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和訳：癌診断・治療剤としての多機能・生体整合磁性ナノ粒子の開発：癌ナノテクノロジーへの応用
Magnetic nanoparticles have garnered a lot of attention in biomedical and industrial applications owing to their biocompatibility, ease of biofunctionalization and its inherent magnetic properties. The development of multifunctional magnetic nanoparticles with biocompatibility can enhance the interaction and can be exploited for both active and passive imaging, drug delivery and magnetic hyperthermia for the destruction of cancer cells.

The thesis entitled submitted by Mr. B. Sivakumar “Multifunctional biocompatible magnetic nanoparticles as theragnostic agents: applications in cancer nanotechnology” has been divided into 9 chapters, which mainly deals with the development of multifunctional magnetic nanoparticles exploiting the naturally occurring biopolymers for in vitro cellular imaging, efficient drug delivery and hyperthermia studies in cancer cells.

In the 1st chapter of the thesis, Mr. Siva provided an introduction to multifunctional magnetic nanoparticles and their theragnostics applications for simultaneous diagnosis and therapeutics (delivering targeted drugs to cancer specific cells). The development of magnetic nanoparticles, the design of multifunctional magnetic nanoparticles, factors rendering multifunctionality and some of the major biomedical applications with special reference to drug targeting (passive, active and magnetic) and magnetic hyperthermia are provided in
this chapter.
In the 2nd chapter Mr. Siva summarized the theoretical details of tools and instruments that are used for the characterization of nano-sized structures.

In the 3rd chapter, Mr. Siva mentioned about the development of Multifunctional carboxymethyl cellulose-based magnetic nanovector as a theragnostic system for folate receptor targeted chemotherapy, imaging, and hyperthermia against cancer. A multifunctional biocompatible nanovector based on magnetic nanoparticle and carboxymethyl cellulose (CMC) was developed. The results indicate that the multifunctional CMC MNPs possess a high drug loading efficiency and high biocompatibility and with low cell cytotoxicity and can be considered to be promising candidates for CMC-based targeted drug delivery, cellular imaging, and magnetic hyperthermia (MHT).

The 4th chapter deals with the development of bacterial exopolysaccharide coated and stabilized magnetic nanoparticles. Microbial exopolysaccharides (EPSs) are highly heterogeneous polymers produced by fungi and bacteria that have garnered considerable attention and have remarkable potential in various fields, including biomedical research. In this study Mr. Siva has coated magnetic nanoparticles (MNPs) with two bacterial EPS- mauran (MR) and gellan gum (GG). The biocompatibility of EPS coated MNPs was enhanced and he provided the multi-functionality by attaching targeting moiety, incorporating imaging moieties and encapsulated with potent anticancerous drug. The biocompatibility of EPS coated MNPs and synergistic effect of magnetic hyperthermia and drug for enhanced antiproliferation of cancer cells was evaluated. The results suggests that MR and GG coated MNPs exhibited excellent biocompatibility with low cell cytotoxicity, high therapeutic potential, and superparamagnetic behavior that can be employed as prospective candidates for bacterial EPS based targeted drug delivery, cancer cell imaging and for magnetic hyperthermia (MHT) for killing cancer cells within short period of time.

In the 5th chapter, Mr. Siva discusses the synthesis of curcumin encapsulated PLGA-magnetic nanoparticles and their augmented cellular uptake and antiproliferation against pancreatic cancer cells induced by nanoformulation. He has developed a multifunctional nanoconjugate based on PLGA that has been proved to be completely safe by FDA to label, specifically target and efficiently treat tumor cells. Superparamagnetic iron oxide nanoparticles (SPIONs) are used for magnetically targeted drug delivery, magnetic hyperthermia and as an image contrast agent. In the present study, the potentiality of SPIONs and curcumin, a natural antitumor agent, encapsulated in PLGA nanoparticles (Cur-SPION-PLGA-NPs) against pancreatic cancer cells was investigated. Specificity to the
nanoconjugate was imparted by conjugating HER2 to Cur-SPION-PLGA that confirmed enhanced uptake by the cancer cells. HER2-Cur-SPION-PLGA NPs establishes a magnetic drug delivery system as observed by the enhanced uptake of nanoparticles by cancer cells under the action of external magnetic field. HER2-Cur-SPION-PLGA demonstrated greater and sustained therapeutic efficiency, however, upon exposure to external magnetic field the anti-proliferative activity of HER2-Cur-SPION-PLGA was augmented owing to superior cellular uptake that resulted in enhanced cytotoxicity. In vitro results support potential clinical applications of HER2-Cur-SPION-PLGA NPs by activating the loss of mitochondrial membrane potential thereby triggering apoptosis in cancer cells.

In the 6th chapter, Mr. Siva demonstrates the development of double targeted and double drug loaded PLGA magnetic nanoparticles. The efficient targeting and therapeutic efficacy of combination of drug (curcumin and 5FU) and magnetic nanoparticles encapsulated PLGA nanoparticles, functionalized with two targeting ligands specific towards cancer are discussed in the work. This multifunctional, highly specific nanoconjugate resulted in superior uptake of nanoparticles by cancer cells. By using magnetic hyperthermia; due to the added advantage of the incorporation of magnetic nanoparticles that synergistically acting with the drugs, destroyed cancer cells within a very short period of time. The remarkable multimodal efficacy attained by this therapeutic nanoformulation offers the potential for targeting, imaging and treatment of the cancer within a short period of time by initiating early and late apoptosis.

In the 7th Chapter the development of aptamer targeted theragnostic multifunctional magnetic nanoparticles as nanoplatform for pancreatic cancer therapy has been discussed. The development of designer multifunctional therapeutics with magnetic nanoparticles (MNP) is a promising approach to selectively deliver therapeutics and image them in cancer cells. This study explored the preparation, characterization and application of a targeted nano-system represented by aptamer conjugated dual drug (Curcumin and Gemcitabine-loaded) and MNP encapsulated PLGA nanoparticles (Apt-Cur-Gem-PLGA-MNPs) for pancreatic cancer therapy. The aptamer targeted NPs exhibited excellent antiproliferative activity leading to apoptosis owing to enhanced cellular uptake. The presence of MNP in nanoconjugate was exploited for magnetic hyperthermia and magnetic ablation of cancer cells by rotational magnetic field. Thus, Apt-Cur-Gem-PLGA-MNPs is able to deliver an effectual and targeted delivery of therapeutics for pancreatic cancer treatment.

The 8th chapter discusses the development of multifunctional hybrid magnetic nanoparticles as theragnostic agents. Gold-coated magnetic nanoparticles was developed and hybrid
nanoparticle was incorporated into PLGA nanoparticles along with two drugs (curcumin and gemcitabine). The nanoconjugate was made specific to pancreatic and breast cancer cells by attaching three specific targeting ligands (folate, transferrin and AS1411 aptamer). He investigated the three different modes of cancer cell destruction with the nanoconjugate. Thus an ‘all in one’ approach for simultaneous chemotherapeutic, photothermal and magnetic hyperthermia mediated destruction of cancer cells was investigated. The concluding remarks of the thesis are discussed in the final 9th chapter.

【審査結果】Summary and decision

The thesis entitled “Multifunctional biocompatible magnetic nanoparticles as theragnostic agents: applications in cancer nanotechnology” focuses on the synthesis of theragnostic agents (Therapy and Diagnosis) by coupling magnetic nanoparticles, drugs and imaging moieties. Aptamer and folate targeting ligands have been used to direct these theragnostic agents to cancer cells. This targeting was highly successful in sparing the normal healthy cells. The results shown in the thesis are outstanding in international point of view and the salient features of this work are summarized below.

• A multifunctional biocompatible nanovector based on magnetic nanoparticle and carboxymethyl cellulose (CMC) has been demonstrated. The results indicate that the multifunctional CMC MNPs possess a high drug loading efficiency and high biocompatibility and with low cell cytotoxicity and can be considered to be promising candidates for CMC-based targeted drug delivery, cellular imaging, and magnetic hyperthermia (MHT).
• Mauran and Gellan gum coated MNPs exhibited excellent biocompatibility with low cell cytotoxicity, high therapeutic potential, and superparamagnetic behavior that can be employed as prospective candidates for bacterial EPS based targeted drug delivery, cancer cell imaging and for magnetic hyperthermia (MHT) for killing cancer cells within short period of time.
• HER2-Cur-SPION-PLGA demonstrated greater and sustained therapeutic efficiency, however, upon exposure to external magnetic field; the anti-proliferative activity of HER2-Cur-SPION-PLGA was augmented owing to superior cellular uptake that resulted in enhanced cytotoxicity. In vitro results support potential clinical applications of HER2-Cur-SPION-PLGA NPs by activating the loss of mitochondrial membrane potential thereby triggering apoptosis in cancer cells.
• Aptamer conjugated dual drug (Curcumin and Gemcitabine-loaded) and MNP
encapsulated PLGA nanoparticles (Apt-Cur-Gem-PLGA-MNPs) used for pancreatic cancer therapy. The aptamer targeted NPs exhibited excellent antiproliferative activity leading to apoptosis owing to enhanced cellular uptake. The presence of MNP in nano-conjugate was exploited for magnetic hyperthermia and magnetic ablation of cancer cells by rotational magnetic field. Thus, Apt-Cur-Gem-PLGA-MNPs is able to deliver an effectual and targeted delivery of therapeutics for pancreatic cancer treatment.

Based on the work, 2 first-authoring and 1 co-authoring papers have been published in international journals such as *Langmuir* and *International Journal of Nanomedicine*.

Judging by the results shown in the thesis and the number of international papers published so far, the level of the present research results is definitely high by international standards and the present results may well make a great contribution to the nano drug delivery against cancer. In conclusion, the thesis is considered as a high quality, high standard one by international standards.