Novel Mg-Al Layered Double Hydroxide-Fe₃O₄ Magnetic Nanohybrids for Efficient Thermo-chemo Therapy of Cervical Cancer

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ABSTRACT SUMMARY

Our objective is to synthesize Mg-Al layered double hydroxide (LDH) decorated with Fe₃O₄ nanoparticles (MNPs) to fabricate magnetic nanohybrids (MNHs). The biocompatible LDH together with the MNPs give rise to a nanosystem with much efficiency as more cargo carriers. The performance of these MNHs to anchor and release doxorubicin (DOX) was assessed in addition to its hyperthermic fatal activity towards the cervical cancer cells. These MNHs were used to combine the classic chemotherapy with hyperthermia to design a combinatorial anticancer therapy having synergistic effects.

INTRODUCTION

Layered double hydroxides (LDHs) are a class of anionic clay minerals with the general formula \([M^{2+}_{1-x}M^{3+}_x(OH)_{2}]\cdot[A^{n-}·mH₂O]\), where \(M^{2+}\) and \(M^{3+}\) are divalent and trivalent metal cations respectively and \(A^{n-}\) is the interlayer anion [1]. LDHs have been immensely used by researchers for various applications viz nanocarrier systems, water purification, catalysis and supercapacitors [2]. Similarly, amongst a vast variety of inorganic nanoparticles, MNPs have proven themselves to be an excellent choice for various biomedical applications due to their specific nanostructure, tailored properties, lower toxicity and immunogenicity [3].

A hybrid nanomatериал comprising of LDH and MNPs is, thus, explored in the current study. The MNHs were fabricated using a simplified approach described earlier by Chen et al [4]. Our objective is to evaluate the performance of these MNHs as drug carrier as well as heating platform towards a more effective cancer therapy.

EXPERIMENTAL METHODS

Mg-Al LDH and citric acid functionalized MNPs were synthesized using methods described in previous literature with minor modifications [4, 5]. To synthesize LDH, Mg:Al nitrates (molar ratio 3:1) were quickly added to 0.3M NaOH and hydrothermally treated at 100 °C for 16 hrs in an autoclave. MNPs were synthesized by co-precipitation method according to the literature [5]. For a typical synthesis, 2:1 molar ratio of \(Fe^{3+}:Fe^{2+}\) were precipitated by ammonia solution under inert atmosphere at 90 °C. The MNPs were functionalized by citric acid in situ. The MNHs were prepared by simple non-covalent electrostatic interactions between the cationic LDH and anionic MNPs (mass ratio 2:1) under sonication.

These MNHs were assessed for their efficacy to anchor and release DOX as model anticancer drug. Various amount of DOX was incubated with different amount of MNHs under shaking conditions and the drug loading efficiency was calculated. The drug release was performed under reservoir-sink conditions mimicking the cellular environment of lysosome and cytoplasm using sodium acetate buffer (pH 5; reservoir) as pH stimulus and phosphate buffer saline (pH 7.4; sink) as release media. The drug release efficacy was calculated by the measurement of fluorescence of aliquots from the sink at different time intervals.

The drug conjugated MNHs were evaluated for their performance for inhibition of cell proliferation against various cell lines (L929 and HeLa). In order to establish the anticancer potential of these DOX loaded MNHs, a dose dependent study was undertaken to evaluate the 50% inhibitory concentration value (IC₅₀) for a period of 24 hrs.

RESULTS AND DISCUSSION

The XRD pattern in Fig. 1 (a) revealed the formation of single-phase Fe₃O₄ nanoparticles, LDH crystal with rhombohedral symmetry and biphasic MNHs. Fig. 1 (b) and (c) show the FEGTEM images of LDH and nanohybrid with their electron diffraction patterns which are in good agreement with XRD studies.

![Figure 1](image-url)

Figure 1. (a) XRD patterns, (b) and (c) FEG-TEM images and SAED patterns (inset) of Mg-Al LDH, Mg-Al LDH-Fe₃O₄ nanohybrids, respectively.

The field dependent magnetization of MNPs and MNHs was recorded at 300 K and the curve exhibited the superparamagnetic behavior with a saturation
magnetization of 69 and 34 emu/g respectively (Fig. 2 (a)). The comparison of the heating ability of MNPs with MNHs was evaluated in the presence of alternating current magnetic field (ACMF: Fig. 2 (b)) and its SAR was calculated to be 98.4, 73.5 W/g. The addition of non-magnetic LDH to the MNPs hinders the orientation of its magnetic spin in the ACMF. This lowers the specific absorption rate of the MNHs which, in turn, increases the time required by the nanomaterials to reach the hyperthermia temperature of 45 °C.

The drug was conjugated onto the formulations by the spontaneous electrostatic interactions as depicted by the Stern-Volmer plot and the loading percentage was calculated to be ~98%. Fig. 3 (a) shows the fluorescence intensity profile of DOX with varying amounts of MNHs. The drug binding was further investigated by the non-linear Gaussian profiles of DOX in presence of MNHs. The ratios of area under the deconvoluted curves show significant difference which confirms the binding of the drug with MNHs (Fig. 3(b)).

The drug release profile suggested that the DOX was released slowly under acidic pH for initial 6 hrs and attained a plateau after 10 h. The maximum drug release observed was 63% over a period of 30 hrs. The release of the drug molecules is initiated due to the weakening of the electrostatic interactions between the drug and the MNHs as the lowering of the pH leads to an increase in the protons in the colloidal solution. The MNHs do not hinder the cell growth and had minimal effect on cellular morphology indicating their biocompatibility towards the cell lines for concentrations as high as 2 mg/ml. The inhibition of cell proliferation is observed for the MNHs on the HeLa cell line. The IC_{50} values of the DOX-loaded MNHs was fitted using the dose dependent sigmoidal curve of Origin 8 software and was calculated to be 0.99 mg/ml (R^2=0.9873).

The cell internalization study of the MNHs by the HeLa cells was done by tagging the nanomaterials with FITC. Fig. 4 shows the laser scanning confocal microscopy images of HeLa incubated with FITC-tagged MNHs after 3 hrs. The scale bar is 20 µm.

The fatal effect of magnetic hyperthermia on HeLa cells was assessed. Fig. 5 shows that with increase in the exposure time of ACMF the live cell population decreases.

**CONCLUSIONS**

An effort has been made in the direction of fabrication of “smart” nanomaterials for cancer therapy. The results confirm the fabrication of MNP decorated LDH nanohybrids which were successfully used for DOX loading and release and exhibited a sustained drug release profile. Evaluation of the SAR efficacies demonstrated the potential of MNHs in cancer therapeutics. The fatal effect of hyperthermia on HeLa cells promises the avenues of much more successful anticancer therapy.

**REFERENCES**


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