

DEVELOPMENT OF TARGETED / SITE-SPECIFIC DRUG-DELIVERY SYSTEMS FOR CANCER

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**DEPARTMENT OF CHEMICAL ENGINEERING
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**by
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**Submitted
in fulfillment of the requirements of the degree of Doctor of Philosophy
to the**



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*Dedicated to my loving parents
who had the highest regard for
education*

Certificate

This is to certify that the thesis entitled “**Development of Targeted / Site-Specific Drug Delivery Systems for Cancer**” being submitted by **Maneesha Pande** to the Department of Chemical Engineering, Indian Institute of Technology, New Delhi, for the fulfillment of the requirements for the award of **Doctor of Philosophy** in Chemical Engineering, is a record of bonafide work carried out by her. She has worked under my guidance and supervision and has fulfilled the requirements, which to my knowledge, have reached the requisite standard for the submission of the thesis.

The research report and results presented in this thesis have not been submitted, in part or full, to any other university or institute for the award of any degree or diploma.

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ABSTRACT

Cancer is one of the top ten leading causes of death globally. Amongst all types of cancers, the head and neck cancer is the most prevalent type in India, as a large section of the population is in the habit of chewing tobacco, betel nut, and areca nut. Presently, the most common treatment protocol for this cancer involves surgical removal of the tumor followed by radiotherapy and/or chemotherapy. A number of targeting strategies are being explored to deliver the chemotherapeutic agent specifically to the tumor. These include active targeting, passive targeting, targeting tumor microenvironment, and direct injection into the tumor. The last strategy, i.e. intratumoral delivery is advantageous in case of solid tumors such as head and neck tumors. Such a delivery enables a significant reduction in dose, improved efficacy, increase in maximum tolerated dose, and elimination of the adverse effects. Systemic delivery of drugs (including targeted delivery) brings the drug to the periphery of the tumor. Most of the solid tumors have a high interstitial fluid pressure, which resists diffusion of these drugs from the periphery into their interior. This convective barrier is also circumvented by way of intratumoral drug delivery. The present work, therefore aims at developing an intratumoral delivery system for head and neck tumor, which is capable of releasing the desired amount of drug over a sufficiently prolonged period of time inside the tumor.

One of the most commonly prescribed drugs of choice for head and neck tumors is cisplatin, either alone, or in combination with other drugs such as docetaxel, paclitaxel, methotrexate, and cetuximab. The usual treatment protocol for cisplatin in a monotherapy involves: i) 15-20 mg/m² of cisplatin given daily for 5 days, every 3-4 weeks, or ii) the administration of cisplatin as a single dose in the range of 50-120 mg/m² every 3-4 weeks. In either case, it is given as a slow intravenous infusion (over a period of 6-8 h) in 2L of chloride-containing infusion fluid. Such a systemic

delivery of the drug is associated with some very serious adverse effects such as nephrotoxicity, low myelotoxicity, tinnitus, and hearing loss in high-frequency range, peripheral neuropathies, hyperuricaemia, nausea, and vomiting, and in some cases, anaphylactic reactions. Direct intratumoral delivery of cisplatin can avoid all these adverse effects, with the added advantage of a reduced dose.

In the present work, temperature sensitive hydrogels and emulsion-biliquid foam based delivery systems have been explored for injectable, intratumoral delivery of model drug sulfadiazine (which is highly hydrophobic, with a very poor aqueous solubility, similar to many anti-cancer drugs) and anti-cancer drug cisplatin. The formulations developed provided a sustained release of the drugs suitable for the two dosage regimens mentioned earlier - i) a plain w/o/w emulsion-biliquid foam system, which provides the release for a period of 24 h, and ii) a w/o/w emulsion-biliquid foam system with a modified oil phase which provides the release for a period of six days. These formulations may serve as a convenient, safe, and affordable treatment option to the conventional intravenous infusion which requires hospitalization and is associated with serious adverse effects. As the rate of gelation of hydrogels is likely to affect the rate of drug delivery, gelation kinetics of hydrogels has been studied (using a model polymer methylcellulose and the polymer chitosan, which has drug delivery applications). An alternative method to the conventional rheological method (involving measurements of viscosity, storage modulus, and loss modulus) for studying gelation kinetics has been proposed. This method is likely to provide a real-time estimation of the gelation process wherein the gel remains undisturbed throughout the study, unlike the oscillatory rheological methods in which the gel is subjected to a shear between two moving plates.

सार

वैश्विक स्तर पर कैंसर मृत्यु के दस प्रमुख कारणों में से एक है। सभी प्रकार के कैंसर के बीच, भारत में सिर और गर्दन का कैंसर सबसे अधिक प्रचलित प्रकार है, क्योंकि आबादी का एक बड़ा वर्ग तंबाकू, सुपारी और पान चबाने का आदि है। वर्तमान में, इस कैंसर के लिए सबसे आम उपचार प्रोटोकॉल में ट्यूमर की शल्य चिकित्सा के बाद रेडियोथेरेपी और / या कीमोथेरेपी शामिल है। विशेष रूप से ट्यूमर को कीमोथेरेप्यूटिक एजेंट देने के लिए कई लक्ष्यीकरण रणनीतियों की खोज की जा रही है। इनमें सक्रिय लक्ष्यीकरण, निष्क्रिय लक्ष्यीकरण, ट्यूमर माइक्रोएन्वायरमेंट को लक्षित करना और ट्यूमर में प्रत्यक्ष इंजेक्शन, शामिल हैं। आखिरी रणनीति, यानी इंटरट्यूमोरल डिलीवरी ठोस ट्यूमर जैसे कि सिर और गर्दन के ट्यूमर के मामले में फायदेमंद है। इस तरह की डिलीवरी खुराक में महत्वपूर्ण कमी, प्रभावकारिता में सुधार, अधिकतम सहनशील खुराक में वृद्धि और प्रतिकूल प्रभावों को समाप्त करने में सक्षम बनाती है। दवाओं की सिस्टेमिक डिलीवरी (लक्षित डिलीवरी सहित) दवा को ट्यूमर की परिधि में लाती है। अधिकांश ठोस ट्यूमर में एक उच्च अंतरालीय द्रव दबाव होता है, जो परिधि से इन दवाओं को ट्यूमर के आंतरिक भाग में प्रवेश करने से रोकता है। इस संवहन बाधा को इंटरट्यूमोरल दवा वितरण के माध्यम से दरकिनार किया जा सकता है। वर्तमान काम, इसलिए सिर और गर्दन के ट्यूमर के लिए एक इंटरट्यूमोरल वितरण प्रणाली विकसित करने के लक्ष्य से किया गया है, जो ट्यूमर के अंदर लंबे समय तक पर्याप्त मात्रा में दवा की वांछित मात्रा जारी करने में सक्षम हो।

सिर और गर्दन के ट्यूमर के लिए पसंद की सबसे सामान्य रूप से निर्धारित दवाओं में से एक सिस्प्लैटिन है। यह या तो अकेले, या अन्य दवाओं के साथ संयोजन में दी जाती है, जैसे कि डोसैटेक्सेल, पैक्लिटैक्सेल, मेथोट्रेक्सेट और सेटुक्ज़िमाब। मोनोथेरेपी में सिस्प्लैटिन के लिए सामान्य उपचार प्रोटोकॉल हैं: i) सिस्प्लैटिन १५-२० मिलीग्राम / मी^२ प्रति दिन, हर ३-४ सप्ताह में या, ii) ५०-१२० मिलीग्राम / मी^२ की सीमा में एकल खुराक के रूप में हर ३-४ सप्ताह में। किसी भी मामले में, इसे २ लिटर क्लोराइड युक्त इन्फ्यूजन द्रव्य के

साथ धीमी अंतःशिरा इन्फ्यूजन (6-8 घंटे की अवधि में) द्वारा दिया जाता है। दवा की ऐसी सिस्टेमिक डिलीवरी कुछ बहुत ही गंभीर प्रतिकूल प्रभावों से जुड़ी हुई है जैसे कि नेफ्रोटीक्सिसिटी, माइलोटीक्सिसिटी, टिनटस और उच्च आवृत्ति रेंज में सुनवाई हानि, परिधीय न्यूरोपैथिस, हाइपर्यूरेसीमिया, मतली और उल्टी, और कुछ मामलों में, एनाफिलेक्टिक प्रतिक्रियाएं। सिस्प्लैटिन की प्रत्यक्ष इंटरट्यूमोरल डिलीवरी इन सभी प्रतिकूल प्रभावों से बचा सकती है, कम खुराक के अतिरिक्त लाभ के साथ।

वर्तमान कार्य में, तापमान संवेदनशील हाइड्रोजेल और इमल्शन-बाइलिक्विड फोम आधारित डिलीवरी सिस्टम को मॉडल ड्रग सल्फैडज़ाइन (जो कि अत्यधिक हाइड्रोफोबिक है, एवं जिसकी अति खराब जलीय घुलनशीलता है- कई कैंसर रोधी दवाओं के समान) एवं कैंसर रोधी दवा सिस्प्लैटिन के लिए खोजा गया है। विकसित किए गए फॉर्म्युलेशन ने पहले बताई गई दो डोज़ रेजिमेंस के लिए उपयुक्त, दवाओं की एक निरंतर रिलीज़ प्रदान करने में सक्षम रही - i) एक सादा w / o / w इमल्शन--बाइलिक्विड फोम सिस्टम, जो २४ घंटे की अवधि के लिए रिलीज़ प्रदान करता है, और ii) w / o / w इमल्शन--बाइलिक्विड फोम सिस्टम, एक संशोधित तेल चरण के साथ, जो छह दिनों की अवधि के लिए रिलीज़ प्रदान करता है। यह सूत्रीकरण पारंपरिक अंतःशिरा इन्फ्यूजन के एक सुविधाजनक, सुरक्षित और सस्ते उपचार विकल्प के रूप में कार्य कर सकते हैं- जिसके लिए अस्पताल में भर्ती होने की आवश्यकता नहीं होती है और गंभीर प्रतिकूल प्रभावों से भी बचा जा सकता है।

जो कि हाइड्रोजेल के जेलेशन का दर दवा वितरण के दर को प्रभावित करने की संभावना रखता है, इस कारण वर्तमान कार्य में हाइड्रोजेल के जेलेशन कैनेटीक्स का अध्ययन किया गया है (एक मॉडल पॉलिमर मिथाइलसेलुलोज, और पॉलिमर चितोसन- जिसका दवा वितरण अनुप्रयोग हैं- का उपयोग करके)। जेलेशन कैनेटीक्स का अध्ययन करने के लिए पारंपरिक रीओलॉजिकल विधि (जिसमें विस्कोसिटी, लॉस मॉड्यूलस और स्टोरेज मॉड्यूलस का मापांक निकाला जाता है) का एक वैकल्पिक तरीका प्रस्तावित किया गया है। इस

विधि में जेल अविकलित रूप में बरकरार रहता है, जिस से जेल प्रक्रिया का एक वास्तविक "रीयल-टाइम" आकलन प्रदान करने की संभावना है। इसके प्रतिकूल, पारंपरिक विधि में जेल को दो विपरीत दिशा में घूमती प्लेटों के बीच रखा जाता है, जिस से जेल स्ट्रक्चर विकृत हो जाता है।

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LIST OF ABBREVIATIONS AND SYMBOLS

ΔG	Free energy, kJ/mol
ΔH	Enthalpy, kJ/mol
T	Temperature, K
ΔS	Entropy, kJ/mol K
n	Number of cross linking loci L
L	Cross linking loci
J	Junction zones
v	Rate of gelation, h^{-1}
[J]	Concentration of J units, k mol
A	Actual absorbance of light measured in spectrophotometer (based on % Transmittance)
A'	Modified absorbance (based on Transmittance)
I	Intensity of transmitted light, Einstein/ m^2
I_0	Intensity of incident light, Einstein/ m^2
α'	Molar extinction coefficient, $m^2/k\text{ mol}$
x	Path length for light absorbance, m
α	Molar extinction coefficient for path length x , $m^3/k\text{ mol}$
C_A	Concentration of polymer chains at time t, k mol
C_B	Concentration of gel at time t, k mol
C_{A0}	Initial concentration of polymer, k mol
k_1	First order rate constant, h^{-1}

C_{Bmax}	Maximum concentration of gel, k mol
E_a	Activation energy, kJ/mol
IFP	Interstitial Fluid Pressure
EPR	Enhanced Permeation and Retention
ECM	Extracellular Matrix
BLF, Blf	Biliquid foam
MC	Methylcellulose
H-gel	Hydrogel
OPDA	o-phenylenedene diamine
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide