CHAPTER 8

SUMMARY AND CONCLUSION

This chapter summarizes the results and describes the conclusion of the study. This also describes the scope for future development in this field. A nove approach for utilization of metallic nanoparticles and its successful demonstration for efficient delivery of biological were investigated.

SUMMARY AND CONCLUSION

8.1 Summary

The work taken up in this thesis relates to the realization of potential biomedical applications of nanomaterials. The nanomaterial considered here are metal nanoparticles, especially gold. Gold nanoparticles have been gaining the fame as unique drug delivery vehicles due to their distinctive shape, size and surface dependent properties. With a multitude of opportunities for gold nanoparticles use in pharmaceutical and medical applications, it becomes necessary to have a system which is water soluble, aggregation resistant, and biocompatible and does not include toxic chemicals in the synthesis protocol. in this context, we have initiated our explorations to synthesize and stabilize gold nanoparticles using a microbial polysaccharide gellan gum. The anticancer drug, doxorubicin hydrochloride, was loaded on gold nanoparticles and evaluated for its cytotoxic effect against various cell lines.

The present study was summarized as follows:

8.1.1 Synthesis of gold nanoparticles using gellan gum as reducing and stabilizing agent

Gellan gum being natural in its origin is widely accepted in Food and Pharmaceutical industry for various applications. In the present study, we took advantage of its biocompatible properties and used it for the synthesis of gold nanoparticles. The gold nanoparticles synthesized were thoroughly characterized by a suite of techniques including UV/Vis, XRD, TEM, zeta potential and stability studies. The gold nanoparticles synthesis was possible even at very low concentration of gellan gum (0.02% w/v) and the band corresponding to the surface plasmon resonance occurred at 520 nm. The nanoparticles were spherical in shape and had a significantly narrow size distribution with an average size of 14 nm. The gellan gum reduced gold nanoparticles were found to be negatively charged. Gold nanoparticles showed no discernible change in the intensity or position of the absorbance at -520 nm in the pH window of 4-12 and even the addition of NaCl upto 0.1 M caused no major aggregation. The stability of nanoparticles monitored over a period of three months showed that gold nanoparticles synthesized at different concentrations of gellan gum (0.02% w/v to 0.1% w/v) had no shift in surface plasmon peak, indicating that nanoparticles formed were stable and without aggregation during the stability period. Moreover, there was no change in the spray dried gold nanoparticles in terms of surface plasmon resonance, TEM and long term stability.

8.1.2 Cellular uptake and *in vivo* oral toxicity studies of gellan gum reduced gold nanoparticles

Both *in vitro* and *in vivo* toxicity studies of nanoparticles prepared by any unprecedented method needs to be thoroughly examined before they are exposed to human beings. This will help to better understand the -correlation between nanoparticles diameter, surface chemistry, interactions and transport, leading to the best possible choice of systems for drug delivery and diagnostic applications. We studied the cellular uptake of gold nanoparticles on human glioma cell line, LN-229. The toxicity of the gold nanoparticles was evaluated by carrying out subacute 28 days oral toxicity studies in rats. The detailed haematology, clinical biochemistry and organ histology of animals were evaluated at different concentrations (75 ppm, 150 ppm and 300 ppm) of the colloidal gold dispersion. In the cancerous cells, gold nanoparticles were localized mainly in the cytoplasm and perinuclear region of the cells. Oral administration of gold nanoparticles into animals did not cause any significant difference in haematological and biochemical assays of the control and gold nanoparticles treated groups. The weight and normal architecture of various organs did not change compared to the control group. These findings while establishing the uptake of nanoparticles into cancerous cells also demonstrates that the gellan gum reduced gold nanoparticles were devoid of toxicity in animals following oral administration.

8.1.3 Development of gellan gum reduced gold nanoparticles as a carrier for delivery of anticancer drug, doxorubicin hydrochloride

Colloidal gold nanoparticles incorporating anticancer agents can overcome resistances to drug action, thus reducing the need for higher doses and therefore reducing their toxicity towards normal cells. We explored gellan gum reduced gold nanoparticles as carrier for delivery of anticancer drug doxorubicin hydrochloride. The doxorubicin loaded gold nanoparticles were characterized in terms of size, zeta potential, drug loading efficiency, fluorescence studies and stability studies. In addition, the in vitro cytotoxicity of the doxorubicin loaded gold nanoparticles was evaluated using two different well established glioma cell lines i.e. LN-18 and LN- 229. The loading efficiency of doxorubicin loaded gold nanoparticles was determined to be 75%. It was observed that the doxorubicin loaded gold nanoparticles were stable between pH 4.0 to 8.0. TEM images recorded from the doxorubicin loaded gold nanoparticles indicated that the overall structure of the nanoparticles and their assemblies were not different in comparison to blank gold nanoparticles. The fluorescence studies indicated that there was no major change in the spectral profile in doxorubicin loaded gold nanoparticles. The spray dried doxorubicin loaded gold nanoparticles were free flowing without any aggregation or change in color.

Cytotoxicity effect of blank gellan gum reduced gold nanoparticles was not observed on LN-18 and LN-229 cell lines. Doxorubicin loaded gold nanoparticles exhibited strongly enhanced cytotoxicity as compared to free doxorubicin solution. In case of LN-18, the highest concentration 15.0 μg/mL of doxorubicin loaded gold nanoparticles was able to decrease the cell viability to 5% at the end of 48 h as compared to free doxorubicin solution (40%o at the end of 48 h). In the case of human glioma cell line LN-229, at the end of 48 h incubation, the cell viability at highest concentration of 12.5 μg/mL of doxorubicin loaded gold nanoparticles was 10% compared to 24% in the case of free doxorubicin solution. Confocal images clearly demonstrated the apoptosis induced cell death by doxorubicin loaded gold nanoparticles on human glioma cell lines LN-18 and LN-229.

8.1.4 Development and evaluation of doxorubicin hydrochloride loaded sophorolipid and poly (ethylene glycol) conjugated gold nanoparticles

To perk up the function of gold nanoparticles as drug delivery vehicle, we studied the bioconjugation of two different capping agents on gold nanoparticles. In the first approach, sophorolipid was conjugated with gold nanoparticles and in the second approach, the gold nanoparticles were first conjugated with sophorolipid and then with poly (ethylene glycol). We studied the efficient manipulation of established human glioma cell line LN-229 and more significantly of the human glioma stem cell line HNGC-2 by sophorolipid conjugated gold nanoparticles, poly(ethylene glycol)- sophorolipid conjugated gold nanoparticles, doxorubicin loaded sophorolipid gold nanoparticles and doxorubicin loaded poly(ethylene glycol)-sophorolipid conjugated gold nanoparticles. The apoptosis induced cell death due to doxorubicin loaded nanoparticles was studied by using confocal microscope. No broadening and red shift of the surface plasmon resonance was observed after conjugation of sophorolipid and poly (ethylene glycol) to gold nanoparticles. The sophorolipid gold nanoparticles dispersion did not show any discernible change in the position at 520 nm in the pH window of 4-12. The addition of electrolyte also did not cause any aggregation in the sophorolipid gold nanoparticles. In case of poly (ethylene glycol)-sophorolipid conjugated gold nanoparticles, the dispersion was stable over a pH range of 2-12 and up to salt concentration of 10^{1}M. Sophorolipid and poly (etnylene glycol)-sophorolipid conjugated gold nanoparticles had an average particle size of 17 nm and 22 nm respectively. Cellular uptake using confocal microscopy showed that the nanoparticles were efficiently internalized in tumor cells within 3 h of incubation and localized in the cytoplasm and perinuclear region of the cells. The loading efficiency of doxorubicin loaded sophorolipid and poly(ethylene glycol)- sophorolipid conjugated gold nanoparticles was determined to be 85% and 92% respectively. There was no major change in the emission profile from doxorubicin in doxorubicin loaded sophorolipid and poly(ethylene glycol)-sophorolipid conjugated gold nanoparticles. The spray dried doxorubicin loaded sophorolipid and poly(ethylene glycol)-sophorolipid conjugated gold nanoparticles was found to be stable during its storage period of 3 months with no change in the fluorescence signature. Sophorolipid conjugated gold nanoparticles decreased the cell viability to 50% after 48 h of incubation in human glioma cell line as well as stem cell line. By the end of 48 h of incubation, at highest concentration of 12.5 μg/mL, the viability of LN-229 cell reached 2% for doxorubicin loaded sophorolipid gold nanoparticles compared to 16% of free doxorubicin solution. In case of HNGC-2, the maximum cell viability achieved via 12.5 pg/mL doxorubicin at the end of 48 h was 32% for free doxorubicin solution and 9% for doxorubicin loaded sophorolipid gold nanoparticles. In case of human glioma cell line LN-229, after 48 h of incubation, doxorubicin loaded poly (ethylene glycol)-sophorolipid conjugated gold nanoparticles (at concentration of 12.5 pig/mL) were able to decrease the cell viability to 9% as compared to 25% for free doxorubicin solution. In case of human glioma stem cell line HNGC-2, at the end of 48 h incubation, the cell viability via free doxorubicin solution at the concentration range studied (1.0 pg/mL to 12.5 pg/mL) was from 62- 28% and for doxorubicin loaded nanoparticles, the decrease was from 53-15%. The doxorubicin loaded sophorolipid and poly(ethylene glycolj-sophorolipid conjugated gold nanoparticies clearly induced apoptosis on both glioma cell lines.

8.1.5 Tumor Microvessel Density (Tumor Angiogenesis) Evaluation of doxorubicin hydrochloride conjugated gold nanoparticles

Mouse Melanoma tumor model was used in order to evaluate antitumor effects of in vivo. After the administration of total 7.2 mg/kg of doxorubicin-gold nanoparticles or doxorubicin alone. there was a significant difference in reduction of tumor growth and ascites formation between doxorubicin-gold nanoparticle and control groups. These results suggest to that although the low dose of Doxorubicin-gold nanoparticles treatment was used in this model, gold nanoparticles could facilitate the potency of doxorubicin. The low dose doxorubicin treatment, however, did not significantly reduce the tumor growth compared to Phosphate buffer control group. Inhibition of ascites formation was observed both in Doxorubicin-Gold nanoparticles, doxorubicin & gold nanoparticles groups that may attribute to the administration of chemotherapeutic agent and the antiangiogenic properties of gold nanoparticles.

The anti-angiogenesis effect was found to be significantly improved (Tukey's HSD test and Dunnett's test, a = 0.05) in the tumors of mice receiving combination drug treatment in comparison to control and individual drug treated animal tumors.

8.2 Conclusion

- A novel approach for utilization of metal nanoparticles and its successful demonstration for efficient delivery of biological molecule were investigated. Highly stabilized gold nanoparticles were obtained by using gellan gum as reducing and stabilizing agent.
- The gellan gum reduced gold nanoparticles were devoid of toxicity in animals following oral administration.
- Doxorubicin hydrochloride loaded gellan gum reduced gold nanoparticles were successfully obtained with good loading efficiency. The doxorubicin loaded gold nanoparticles were effectively cytotoxic against human glioma cell lines LN-18 and LN-229. Doxorubicin loaded gold nanoparticles induced apoptosis on both cell lines.
- Surface functionalization of gold nanoparticles by biosurfactant sophorolipid and poly(ethylene glycol) was successfully attained. The doxorubicin loaded functionalized gold nanoparticles were cytotoxic against human glioma cell line LN-229 as well as human glioma stem cell line HNGC-2.
- We have evaluated the antiangiogenic properties of gold nanoparticles in mouse model. With the combination of doxorubicin chemotherapy, doxorubicin-conjugated gold nanoparticles might provide a synergistic effect for cancer therapeutics. Hence doxorubicin gold nanoparticles provide an alternative drug formulation of conventional doxorubicin for cancer therapy. By using nanoparticles as a drug carrier, Doxorubicin was efficiently transported into cells. With antiangiogenic properties and non toxicity to cells, gold nanoparticles are suitable for delivery of chemotherapeutic agents.

8.3 SCOPE OF FUTURE WORK

These results warrant further investigation to reveal the *in vivo* cytotoxic capacity of doxorubicin loaded gold nanoparticles in cancer therapy.

The carbohydrate rich gold nanoparticles surface may facilitate better transportation of the loaded drug across blood brain barrier. To demonstrate this hypothesis, brain uptake studies need to be investigated. Further to explore the application for oral delivery, *in vitro* absorption studies on Caco-2 cells needs to be investigated.

As such, these findings are very generic and can be expanded to include the delivery of other biologically active molecules as well.

Specificity of the tumor targeting with gold nanoparticles can be evaluated through integrin targeted peptides attached on the surface of the gold nanoparticles for better therapeutic efficacy.