Controlled Drug Delivery using Functionalized Layered Double Hydroxides for Tumor Treatment

ABSTRACT

Thesis submitted in partial fulfillment for the Award of Degree

Doctor of Philosophy

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This is to certify that the thesis entitled ***"Controlled Drug Delivery using Functionalized Layered Double Hydroxide for Tumor Treatment"*** by ***Swapan Maity*** has been conducted under my supervision. I confirm that this work is original and has not been submitted elsewhere for the award of any degree. Furthermore, it is certified that the candidate has successfully completed all necessary requirements, including the Comprehensive Examination, Candidacy, and State of the Art (SOTA), for the conferment of the Ph.D. degree.

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Dedicated to

My parents and Family

DECLARATION OF THE CANDIDATE

I, Swapan Maity, hereby certify that the work presented in this thesis is my own original and bona fide effort, conducted under the esteemed guidance of Prof. Pralay Maiti from July 2019 to July 2024, at the School of Materials Science & Technology, Indian Institute of Technology (BHU), Varanasi. The content of this thesis has not been submitted for the award of any other degree or diploma.

I declare that due recognition has been given to all research workers whose contributions have been referenced throughout this thesis. I have diligently acknowledged their work and ensured proper citation wherever required. Furthermore, I affirm that I have not deliberately replicated any other individual's work, including paragraphs, texts, data, results, or any material published in journals, books, magazines, reports, dissertations, theses, or accessible through websites, without proper attribution, and I have not presented such material as my own.

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I hereby certify that the above statement provided by the student is accurate and true to the best of my knowledge.

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c) SiHa cells were treated with 20 µg/ml of material (P-L), doxorubicin drug (Dox) and design formulation (P-L@Dox) for 72 hours. The expression of caspase 3, Bax, Cleaved caspase 9, Bcl2, p53, and PARP proteins were assessed by Western blot analysis; d) The relative expression of the specified protein markers has been meticulously quantified through western blot analysis**; e)** The relative expression levels of **mRNA** for **p53, Bax, PUMA**, and **Bcl-2** were quantitatively assessed through a detailed **RT-PCR** analysis; **f)** Photographic images of the colony study for Material, Dox, and P-L@Dox were captured after 72 hours of incubation for each sample, using a concentration of 20 µg/ml**; g)** Plot of number of colonies for material P-L, drug Dox and formulation P-L@Dox reflecting the potent efficacy towards cancer of the designed formulation; **h)** Microscopic images capturing the migration of SiHa cancer cells treated with material P-L, Doxorubicin Dox, and a specially designed formulation P-L@Dox after 72 hours incubation with samples concentration 20 µg/ml reveal striking evidence of enhanced efficacy. These images illustrate the profound impact of the treatment, showcasing a higher rate of apoptosis, as the cancer cells are visibly impaired in their movement, a testament to the potent therapeutic action of the formulation; **i)** The bar diagram illustrating the wound area across different groups such as, material P-L, Dox, and our designed formulation P-L@Dox elegantly reveals the potent efficacy of our novel formulation against cancer. The clear distinction between the groups highlights the remarkable impact of our formulation, emphasizing its therapeutic potential in comparison to the others. 217

Figure 5.5: The effectiveness of the injectable formulation (P-L@Dox-MC) for *in vivo* therapy in mice with melanoma that contain luciferase. **a)** Images capturing the tumor sizes in mice, both before and after treatments, reveal the effects of various interventions. The treatments include Dox-MC (Dox encapsulated within an MC gel), P-L@Dox-MC (Dox incorporated into P-L, further embedded in an MC gel), and a control group receiving only saline. These visual comparisons offer a glimpse into the transformative impact of each treatment, providing a clear distinction between the progression or regression of tumors across the experiment; **b)** Photographic images showcasing the measured tumor volumes, accompanied by a scale, were captured after treatment across various groups i.e. Control, Dox-MC and P-L@Dox-MC. At the conclusion of the 22-day experiment, tumors from the different groups of animals were carefully collected and measured; **c)** Measured tumor volume over time as influenced by the therapy, with different approaches applied where appropriate, **d)** Percentage of relative body weight as a function of treatment time across all groups;

List of Tables

List of Schemes

Abbreviations

DSC	Differential Scanning Calorimetry
FTIR	Fourier Transformation Infrared
h	Hour
LDH	Layered Double Hydroxide
MTT	4, 5- dimethylthiazole-2yl-2, 5-Diphenyltretrazolium Bromide
^{o}C	Degree Centigrade
SEM	Scanning Electron Microscope
t	Time
T	Temperature
TEM	Transition Electron Microscope
TGA	Thermal gravimetric Analysis
UTM	Universal Testing Machine
XRD	X-ray diffraction
DFT	Density function