Biocompatible FePO₄ Nanoparticles: Drug Delivery, RNA Stabilization, and Functional Activity

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Abstract
FePO₄ NPs are of special interest in food fortification and biomedical imaging because of their biocompatibility, high bioavailability, magnetic property, and superior sensory performance that do not cause adverse organoleptic effects. These characteristics are desirable in drug delivery as well. Here, we explored the FePO₄ nanoparticles as a delivery vehicle for the anticancer drug, doxorubicin, with an optimum drug loading of 26.81% ± 1.0%. This loading further enforces the formation of Fe³⁺ doxorubicin complex resulting in the formation of FePO₄-DOX nanoparticles. FePO₄-DOX nanoparticles showed a good size homogeneity and concentration-dependent biocompatibility, with over 70% biocompatibility up to 80 µg/mL concentration. Importantly, cytotoxicity analysis showed that Fe³⁺ complexation with DOX in FePO₄-DOX NPs enhanced the cytotoxicity by around 10 times than free DOX and improved the selectivity toward cancer cells. Furthermore, FePO₄ NPs temperature-stabilize RNA and support mRNA translation activity showing promises for RNA stabilizing agents. The results show the biocompatibility of iron-based inorganic nanoparticles, their drug and RNA loading, stabilization, and delivery activity with potential ramifications for food fortification and drug/RNA delivery.

Keywords: Iron phosphate nanoparticles, Doxorubicin, Drug delivery, Drug loading, RNA stabilization, Biocompatible nanoparticles

Introduction
Among various inorganic nanoparticles such as gold, silica, and quantum dots, iron-based nanoparticles (Fe-NPs) are widely explored for biomedical applications like contrast agents, drug delivery vehicles, and thermal-based therapeutics [1–3]. Owing to the magnetic property, high bio-adaptability, and known endogenous metabolism of iron, Fe-NPs are desirable candidates for biomedical applications. As such, Fe-NPs make the majority of FDA-approved inorganic nanomedicine [1, 2]. These include INFeD, DexFerrum, Ferrelecit, Venofer, Feraheme, and Injectafer which are commercially available for their application in iron-deficient anemia and iron deficiency in chronic kidney disease [1]. Similarly, intravenous administration of the chelate iron gluconate is a well-tolerated intervention for anemia [4]. Anemia is one of the most prevalent nutritional deficiency in the world and Fe-based nanoparticles like FePO₄ and FeSO₄ has been used in food fortification to prevent anemia. Food fortification is the process of adding micronutrients to the food with an aim to overcome the nutritional deficiency in a population [5]. FePO₄ NPs are of special interest in food fortification because of their biocompatibility, high bioavailability, and superior sensory performance that do not cause adverse organoleptic effects [6–9]. Perfecto et al. have demonstrated the FePO₄ NPs
internalization in human intestinal cells occurs primarily through divalent metal transporter-1 (DMT-1) and therefore can be readily absorbed [9, 10]. Iron-based Feridex® and Reovisit® are widely used magnetic resonance imaging (MRI) contrast agents for contrast enhanced MRI [11–16]. In light of these outstanding reports, FePO4 NPs present themselves as a good delivery vehicle. Here, we explored FePO4 as a drug-delivery vehicle by loading an anticancer drug, Doxorubicin (DOX). Ferric ion (Fe^{3+}) can form complex with DOX molecule facilitated by electrostatic interaction between electron deficient Fe in FePO4 and electron rich –OH group in DOX to form DOX loaded FePO4 NPs: FePO4−DOX NPs. We evaluated the physicochemical properties of FePO4 and FePO4−DOX NPs and assessed their biocompatibility and cytotoxicity profile, respectively, in mouse osteosarcoma K7M2 and fibroblast NIH/3T3 cell-line.

Along with that, the inorganic nanoparticle has shown promises in nucleic acid stabilization and delivery [17–19]. In this regard, the gold nanoparticle has been widely studied because of their ability to immobilize oligonucleotides in their surface resulting in the prevention of molecular aggregation and degradation [17, 20]. However, gold is not an endogenous element and thereby may limit its translational application. Here, Fe-based nanoparticles like FePO4 nanoparticles can be of prime interest for RNA stabilization study because of their endogenous nature and established biocompatibility profile. There is two proposed mechanism of interaction of nucleic acid (RNA/DNA) with Fe-NPs for the stabilization—(1) formation of hydrogen bonds and electrostatic interaction between the phosphate group of nucleic acid backbone and Fe-NPs resulting in adsorption of nucleic acid in Fe-NPs, and (2) nucleic acid can adsorb to the Fe-NPs surface via nucleotide base pair interaction [19, 21, 22]. A study has shown the potential of calcium phosphate nanoparticles for DNA vaccine stabilization and delivery [23]. In this regard, here we have explored the RNA stabilization and functional activity of another phosphate-based nanoparticle, FePO4, to investigate the multifunctional potential of FePO4 based nanoparticles, in the delivery and stabilization of cargo.

With rapid approval of mRNA vaccine against COVID19, mRNA vaccine nanoparticles are of great interest, RNA being subject to rapid hydrolysis and loss of functional expression, it is incumbent upon the nanoparticle to improve these critical characteristics. Here we show FePO4 NPs stabilize RNA and support functional mRNA translation. Given these excellent characteristics, FePO4 NPs may merit consideration for food fortification, drug, and RNA delivery, opening up exciting biomedical applications.

Results and Discussion

FePO4 NPs Synthesis, Characterization, and Biocompatibility Analysis

A simple one-step chemical reaction between (NH₄)₃PO₄ and Fe(NO₃)₃ gives FePO₄ as precipitate which is dispersed in biocompatible lipid-PEG surfactant that helps to stabilize FePO₄ nanoparticles and prevent aggregation. FePO₄ NPs showed a hydrodynamic size of 175±5 nm with a polydispersity index (PDI) of 0.150±0.01 suggesting good particle homogeneity and narrow size distribution. Zeta potential analysis showed a negative surface charge of FePO₄ NPs with −19.1±8 mV zeta potential. The negative surface charge further helps to stabilize particles in colloids thereby preventing protein opsonization, a mechanism that prevents cellular targeting and alters pharmacokinetics [24–26]. FePO₄ was further characterized by FTIR. Figure 1c shows the spectral characteristic of FePO₄ nanoparticles and their precursor—Fe(NO₃)₃ and (NH₄)₃PO₄. FePO₄ spectra show a distinct sharp peak on 1030 cm⁻¹ which can be attributed to the P−O stretching band, a small peak at 520 cm⁻¹ corresponds to the O−P−O antisymmetric bending, and a broad range from 3000 to3500 cm⁻¹ represents water bending and stretching vibrations from adsorbed water molecules [27, 28]. The FePO₄ spectra showed the presence of PO₄³⁻ group and are similar to the FTIR peak reported by other studies thus confirming the formation of FePO₄ nanoparticles [27–29]. Fe(NO₃)₃ spectra showed characteristic peaks for N−O stretching bands at 1326 and 1030 cm⁻¹ which can be attributed to the P−O stretching band, a small peak at 520 cm⁻¹ corresponds to the O−P−O antisymmetric bending, and a broad range from 3000 to3500 cm⁻¹ represents water bending and stretching vibrations [30]. Likewise, (NH₄)₃PO₄ showed characteristic peaks for the ammonium group around 1500 cm⁻¹ and phosphate group around 1000 cm⁻¹ [31].

With the assurance of successful synthesis, purity, good size homogeneity, and stable surface charge of FePO₄ NPs, we went on to analyze the biocompatibility of FePO₄ NPs. For this purpose, we used cancer and non-cancer cells: mouse osteosarcoma K7M2 and mouse fibroblast NIH/3T3 and analyzed the biocompatibility of FePO₄ NPs in the concentration range of 20 to 600 µg/mL (Fig. 1d). FePO₄ NPs showed good biocompatibility up to 80 µg/mL concentration with cell viability greater than 70%. Biocompatibility was relatively higher in non-cancer cell NIH/3T3 compared to cancer cell K7M2.
Doxorubicin Loading in FePO₄ and Cytotoxicity of FePO₄-DOX

Doxorubicin is loaded in FePO₄ through the co-incubation-precipitation method in which doxorubicin solution is mixed with the precursor of FePO₄ that results in the formation of DOX loaded FePO₄. Three different formulations to load DOX are employed as discussed in the methods. Formulation 1 showed the best loading efficiency of 26.81% ± 1 whereas formulation 2 showed a loading efficiency of 8.83% ± 2 and formulation 3 did not show any loading (Fig. 2a). For loading, we added DOX solution to the precursor Fe(NO₃)₃ in formulation 1 and to (NH₄)₃PO₄ in formulation 2, whereas, in formulation 3, we added DOX solution to FePO₄ NPs directly. The loading data clearly showed that adding DOX to the FePO₄ NPs does not retain the DOX whereas adding DOX to either precursor: Fe(NO₃)₃ and (NH₄)₃PO₄ solution helps in the loading and retention of DOX. This can be explained by the fact that Fe³⁺ from Fe(NO₃)₃ can form a complex with the electron-rich oxygen group present in Doxorubicin [32, 33]. The Fe³⁺-DOX complex is then precipitated by the addition of (NH₄)₃PO₄ resulting in FePO₄-DOX, which is characterized by a change of color from faint yellow to faint brown (Fig. 2b). Despite the color change, there was no change in the emission spectra of FePO₄-DOX which showed emission maxima at 590 nm similar to that of Free DOX, when excited at 480 nm (Fig. 2c). FePO₄-DOX NPs showed a hydrodynamic size of 187 ± 7 nm and PDI of 0.143 ± 0.02, similar to that of FePO₄ (Fig. 2d). However, there was a significant difference in the surface charge of FePO₄-DOX NPs (-8.89 ± 5 mV), compared to FePO₄ NP (-19.1 ± 8 mV) (Fig. 2e). Change in zeta potential suggests functional changes in the surface property of nanoparticles. Here, the reduction of zeta potential from −19.1 to −8.89 mV can be attributed to the DOX complexation which adds cationic property in the complex.

Following the physicochemical characterization, the cytotoxicity of FePO₄-DOX was analyzed in K7M2 and NIH/3T3 cells and compared with free DOX (Fig. 3). FePO₄-DOX showed higher cytotoxicity compared to Free DOX at equivalent DOX concentration in both cell lines. IC50 value showed around 10 times reduction with FePO₄-DOX treatment, from 2.61 to 0.248 µM in NIH/3T3 and 1.01 to 0.107 µM in K7M2 cells. This drastic reduction in IC50 value in both cell lines suggests
an enhanced cytotoxicity profile of FePO₄-DOX NPs. The equivalent FePO₄ concentration in the IC50 concentration range of FePO₄-DOX is 40 µg/mL (0.107 µM in K7M2 cells) and 100 µg/mL (0.248 µM in NIH/3T3 cells), which are both within the biocompatible range of FePO₄ concentration, with more than 70% cell viability. Hence, the elevation of FePO₄-DOX cytotoxicity can be attributed to the Fe³⁺-DOX complex formation and not to the individual contribution of FePO₄ and DOX. Literature has shown the elevated cytotoxic effect of anthracycline like doxorubicin in presence of iron [34–37]. These reports are further supported by the alleviation of Fe-DOX cytotoxicity by the use of iron chelators [35–37]. One proposed mechanism is Fe-DOX complex potentiates the toxicity of DOX-derived reactive oxygen species (ROS) transforming relatively safe ROS (O²⁻ and H₂O₂) into much more toxic ROS leading to elevated DNA damage and cell death [34, 36]. Another proposed mechanism is the interaction of DOX with the function of iron regulatory proteins and ferritin in presence of excess Fe thereby affecting iron homeostasis leading to ROS-dependent and independent damage and apoptotic cell death [36, 38].

Along with the elevated cytotoxicity, FePO₄-DOX showed selectivity toward cancer cells with higher cytotoxicity behavior similar to that of Free DOX. Figure 3c shows 0.1 µM DOX equivalent FePO₄-DOX showed 53% cell viability for cancer cell K7M2 compared to 72% cell viability for non-cancer NIH/3T3. Likewise, Free DOX also showed higher cytotoxicity behavior toward cancer cells, with 54% cell viability in K7M2 cells compared to 66% in NIH/3T3. However, the differences have increased in the case of FePO₄-DOX, with 19% differences in cell viability among cancer and non-cancer cells compared to 12% in Free DOX. Cytotoxicity analysis has shown that Fe complexation with DOX in FePO₄-DOX NPs has significantly enhanced the cytotoxicity and improved the selectivity toward cancer cells.

**Cellular Internalization of FePO₄-DOX NPs**

The internalization behavior of FePO₄-DOX NPs was analyzed using confocal microscopy following a time-dependent internalization study (Fig. 4). Free DOX was used as a positive control. Both FePO₄-DOX NPs and Free DOX did not show significant internalization in the initial 0.5 and 1 h incubation time points. However, at 3 h incubation, both showed internalization as depicted by red DOX fluorescence in the confocal image. The blue color comes from nucleus staining by DAPI. The analysis shows that within 3 h, FePO₄-DOX NPs internalize to cells following similar internalization behavior as that of Free DOX. It is important to note that, due
to the change of color of FePO₄-DOX, which is brownish compared to the red color of Free DOX, we may not quantitatively compare the relative internalization profile of FePO₄-DOX. Nonetheless, the internalization assay confirmed that FePO₄-DOX is uptake by the cells within 3 h. Given the well-understood mechanism of handling iron by our body, proposed NPs could hold promises in the development of iron-based anticancer therapeutics with an ability to monitor therapeutic response in a single therapy session.

RNA Stabilization and mRNA Expression
As can be seen in Fig. 5a, whereas copper nanoparticles (Cu NP) and carbon nanotubes (CNTs) accelerate RNA hydrolytic degradation (lower band intensity than control), the FePO₄ and control silver (Ag) nanoparticle stabilize the RNA as shown by relatively strong band intensity in RNA agarose gel electrophoresis (RAGE). The FePO₄ and control zinc oxide nanoparticle (ZnO NP) also impart some resistance to degradation in serum, as depicted by the band intensity which is slightly higher than controls (Fig. 5b). Importantly the functional activity, mRNA expression is higher than non-nanoparticle controls, whereas the RNA-degrading Cu NP causes loss in mRNA expression as measure by relative light units (Fig. 5c). These results show that FePO₄ NPs helps to stabilize RNA and can be used as a stabilizing delivery agent for therapeutic RNA delivery. Earlier preliminary experiments had indicated a normal working range of translation shown are two independent experiments for the control non-nanoparticle treated samples showing 2393 and 2630 RLU/well which is representative. A twofold increase consistent with the above data suggests FePO₄ NP supports translation whereas consistent with RNA denaturation/degradation above, the Cu NP suppresses translation. A variety of inorganic nanoparticle systems have been exploited for therapeutic RNA stabilization and delivery including; gold, silver, copper, iron oxide,
mesoporous silica nanoparticle (MSN), carbon-based polymers, composites, and others [39–45]. For example our group had reported nanoparticle complexation to macromolecular RNA can cause it to resist degradation by RNase, or nucleases present in serum and tissues. The COVID-19 mRNA vaccine has renewed interest in such macromolecular RNA therapies extending beyond vaccines, where it is incumbent upon the nanoparticle to not only protect RNA from hydrolysis and nuclease-mediated digestion, but complexation to the NP must preserve RNA function, for example, mRNA expression. Previously we had seen copper nanoparticle complexation macromolecular RNA causes RNA denaturation [46] Thus we investigated the effects of NP complexation to

![Fig. 4](image-url) Cellular internalization study. Cellular internalization of FePO₄-DOX NPs and Free DOX on K7M2 cells after 3 h, 1 h, and 0.5 h treatment. Cells were treated with 200 µL of 5 µg/mL DOX concentration. The red color observed in nanoparticle treated cell line signify successful internalization of the nanoparticles. The red color is due to the fluorescence characteristic of DOX. No red signal is observed in the untreated control cell.

![Fig. 5](image-url) RNA hydrolysis. a A model RNA we have used in a number of our publications similar in size and sequence composition to most mRNAs from torula yeast (TY-RNA) was used. The RNA was incubated in double-distilled water over time in the presence or absence of nanoparticles either copper (Cu NP), iron phosphate (FePO₄), silver (Ag NP) or carbon nanotube (CNT) at 37 degrees celsius and samples removed at the same time point and assayed by RNA agarose gel electrophoresis (RAGE). Loss of band staining intensity indicates RNA degradation whereas maintenance of RNA band staining intensity indicates stabilization. b Similar to above, RNA was incubated in 10% FBS/DMEM at room temperature in the presence of zinc oxide (ZnO) NP or FePO₄ NP versus control which was RNA alone in the absence of nanoparticle. Again samples were removed over time and assayed by RAGE, presence of the stained RNA band over time again indicates stability and resistance from nuclease or RNase degradation from the serum. c mRNA encoding Luciferase was translated in vitro from standard rabbit reticulocyte and the relative luminescence standardized to RNA in the presence or absence of either iron phosphate (FePO₄) or copper (Cu) nanoparticle.
macromolecular RNA using Torula yeast RNA (TY-RNA) or a reporter construct mRNA expressing Luciferase.

**Conclusion**

FePO₄ nanoparticles were successfully synthesized following a simple co-incubation-precipitation technique resulting in the formation of homogenous-sized particles of 175±5 nm. FTIR analysis confirmed the presence of phosphate group and the absence of precursor impurities in the nanoparticle. Biocompatibility analysis revealed concentration-dependent biocompatibility with more than 70% cell viability up to 80 µg/mL. Further, DOX was effectively loaded in FePO₄ resulting in FePO₄-DOX NPs which showed similar physicochemical properties to that of FePO₄. Cytotoxicity analysis revealed that Fe complexation with DOX in FePO₄-based NPs enhanced the cytotoxicity, with around 10 times improvement in IC₅₀, and improved the selectivity toward cancer cells. Additionally, the internalization assay showed FePO₄-DOX NPs were efficiently internalized in cells at a 3 h incubation time point. RNA stabilization study showed that FePO₄ nanoparticles efficiently stabilize RNA, prevent rapid degradation, and maintain the functional activity demonstrating promises for delivery of therapeutic RNA. Given the good size homogeneity, biocompatibility range, drug loading efficiency, enhanced cytotoxicity profile, RNA stabilizing property, and efficient cellular uptake, FePO₄ NPs showed desirable characteristics for drug and RNA delivery vehicles. Furthermore, the results have shown promising prospects of using FePO₄-drug NPs in food fortifications for the development of a food-based drug platform.

**Methods**

**Synthesis and Characterization of FePO₄ Nanoparticles**

FePO₄ nanoparticles were synthesized by chemical precipitation technique optimizing protocol by Sokolova et al. [47]. Briefly, Ammonium phosphate ((NH₄)₃PO₄, 16 mg/mL) and iron nitrate (Fe(NO₃)₃, 8 mg/mL) solution was prepared. To the 1 mL of Fe(NO₃)₃, 1 mL of (NH₄)₃PO₄ was added dropwise under constant stirring resulting in precipitation of iron phosphates (FePO₄). Excess of (NH₄)₃PO₄ was added dropwise under constant stirring resulting in precipitation of iron phosphates (FePO₄). FePO₄ precipitate was dispersed with DSPE-PEG-COOH solution (10% w/w) in water to formulate FePO₄ nanoparticles. FePO₄ NPs were characterized for size and surface property using dynamic light scattering (DLS) and spectral characteristics using Fourier Transform Infrared Spectroscopy (FTIR).

**Doxorubicin (DOX) Loading on FePO₄ Nanoparticles**

Doxorubicin was loaded in FePO₄ nanoparticles by the co-incubation-precipitation method. Three different DOX-FePO₄ NPs formulations were explored to optimize the best loading efficiency. In the first formulation, DOX-FePO₄ NPs were formulated by adding 100 µg DOX in 1 mL of Fe(NO₃)₃ (8 mg/mL) followed by the addition of 1 mL of (NH₄)₃PO₄ (16 mg/mL) dropwise under constant stirring. In the second formulations, 100 µg DOX was first added to 1 mL of (NH₄)₃PO₄ (16 mg/mL) followed by the addition of 1 mL of Fe(NO₃)₃ (8 mg/mL) dropwise under constant stirring. In the third formulations, 100 µg DOX was added to FePO₄ NP solution. Thus formulated FePO₄-DOX NPs were washed three times with water and the amount of doxorubicin in FePO₄-DOX was quantified spectrofluorimetrically by measuring DOX excitation and emission at 490 nm and 595 nm.

DOX loading efficiency was calculated by the following equation:

\[
\%
\text{Loading efficiency} = \left( \frac{\text{DOX present in FePO}_4 - \text{DOX NP}}{\text{Initial input of DOX}} \right) \times 100
\]

**Biocompatibility of FePO₄ NPs and Cytotoxicity of FePO₄-DOX NPs**

Biocompatibility of FePO₄ NPs and cytotoxicity of FePO₄-DOX NPs were assayed in mouse osteosarcoma K7M2 and mouse fibroblast NIH/3T3 using MTT assay following established protocol [48, 49]. Briefly, 10,000 cells were seeded in 96 well plates and incubated for 24 h in a 37 °C 5% CO₂ incubator. Then, media was removed and fresh media with varying concentrations of nanoparticles were treated to cells and left for incubation for 48 h. After NP incubation, media was removed and cells were incubated with MTT solution (0.5 mg/ml) in serum-free media for 2 h to allow for the formation of formazan crystal. MTT solution was removed and formazan crystal was dissolved in DMSO and left for 15 min at room temperature for proper mixing. Then the absorbance of DMSO solution was measured at 550 nm using a microplate reader (BioTek, Synergy H1 Hybrid Reader) and percentage cell viability was calculated.
Cellular Internalization via Confocal Microscopy

Cellular internalization of FePO₄-DOX NPs was analyzed in mouse osteosarcoma K7M2 cells using confocal microscopy [49–51]. Briefly, 12,000 cells were seeded in 8-well plates and incubated for 24 h in 37 °C 5% CO₂ incubator. Then, 200 µL of 5 µg/mL DOX concentration in media were treated for 3 h, and cells were fixed with 4% Paraformaldehyde for imaging. The nucleus was stained by DAPI and cells were observed under a Confocal Laser Scanning Microscope (Carl Zeiss, LSM-700). Here, the emission maximum of DOX at 560 nm can be exploited to track its internalization which gives red color in confocal microscopy. Using the same protocol, a time-dependent internalization assay was performed by incubating FePO₄-DOX NPs and Free DOX for 0.5, 1, and 3 h respectively.

RNA Stability and Expression

Torula yeast RNA (Sigma-Aldrich) was dissolved at 1 mg/ml in sterile deionized water and 2 µg aliquots exposed to 20 µg/mL nanoparticle (CNT, Cu, Ag, ZnO NP or FePO₄) incubated at 37 °C and assayed over time by RNA agarose gel electrophoresis as we have previously reported [42, 52]. Timepoint shown in Fig. 5 is overnight. Similarly, the RNA with/without nanoparticles was exposed to 10% FBS/DMEM and again assayed by RAGE as above. mRNA fluc was obtained from Trilink Biotechnologies, 2 µl were incubated in rabbit reticulocyte supplemented with Methinine, Cysteine and Leucine (ProMega Corp) for 30 degrees for 1.5 h with or without nanoparticle at 20 µg/ml, standard Luciferin reagent added, and luminescence measurement taken on a Biotek Synergy H1 plate reader under standard conditions.

Statistical Analysis

All data represents at least three independent replicates and expressed as mean ± s.d. whenever possible. Cell viability data includes six replicates.

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Authors’ Contributions

SA and RD conceived the study and discussed with SR and SW. SR and SW conducted all the experiment in supervision of SA and RD. SR and SA prepared the manuscript. RD finalized RNA part. SA and RD edited the final manuscript with input from all authors. All authors read and approves the final manuscript.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

Authors declare no conflict of interest.
16. Bulte JWM, Kraitchman DL (2004) Iron oxide MR contrast agents for molecular and cellular imaging. NMR Biomed 17:484–499. https://doi.org/10.1002/nbm.924
17. Jiang Y, Hsu S, Hardie J, Jiang X-J, Rotello VM (2016) Progress and perspective of inorganic nanoparticle-based sRNA delivery systems. Expert Opin Drug Deliv 13:547–559. https://doi.org/10.1517/17425247.2016.1134486
18. Shu Y, Pi F, Sharma A, Rajabi M, Haque F, Shu D, Leggas M, Evers BM, Guo P (2014) Stable RNA nanoparticles as potential new generation drugs for cancer therapy. Adv Drug Deliv Rev 66:64–89. https://doi.org/10.1016/j.addr.2013.11.006
19. Storhoff JJ, Elghanian R, Mirkin CA, Letsinger RL (2002) Sequence-dependent stability of DNA-modified gold nanoparticles. Langmuir 18:6666–6670. https://doi.org/10.1021/la0202428
20. DeLong RK, Reynolds CM, Malcolm Y, Schaeffer A, Severs T, Wanekaya A (2012) Synthesis of amorphous iron phosphonate nanoparticles and on ironic liquid-modified Fe3O4 nanoparticles. Microchim Acta 181:45–53. https://doi.org/10.1007/s00604-013-1040-5
21. Abarca-Cabrera L, Fraga-García P, Berensmeier S (2021) Bio-nano interactions: binding proteins, polysaccharides, lipids and nucleic acids onto magnetic nanoparticles. Biomater Sci 25:12. https://doi.org/10.1039/s40824-021-00212-y
22. Vollmer N, Ayers R (2012) Decomposition combustion synthesis of calcium phosphate powders for bone tissue engineering. J Mater Chem B 9:4793–4803. https://doi.org/10.1039/D1TB00551K
23. Bhatia J, Jakesova M, Kroutilova V, Zlokarnik J, Kvasnicka B (2010) Functionalized gold nanoparticles for the binding, stabilization, and delivery of therapeutic DNA, RNA, and other biological macromolecules. Nasa 3:53–63. https://doi.org/10.2147/NSA.19984
24. Gammella E, Maccarini F, Buratti F, Recalcati S, Wang M (2014) The role of iron in anthracycline cardiotoxicity. Front Pharmacol 5:25. https://doi.org/10.3389/fphar.2014.00025
25. Hereshko C, Link G, Tzahor M, Kaltwasser JP, Athias P, Grynberg A, Pinson P (1993) Anthracycline toxicity is potentiated by iron and inhibited by deferoxamine: Studies in rat heart cells in culture. J Lab Clin Med 122:245–251. https://doi.org/10.1016/0022-3495(93)00707F
26. Chen W, Glackin CA, Horvitz MA, Zlokarnik J (2019) Nanomachines and other caps on mesoporous silica nanoparticles for drug delivery. Acc Chem Res 52:1531–1542. https://doi.org/10.1021/acs.accounts.8b00309
27. Friedman AD, Kim D, Liu R (2015) Highly stable aptamers selected from a 2'-fully modified GmNH RNA library for targeting biomaterials. Biomaterials 36:110–123. https://doi.org/10.1016/j.biomaterials.2014.08.046
28. Reyes-Reveles J, Sedaghat-Herati R, Gahm MG, Ghosh KC, Greene TD, Gann HE, Dowler WA, Kramer S, Dean JM, DeLong RK (2013) mPEG-PAAMAM-G4 nucleic acid nanocomplexes: enhanced stability, in vivo protection, and activity of splice switching oligomer and poly I:C RNA. Biomacromol 14:4108–4115. https://doi.org/10.1021/bm4012425
29. Jiang S, Eltoukhy AA, Love KT, Langer R, Anderson DG (2013) Lipid-coated iron oxide nanoparticles for efficient DNA and sRNA delivery. Nano Lett 13:1059–1064. https://doi.org/10.1021/nl304287a
30. Jiang S, Eltoukhy AA, Love KT, Langer R, Anderson DG (2013) Lipid-coated iron oxide nanoparticles for efficient DNA and sRNA delivery. Nano Lett 13:1059–1064. https://doi.org/10.1021/nl304287a
31. Johnson BJ, Melde BJ, Dinderman MA, Lin B (2012) Stabilization of RNA through absorption by functionalized mesoporous silicate nanospheres. PLoS ONE 7:e50356. https://doi.org/10.1371/journal.pone.0050356
32. Hentze MW, Muckenthaler MU, Andrews NC (2004) Balancing acts: Changes in aprotic solvent systems. Inorg Chem 40:5324–5333. https://doi.org/10.1021/ic0002723
33. Hershko C, Link G, Tzahor M, Athias P, Grynberg A, Pinson P (1993) Anthracycline toxicity is potentiated by iron and inhibited by deferoxamine: Studies in rat heart cells in culture. J Lab Clin Med 122:245–251. https://doi.org/10.1016/0022-3495(93)00707F
34. Rayamajhi S, Nguyen TDT, Marasini R, Aryal S (2019) Macrophage-derived exosome-mimetic hybrid vesicles for tumor targeted drug delivery. Adv Mater 31:110804. https://doi.org/10.1002/adma.2019110804
35. Johnson BJ, Melde BJ, Dinderman MA, Lin B (2012) Stabilization of RNA through absorption by functionalized mesoporous silicate nanospheres. PLoS ONE 7:e50356. https://doi.org/10.1371/journal.pone.0050356
36. Rayamajhi S, Marasini R, Aryal S (2019) Macrophage-derived exosome-mimetic hybrid vesicles for tumor targeted drug delivery. Acta Biomater 94:2482–2491. https://doi.org/10.1016/j.actbio.2019.05.054
37. Rayamajhi S, Marasini R, Nguyen TDT, Plattner BL, Biller D, Aryal S (2020) Effective tumor delivery of macromolecules: a review of nanotechnology. Adv Drug Deliver Rev. https://doi.org/10.1016/j.addr.2020.12.009
38. Rayamajhi S, Marasini R, Nguyen TDT, Plattner BL, Biller D, Aryal S (2020) Effective tumor delivery of macromolecules: a review of nanotechnology. Adv Drug Deliver Rev. https://doi.org/10.1016/j.addr.2020.12.009
39. Rayamajhi S, Marasini R, Nguyen TDT, Plattner BL, Biller D, Aryal S (2020) Effective tumor delivery of macromolecules: a review of nanotechnology. Adv Drug Deliver Rev. https://doi.org/10.1016/j.addr.2020.12.009
30. Chen W, Glackin CA, Horvitz MA, Zlokarnik J (2019) Nanomachines and other caps on mesoporous silica nanoparticles for drug delivery. Acc Chem Res 52:1531–1542. https://doi.org/10.1021/acs.accounts.8b00309
31. Friedman AD, Kim D, Liu R (2015) Highly stable aptamers selected from a 2'-fully modified GmNH RNA library for targeting biomaterials. Biomaterис 36:110–123. https://doi.org/10.1016/j.biomaterials.2014.08.046
32. Reyes-Reveles J, Sedaghat-Herati R, Gilley DR, Schaeffer AM, Ghosh KC, Greene TD, Gann HE, Dowler WA, Kramer S, Dean JM, DeLong RK (2013) mPEG-PAAMAM-G4 nucleic acid nanocomplexes: enhanced stability, in vivo protection, and activity of splice switching oligomer and poly I:C RNA. Biomacromol 14:4108–4115. https://doi.org/10.1021/bm4012425
33. Jiang S, Eltoukhy AA, Love KT, Langer R, Anderson DG (2013) Lipid-coated iron oxide nanoparticles for efficient DNA and sRNA delivery. Nano Lett 13:1059–1064. https://doi.org/10.1021/nl304287a
34. Nguyen TDT, Masrini R, Rayamajhi S, Aparicio C, Biller D, Aryal S (2020) Erythrocyte membrane-conjugated paramagnetic polymeric nanoparticle for contrast-enhanced magnetic resonance imaging. Nanoscale 12:4137–4149. https://doi.org/10.1039/DONR00039F
35. Delong RK, Dean J, Glaspell G, Jaberi-Douraki M, Ghosh K, Davis D, Monteiro-Riviere N, Chandran P, Nguyen T, Aryal S, Siddiqua CR, Chan Park S, Choi S-O, Ramani M (2020) Amino/amido conjugates from to nanoscale cobalt phiosystemeocomposite (PMC) materials functionally delivering nucleic acid therapeutic to nucleus enhancing anticancer activity via Rasa-targeted protein inference. ACS Appl Biol Mater 3:175–179. https://doi.org/10.1021/acsabm.9b00798

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