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学 位 の 種 類	博士（バイオ・ナノサイエンス融合）		
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【論文審査】Review of the thesis

In the thesis, **“Regime of gene silencing: Efficient siRNA delivery into cancer cells using nanocapsules”**, Ms. Archana Raichur presents siRNA delivery into cancer cells by using polymeric hollow nanocapsules (NPs). She used siRNA to downregulate the *MYC* protooncogene in cancer cells using functionalized PLGA NPs. These siRNA encapsulated hollow PLGA nanoparticles were functionalized by cell penetrating peptide and peptide nucleic acid for enhanced cellular uptake. The thesis is divided into six chapters.

In chapter one, **“History of techniques used in siRNA delivery in the field of nanomedicine”** is discussed. The potential ability of siRNA to silence abnormal gene expression created a new horizon in the field of gene therapy. Until now, different types of nanoparticles like organic, inorganic and hybrid nanoparticles were used in siRNA delivery. These nanoparticles have proved effective in siRNA delivery. The major hindrances in the application of siRNA delivery are its stability and its effective *in vivo* delivery. Apart from these, renal clearance, uptake by phagocytic cells, aggregation with serum proteins and enzymatic degradation are certain other factors that affect systemic siRNA delivery. To overcome these barriers, certain techniques like layer-by-layer (LBL) assembly, encapsulation and PEGylation techniques are used for enhanced siRNA delivery.

In chapter two, Archana discusses the **"Techniques for characterization of nanomaterials."** In this chapter she discussed the principle of the instrumentation used for characterization of the nanomaterials in her research. The basic understanding and analysis of the physiochemical properties of the nanomaterials are essential to acquire insight into the unique properties of nano-scaled materials when compared to bulk counterparts. In addition to their material properties, the application in bio-nano interface was also analyzed. She has used electron microscopy, spectroscopic studies and molecular biology techniques for characterization of the nanoparticles.

In chapter three, **"Importance of gene therapy and use of polymeric nanoparticles in siRNA delivery"** is described. RNAi (RNA interference) is a fundamental pathway in eukaryotic cells by which sequence of specific siRNA is able to cleave complementary mRNA. RNAi is triggered by the presence of long strands of double stranded siRNA, which are cleaved into fragments by enzyme DICER. In practice, these double stranded RNA are synthetically produced and introduced directly inside the nucleus of cell. Therefore, the use of siRNA will not affect the other genes and their functions. Owing to this potential, siRNA delivery technique is the future of upcoming scientific and therapeutic studies. It is one of the reliable and effective tools that can be used for treatment of various diseases. The *in vivo* delivery of siRNA in its active form is complex and multistep process. The therapeutic siRNAs have to overcome RNase mediated degradation and renal clearance, and enter the target cells by endocytosis, followed by nuclear uptake. Nanotechnology has been adopted to overcome these barriers faced by unprotected siRNA when administered intravenously. Also, nanotechnology enhances siRNA delivery to become more efficient. Polymeric nanoparticles are biodegradable, biocompatible and have the capacity to encapsulate siRNA. Therefore polymeric nanoparticles are widely used in siRNA delivery oriented research. The application of nanotechnology and gene delivery together conquers many hurdles in curing various diseases. The chapter three deals with the significance of gene delivery in cancer cells and polymeric nanoparticles used in gene delivery. The need for siRNA delivery is also highlighted in this chapter.

In chapter four, **"Hollow polymeric PLGA nanocapsules synthesis by solvent emulsion evaporation method for enhanced drug encapsulation and release efficiency"** is reported. Nano-hollow polymer shells have captured the attention of many researchers in the field of pharmaceutical and medical therapeutics. In the field of controlled drug/gene release; nano-capsules in colloidal solutions; i.e., particles with hollow piths play an important role in cargo encapsulation. These NPs are synthesized using a variety of procedures like emulsion polymerization, phase separation, crosslinking of micelles, inner core etching and

self-assembly. The present work proposes a novel route to prepare hollow PLGA NPs (HNPs) , which showed increased drug-encapsulation and release efficiency. Simple emulsion solvent evaporation technique was adopted to synthesize nano- hollow shells. The hollow characteristics of NPs were studied using SEM, TEM and Confocal microscopy analyses. The particle size was analyzed by DLS. Drug (paclitaxel) loading, encapsulation and release efficiency in *in vitro* were assessed by ultraviolet spectroscopy. The developed NPs were hollow and spherical in shape with approximately 80nm in size. The drug encapsulation efficiency is 99.4% and drug was released in controllable manner during *in vitro* analysis. Hollow NPs have great efficiency for gene and drug release as compared with non-hollow NPs. Using targeted moiety and due to their tailored porous structure, high cargo loading and encapsulating efficiency, zero order drug release kinetics and material reliability have been obtained. In chapter four, she reports the synthesis of PLGA hollow nanoparticles which released the drug in controllable manner that can be used as a nano vehicle to deliver gene and drug in cell organelles. The synthesis of hollow PLGA NPs by the single emulsion solvent method is an economical way of synthesis with enhanced drug encapsulation and release efficiency.

In chapter five, she describes "**Strategist PLGA nanocapsules to deliver siRNA for inhibition of carcinoma and neuroblastoma cell lines by knockdown of MYC proto-oncogene using CPP and PNA**". RNA interference and the therapeutic applications using siRNA were discovered more than 10 years ago and currently are used in various applications including in the therapeutic field. However, the research in this field is still in its infancy. Many challenges like safe delivery of targeted siRNA to nucleus and cytosol of cancerous cells without compromising the activity of siRNA need to be addressed. She overcame this hurdle with the help of nanotechnology using PLGA hollow nanoparticles and suppressing the oncogene of *MYC* transcription factors by using anti myc-siRNAs in human cancer cell lines. siRNA was encapsulated in PLGA hollow nanoparticles. These spherical PLGA hollow nanoparticles (PLGAHNPs) of size 70 nm had high efficiency of gene release at pH 4.2 under *in vitro* conditions. Cell penetrating peptide (CPP) - Tat peptide (TAT) and peptide nucleic acid- nucleolus localizing signal (PNA-NLS) were used for functionalizing the nanoparticles without affecting the therapeutic activity of siRNA. In this study, she used layer-by-layer technique to formulate functionalized nanoparticles. The advantages of LBL technique include ease of preparation, versatility, and capability of incorporating controlled high loadings of different types of biomolecules in layers. By taking advantage of this technique, she incorporated layers of TAT peptide and PNA-

NLS on PLGAHNPs of homogenous size below 100 nm (68-70 nm) . These targeting moieties layered on siRNA encapsulated PLGAHNPs enhanced the uptake of siRNA delivery to the targeted cells. The siRNA duplex was prepared using T7 polymerase and double stranded DNA through *in vitro* transcription. Incubation of the siRNA encapsulated PLGAHNPs functionalized with TAT and PNA-NLS (TAT-siRNA-PNA-PLGAHNPs-siRNA) with cancer cells resulted in reduced cell proliferation. A downregulation of gene expression by 90 % was observed even with a low concentration of siRNA. She found complete arrest of cell division which was mediated by downregulation of *MYC* expression. In chapter six, she presents the "**Conclusion**" of her work. In this thesis, "**Regime of gene silencing: Efficient siRNA delivery into cancer cells using nanocapsules**", the polymeric nanoparticle mediated siRNA delivery for the downregulation of protooncogene in cancer has been discussed. She presented the technique of synthesizing hollow PLGA nanoparticles and its capacity for enhancing drug encapsulation and release efficiency. As these hollow PLGA nanoparticles have the potential of encapsulating the drug, she performed experiments on siRNA encapsulation and its release efficiency. These siRNA encapsulated hollow PLGA nanoparticles used in siRNA delivery were able to reduce cancer cell proliferation and downregulate the *MYC* proto-oncogene. These PLGAHNPs may well be used in therapeutic applications as ideal nanocapsules for drug delivery, gene delivery and protein delivery in the future.

【審査結果】Summary and decision

The doctoral thesis entitled "**Regime of gene silencing: Efficient siRNA delivery into cancer cells using nanocapsules**" focuses on the development of hollow polymer nanoparticles to encapsulate SiRNA to deliver into cancer cells for gene therapy. For this, Archana Raichur developed a novel layer by layer technique for safe delivery of SiRNA, first she incorporated SiRNA into hollow PLGA polymer cages and then she used the LBL technique to coat cell penetrating peptide (CPP) TAT peptide and peptide nucleic acid-nucleolus localizing signal (PNA-NLS) for targeted deliver of SiRNA.

The results shown in the thesis are outstanding from an international point of view and the significant points in the present study are summarized below;

(1) A very simple technique has been developed to synthesize hollow PLGA nanoparticles.
(2) Hollow PLGA nanoparticles have been used to encapsulate SiRNA for gene delivery.

Approximately 99% encapsulation efficiency of SiRNA was achieved by this method.

(3) New LBL technique was developed to coat on polymer hollow nanoparticles; Cell Penetrating Peptide and peptide nucleic acid- nucleolus localizing signal (PNA-NLS) so that the SiRNA can be delivered to the target.

(4) It has been found that the developed system could suppress the gene expression, which is the cause of the cancer, by 90% . The result is remarkable.

Archana Raichur has published two first-authoring papers in international journals; Materials Research Express and NanoWorld Journal. She won the best presentation award for her work in an international seminar in USA. Judging by the quality of the research results shown in the thesis, we can assure that the new drug can be a great achievement to fight against the cancer. In conclusion, the quality of the present study and thesis is considered to be very high by international standards and therefore the thesis can be accepted for awarding a doctoral degree to Archana Raichur.