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Dr. Pranjal Chandra
(Supervisor)
School of Biochemical Engineering
Indian Institute of Technology
(Banaras Hindu University),
Varanasi-221005

Dr. Pranjal Chandra, Ph.D.
Assistant Professor
School of Biochemical Engineering
Indian Institute of Technology(BHU)
Varanasi-221005, U.P., INDIA

Prof. Vikash Kumar Dubey
(Co-supervisor)
School of Biochemical Engineering
Indian Institute of Technology
(Banaras Hindu University),
Varanasi-221005

डा0 विकास कुमार दूबे
Dr. Vikash Kumar Dubey
आचार्य
Professor
जैव रासायनिक अभियांत्रिकी स्कूल
School of Biochemical Engineering
भारतीय प्रौद्योगिकी संस्थान
Indian Institute of Technology
(काहिविवि) वाराणसी-221005
(B.H.U.) Varanasi-221005

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DECLARATION BY THE CANDIDATE

I, **Rahul Kumar**, certify that the work embodied in this thesis is my own bona fide work and carried out by me under the supervision of **Dr. Pranjal Chandra** from **"July, 2016** to **June, 2022"**, at the School of Biochemical Engineering, Indian Institute of Technology (Banaras Hindu University), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma.

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Date: 30/06/2022

Place: Varanasi

Rahul Kumar

Signature of the Student

(Rahul Kumar)

CERTIFICATE BY THE SUPERVISOR

It is certified that the above statement made by the student is correct to the best of our knowledge.

30/6/2022

(Dr. Pranjal Chandra)
Supervisor
Assistant Professor
School of Biochemical Engineering
Indian Institute of Technology
(Banaras Hindu University)
Varanasi - 221005

Dr. Pranjal Chandra, Ph.D.
Assistant Professor
School of Biochemical Engineering
Indian Institute of Technology (BHU)
Varanasi-221005, U.P., INDIA

(Prof. Vikash Kumar Dubey)
Co-supervisor
Professor
School of Biochemical Engineering
Indian Institute of Technology
(Banaras Hindu University)
Varanasi- 221005

(Prof. Vikash Kumar Dubey)
Coordinator
Professor
School of Biochemical Engineering
Indian Institute of Technology
(Banaras Hindu University)
Varanasi - 221005

समन्वयक
Coordinator
जैव रासायनिक अभियांत्रिकी स्कूल
School of Biochemical Engg
भारतीय प्रौद्योगिकी संस्थान
Indian Institute of Technology
(काठहिविवि) वाराणसी-221005
(B.H.U.) Varanasi-221005

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DEDICATED TO MY LOVING PARENTS AND SISTER

FOR THEIR LOVE, SUPPORT AND ENCOURAGEMENT

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Date: 30.06.2022

Rahul Kumar

Place: IIT (BHU), Varanasi (***Rahul Kumar***)

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List of abbreviations and symbols

PEG-PE	Polyethylene glycol-phosphatidyl ethanolamine
DPPC	Dipalmitoyl phosphatidylcholine
DOPE	Dioleoyl phosphatidyl ethanolamine
DOPC	1,2-dioleoyl-sn-glycero-3-phosphocholine
DSPE	1,2-distearoyl-sn-glycero-3-phosphoetanolamine
PEG	polyethylene glycol
HSPC	L-α-phosphatidylcholine hydrogenated (Soy)
DOTA	1,4,7,10- Tetraazacyclododecane-1,4,7,10-tetraacetic acid
DEPC	1,2-Dierucoyl-snglycero-3-phosphocholine
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
DPPE	dipalmitoyl-phosphatidylethanolamine
EPOPC	1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine
FITC	Fluorescein isothiocyanate
EDC	ethyl-3-(3-dimethylamine propyl carbodiimide
NHS	N-hydroxy succinimide
DOTAP	1,2-Dioleoyl-3-trimethylammonium propane
QDs	Quantum dots
MSPC	Monostearoyl phosphatidylcholine
CHS-ED-LA	5-cholesten-3β-yl) 4-oxo-4-[2-(lactobionyl amido) ethylamido] butanoate
	Eyeblink

NADG	N-acetyl-d-glucosamine
NLBCs	Nano-lipid-based carriers
Q	Quercetin
XRD	X-ray diffractometry
SEM	Scanning electron microscopy
FT-IR	Fourier transformed infrared spectroscopy
DLS	Direct light scattering
MTT	3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide
BSA	Bovine serum albumin
LNs	Lipid nanocarriers
DMAP	4-(dimethyl amino) pyridine
LLPBNPs	Lactoferrin conjugated lipid polymer-based nanoparticles
LPBNPs	Lipid polymeric based nanoparticles
MTX	Methotrexate
PCL	Polycaprolactone

PREFACE

In view of drug delivery, diverse delivery vehicles including natural and synthetic polymeric nanoparticles have been greatly investigated till date. In general, micelles, dendrimers, cellulose, gelatin, lipid, chitosan, alginate, poly (D, L-lactide), poly (D, L-glycoside), poly(lactide-co-glycoside), and poly-caprolactone have been employed as drug delivery vehicles. However, we are interested to select lipid-based nanoparticle/nanocarrier for targeted anticancer drug delivery. Cancer is one of the major health-related issues affecting the population worldwide and subsequently accounts for the second-largest death. Genetic and epigenetic modifications in oncogenes or tumor suppressor genes affect the regulatory systems that lead to the initiation and progression of cancer. Therefore, in view of cancer burden worldwide, we have targeted the cancer cells for drug delivery. Conventional methods, including chemotherapy/radiotherapy/appropriate combinational therapy and surgery, are being widely used for theranostics of cancer patients. Surgery is useful in treating localized tumors, but it is ineffective in treating metastatic tumors, which spread to other organs and result in a high recurrence rate and death. Also, the therapeutic application of free drugs is related to substantial issues such as poor absorption, solubility, bioavailability, high degradation rate, short shelf-life, and low therapeutic index. Therefore, these limitations can be sorted out using NLBCs as promising drug delivery carriers. Still, at most, they fail to achieve site targeted drug delivery and detection. This can be achieved by using the concept of theranostics which is a combination of diagnostic and therapeutic agents. Selecting a specific ligand/antibody as a diagnostic tool is being highly utilized since its cognate receptor molecule is expressed on the surface of the cancer cell.

We have designed the study in four different sections, where in the first part; we have developed N-acetyl-D-glucosamine (NADG) coupled quercetin-loaded (Q) nano-lipid-based carriers (NADG-Q-NLBCs) where NADG has been covalently conjugated on the surface of NLBCs containing quercetin as anti-cancer drugs. The constructed nano-bioconjugate was

characterized by various techniques, and the *in vitro* drug release profiles were examined using zero and first-order kinetic models. The characterization data confirmed the morphology, size, charge distribution, crystallinity, and chemical interactions among the various moieties of the nano-bioconjugate. Further, the synthesized NADG-Q-NLBCs were applied to target the human breast cancer cells (MCF-7), which interestingly showed a more cytotoxic effect compared to the lone NLBCs and free Quercetin. The flow cytometry study confirmed that NADG-Q-NLBCs induced apoptosis in MCF-7 cells in a targeted manner. The percentage of early apoptotic cells was found to be 25% in the case of NADG-Q-NLBCs, which is almost 2.5 times higher than the Q-NLBCs. However, the number of viable cells reached the maximum when treated with NLBCs. The present investigation suggests that the constructed nano-bioconjugate could be a capable carrier of drugs with sustained pharmacokinetics and improved physicochemical properties.

In the next section, we have attempted to conjugate BSA to maleimide functionalized lipid surface for cancer theranostics. The BSA was conjugated with C1 carbon of maleimide through a thiol reaction. The BSA conjugated quercetin-loaded lipid nanocarriers (BSA-Q-LNs) were spherical in structure with a shell size of 296.43 ± 4.90 nm. The encapsulation efficiency of BSA-Q-LNs was found to be 76 ± 0.3%. Further, BSA conjugation on carrier surface was confirmed from the shift in FT-IR, XRD peak. The release kinetic of Q- loaded LNs formulation was best fitted in a first-order kinetic model suggesting an early burst of Q followed by sustain rate of release. The Q-loaded LNs and BSA-Q-LNs displayed improved cytotoxicity in the human breast cancer cell line (MCF-7) as compared to free Q.

Further, we have designed and developed a new system that further improves the stability, and crystallinity, and releases the kinetic profile of the free drugs. In this system, lipid and polycaprolactone (PCL) were blended and then lactoferrin as a diagnostic agent has been conjugated on the surface of the hybrid system using covalent bonding. A lactoferrin-conjugated lipid polymer-based nanoparticles (LLPBNPs) encapsulating methotrexate (MTX)

as a potential anticancer drug candidate was constructed via a facile one-step precipitation method. The designed hybrid-nano-bioconjugate exploits both the characteristic features of natural lipids and the biocompatible polymer. The physiochemical properties of the constructed hybrid-nano-bioconjugate were thoroughly characterized by Infrared Spectroscopy, Scanning Electron Microscopy, EDX, Dynamic Light Scattering, and X-ray Diffraction techniques. The general sizes of the particles are obtained in the range of 520-650 nm with a polydispersity of 0.140-0.163 that does not possess a broad size distribution. Further, the encapsulation efficiency of the MTX in LLPBNPs systems was assessed, which was found to be 84.0 ± 1.5 %. The *in vitro* drug release kinetics were analytically examined using the zero and first-order kinetic models. These models revealed that the drug dissociation initially shows the first-order model followed by a sustained rate of drug delivery. The morphological changes of the nucleus and F-actin cytoskeleton of the cancer cells were studied using molecular binding probes DAPI and rhodamine-conjugated phalloidin, respectively.

Thus, this work is a concept-based comparative investigation of N-acetyl-d-glucosamine decorated nano-lipid-based carriers, BSA conjugated quercetin-loaded lipid nanocarriers, and lactoferrin conjugated lipid-polymer nano-bio-hybrid for cancer theranostics. In the future, the constructed system may overcome the problem of multiple drug resistance.