Chapter 6

Conclusion and Future Direction

This chapter describes brief conclusion of my whole research work presented in the thesis and end emphasizes the implications of these works towards future direction.

6.1 Conclusion

The thesis presents synthesis, characterization and evaluation of various layered double hydroxides based nanocarriers for drug and gene delivery to treat cancer. Layered double hydroxides (LDHs), also known as anionic or hydrotalcite-like clays, are a class of two-dimensional (2-D) dimensional nanostructured inorganic materials consisting of positively charged layers of metal hydroxides with charge-balancing hydrated anions located in the interlayer gallery. The chemical composition of LDHs are represented by the general formula $[M^{II}_{1-x}M^{III}x\ (OH)_2][A^{n-}_{x/n}\ .\ yH_2O]$, where M^{II} and M^{III} represent di- and trivalent metal cations, and A^{n-} is an interlayer anion. The properties which make LDHs as an excellent delivery vehicle are their high anion-exchange capacity, tunable interior architecture, two-dimensional structure, high surface area, pH responsive release of biomolecules, positive surface charge on the surface, and resistant to changes upon heating. Conventional chemotherapy strategies hold great promise for cancer treatment. However, efficient intracellular delivery of these drugs still remains a major challenge to clinical translation. During my PhD work I was trying to design and develop layered double hydroxides based nanocarriers to advance drug and gene delivery by overcoming the drawbacks of conventional chemotherapy.

We have synthesized a series of Mg-Al based LDHs with varying interlayer anions (NO_3^-, CO_3^{2-} and PO_4^{3-}) by using coprecipitation technique. A model antitumor drug, raloxifene hydrochloride (RH) has been intercalated into the gallery space of LDHs using anion-exchange technique. *In vitro* controlled drug delivery has been achieved with very fast rate in phosphate bound LDH-drug (LP-R) while sustained profile is obtained with nitrate based LDH (LN-R). This variation in drug delivery rate has been understood from the interactions between drug molecule and LDHs through XPS and UV-vis analysis and ordered-disordered structure of drug-LDH nanohybrids, obtained using XRD analysis.

Stronger interactions between drug molecule and LDHs host layers lead to sluggish delivery in LN-R against relatively weak interaction in LP-R results fast release. *In vitro* anticancer performance studies exhibited that drug intercalated LDHs efficiently inhibit the growth of HeLa cells as compared to pure drug. Again, amongst the drug intercalated LDHs, LP–R shows better *in vivo* tumor suppression efficacy while body weight index indicates the damage of organs. On contrary, LN-R exhibits relatively slow healing of tumor while it has minimum body weight loss indicating a better drug delivery vehicle. Histopathological analysis of major organs strongly suggest damaged liver of mice treated with fast release vehicles (pure drug and LP-R) while no obvious damage has been found in mice liver cell treated with LN-R, slow release vehicle. Moreover, analyses of biochemical parameters also suggest that drug intercalated LDHs have less toxic effects compared to pure drug. Thus, the developed LDH based drug delivery vehicle has the potential to release the drug in a controlled manner without having any adverse side effects of anti-cancer drug.

In another study, a new anticancer drug delivery vehicle has been developed after intercalating the drug within the interlayer galleries of two-dimensional layered double hydroxide through anion exchange technique followed by embedding them in a polymer matrix, called polymer nanoconjugate. The dispersion of drug intercalated LDH in polymer matrix is found to be uniform and surface charge (ζ-potential) and roughness of the polymer nanoconjugate has been adjusted with the help of LDH. Drug release rate has been found to more sustained with decreasing charge density of LDH intercalants from PO_4^{-3} to CO_3^{-2} to NO_3^- and significant sustained profile is achieved from the polymer nanoconjugate. The gradual increase of interaction between the drug molecules and LDH host layers in three different LDHs (ZP-R < ZC-R < ZN-R) is examined using XPS, UV-Vis, FTIR and DSC measurements and this increased amount of interaction is

reflected in their respective sustained drug delivery profile (higher amount of interaction leads to more sustained drug release). Polymer nanoconjugate (PN–R) demonstrates the best *in vitro* anticancer efficiency as compared to pure drug or drug embedded polymer complex (PCL-RH) as evident from *in vitro* cytotoxicity experiment like cell viability. Cell adhesion behavior in PN–R is found to be superior compared to pure polymer or even from pure LDH systems. Polymer nanoconjugate exhibits better cellular uptake compared to pure drug and pure LDH. Sustained release of drug for prolonged period of time has been obtained *in vivo* system using albino rats showing healthy lever and other body parts using polymer nanohybrid against damaged lever using pure drug as evident from the histological, lever and renal functional tests. Thus, the developed polymer LDH nanoconjugate vehicle has been found to enhance the therapeutic efficacy of anti-cancer drug while reducing its adverse side effects.

We have synthesized Li-Al based LDH by a facile coprecipitation technique for the delivery of plasmid gene into mammalian cancer cell. Li-Al based LDH showed high loading capacity of DNA and can release the loaded DNA in controlled manner. The developed gene delivery carrier showed remarkable protection against DNase I and also provide protection against thermal damage. This vehicle also demonstrated efficient cellular uptake performances. Transfection and expression of plasmid gene encoding GFP proteins was successfully achieved by this LDH based vehicle. Again, it is found that the developed Li-Al LDH efficiently induced GFP-p53 mediated apoptosis in HeLa cells.

6.2 Future Direction:

Implications and future direction of my work are as follows:

(1) Synthesis of different LDHs with varying particle size and morphology to evaluate the effect of these parameters on therapeutic efficacy.

(2) *In vivo* anticancer efficacy study of the polymer LDH nanoconjugate system can be investigated further using animal model to validate the results obtained from cell line studies.

(3) These nanocarriers can be modified with active targeting ligands such as folic acid, biotin, monoclonal antibodies etc. to achieve more site specific delivery.

(4) The developed novel delivery carriers can be taken further for clinical trials.