)-AuNPs
Figure S2 UV-Visible spectra of (a) Fe₃O₄-Poly(ST-DVB-VBC)-PPI-G(3)-PdNPs (b) Fe₃O₄-Poly(ST-DVB-VBC)-PPI-G(3)-AuNPs

Figure S3 XRD pattern of (a) Fe₃O₄, (b), (c) Fe₃O₄-Poly(ST-DVB-VBC)-PPI-G(2)/PPI-G(3)-PdNPs & (e), (f) Fe₃O₄-Poly(ST-DVB-VBC)-PPI-G(2)/PPI-G(3)-AuNPs

Figure S4 Raman spectra of (a), (b) Fe₃O₄-Poly(ST-DVB-VBC)-PPI-G(2)/PPI-G(3)-PdNPs & (e), (d) Fe₃O₄-Poly(ST-DVB-VBC)-PPI-G(2)/PPI-G(3)-AuNPs

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Figure. S5 TGA curves of (a), (b) Fe₃O₄-Poly(ST-DVB-VBC)-PPI-G(3)-AuNPs/PdNPs & (c), (d) Fe₃O₄-Poly(ST-DVB-VBC)-PPI-G(2)-AuNPs/PdNPs

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