SRI VENKATESWARA INTERNSHIP PROGRAM

SRI-VIPRA

Project Report of 2023: SVP-2335

"Nanoparticle kinetics in a cell"

IQAC
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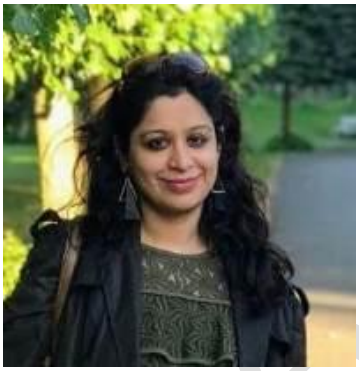
SRIVIPRA PROJECT 2023

Title: Nanoparticle kinetics inside a cell

Name of Mentor: Dr. Mohita Bhagat Name of Department: Zoology Designation: Assistant Professor	Photo: 

List of students under the SRIVIPRA Project

S. No.	Photo	Name of the student	Roll number	Course	Signature
1		Kritika	2021016	B.Sc. (hons.) zoology	

Signature of Mentor

Certificate of Originality

This is to certify that the aforementioned student from Sri Venkateswara College have participated in the summer project SVP-2335 titled **"Nanoparticle kinetics in a cell"**. The

participant has carried out the research project work under my guidance and supervision from 15 June, 2023 to 15th September 2023. The work carried out is original and carried out in a hybrid mode.

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Signature of Mentor

Acknowledgements

I would like to convey my heartfelt gratitude to college for organising SRI-VIPRA which provides this wonderful opportunity.

I would like to express my special thanks to our mentor Dr. Mohita Bhagat for her time and efforts she provided throughout the project. Your useful advice and suggestions were helpful to me during the project's completion. In this aspect, I am eternally grateful to you.
I would like to express my profound gratitude to Prof. Parthaprasad Chattopadhyay of biochemistry department at AIIMS Delhi for providing the opportunity of observership in your lab.

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OVERVIEW OF THE PROJECT

Nanoparticle-based therapies and diagnostics are commonly known as nanomedicine and hold the potential to profoundly influence the future of healthcare. Nevertheless, the application of these advancements in a clinical setting presents notable challenges.

Among these challenges, one stands out—the effective transportation of nanoparticles to specific groups of cells and precise locations within cells in the body. This targeted delivery is crucial to achieve the desired biological and therapeutic outcomes. Thus, it is important to gain a deep grasp of the fundamental principles governing the interactions between nanoparticles and biological systems. This knowledge is crucial in anticipating and controlling how nanoparticles move within the body to enhance their clinical efficacy.

The SRI-VIPRA project comprises of the observership at AIIMS delhi and review on the trafficking of nanoparticle in a cell.

In review, we studied various research articles and papers online and tried to find out about the pathways through which nanoparticles are taken up by cells, how the physical and chemical traits of nanoparticles influence their interactions with cells, and investigate the intricate dynamics of how nanoparticles move within cells.

Observership at AIIMS, Delhi: There was a 5-week informal observership at department of Biochemistry under the guidance of Prof. Parthaprasad Chattopadhyay recommended by mentor Dr. Mohita Bhagat. One part of the observership or training includes about nanoparticles properties and its various aspects. A letter of recommendation was also provided by Prof. Parthaprasad Chattopadhyay.

The research in the field of nanoparticle in medicine was observed. PLGA nanoparticles were used to deliver the drug into the cell.

PLGA Nanoparticles

PLGA nanoparticles are a type of nanoparticle composed of a biodegradable and biocompatible polymer known as poly (lactic-co-glycolic acid), abbreviated as PLGA.

These nanoparticles are used in various fields, particularly in medicine and drug delivery, because of their unique properties and versatility.

PLGA is produced by the catalyzed ring-opening copolymerization of lactic acid and glycolic acid. In the process of polymerization, the individual monomer units are connected in a sequential manner via ester bonds, leading to the creation of the PLGA copolymer.

PLGA have important application in nanomedicine because of its biocompatibility and biodegradability, which happens when the ester bonds of lactate and glycolate undergo hydrolysis. On hydrolysis in cell, PLGA gets converted into D, L lactic acid and glycolic acid. Subsequently, these monomers can be processed through the Krebs cycle, producing harmless end-products in the form of water (H2O) and carbon dioxide (CO2).

These properties of nanoparticle make it toxic free and hence used as a delivery agent.
Many anticancer drugs are encapsulated with PLGA nanoparticles which are as follows:

- Vincristine sulphate
- Paclitaxel
- 9 -Nitrocamptothecin
- Doxorubicin
- Cisplatin
- Rapamycin
- Xanthones
- Dexamethasone

PREPARTION OF PLGA NAOPARTICLES

For preparing the PLGA nanoparticles, single Emulsification–Solvent Evaporation (ESE) Method was used:

- Firstly, the PLGA polymer is dissolved in a volatile organic solvent. Dichloromethane (DCM) was used.
- Then dissolved PLGA is mixed with polyvinyl alcohol (PVA) which acts as the surfactant.
- Later, Sonication is done, and emulsion is produced. This leads to formation of nanoparticles.

- Afterwards, the mixture is kept at continuous stirring overnight, which leads to the evaporation of the DCM.
- After the evaporation of DCM, the remaining are centrifuged. After centrifugation the supernatant is removed and pallete is obtained
- Later, the pallete is again washed with double distilled water and again centrifuged, the pallete obtained contains the desired nanoparticles.
- Later, the nanoparticles are dissolved in water and the size and potential is checked using ZETA sizer.
- To load the desired drug, the drug is dissolved in the organic solvent before the emulsion is formed.

Later, these nanoparticles are introduced in the cells. And the cell cytotoxicity is studied to see the effect of the drug in the cell.

LETTER OF RECOMMENDATION

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Letter of Recommendation

To whom so ever it may concern

I am writing this letter to express my utmost appreciation for Kritika's exceptional performance during her 5-week informal observership under my supervision in the Dept. Of Biochemistry, All India Institute of Medical Sciences, New Delhi.

Throughout the training, she exhibited remarkable proficiency in various laboratory techniques such as PAGE, AGE, RT-PCR, and Isolation of DNA. Her meticulous attention to detail and quick grasp of complex concepts were evident in the successful preparation of numerous Nanoparticles crucial to the progress of our project. Her dedication, sincerity, and curiosity have left a lasting impression on me.

Beyond her technical skills, her eagerness to learn and explore new frontiers in the field of research is truly commendable. Her active engagement and insightful contributions during discussions enriched the research environment in our lab.

Based on her performance, I have no doubt that she possesses the potential to excel in her future endeavours. I wholeheartedly recommend her to pursue further studies or research opportunities, as I firmly believe she will make significant contributions to the scientific community.

I wish her the very best in all her future pursuits. May her passion for research continue to drive her towards great success and fulfilment.

Warm regards,

P. Chattopadhyay 14/07/2023

Parthaprasad Chattopadhyay

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POINTS COVERED IN THE REVIEW

Cellular uptake of nanoparticles

- Clathrin-dependent endocytosis
- Caveolin-dependent endocytosis
- Clathrin- and caveolin-independent endocytosis
- Phagocytosis
- Macropinocytosis

Effect of various Properties of NP on Cellular Uptake

- Effect of Size
- Effect of Shape
- Effect of Surface Charge
- Effect of Hydrophobicity
- Effect of Elasticity

Intracellular Trafficking of NPs

CELLULAR UPTAKE OF NANOPARTICLES

Clathrin-dependent endocytosis

Clathrin-dependent endocytosis is a significant route for the cellular entry of nanoparticles, initiated by the binding of surface ligands on the nanoparticles to corresponding receptors on the cell membrane. Various cell membrane receptors are involved in different cell types and play a role in clathrin-dependent endocytosis.

The clathrin-dependent endocytosis process involves multiple steps:
1. Nucleation of Coated Pit Formation: Cytosolic proteins involved in endocytosis come together to initiate the formation of a coated pit.
2. Plasma Membrane Bending and Invagination: The cell membrane bends and forms an invagination at the coated pit site.
3. Scission: The neck of the invagination is cut and separated from the plasma membrane, resulting in the creation of an intracellular vesicle.

4. Uncoating and Protein Recovery: The endocytotic proteins are released from the vesicle through uncoating and are recovered within the cell.

The clathrin-dependent endocytosis pathway leads to the capture of nanoparticles within intracellular vesicles, typically ranging in size from approximately 100 to 500 nm. After detachment from the membrane, these vesicles are transported, often aided by intracellular actin filaments, to endosomes. Endosomes can be recycled or eventually fuse with lysosomes, resulting in the enzymatic breakdown of the vesicular contents and payloads.

Caveolin-dependent endocytosis

Caveolin-dependent endocytosis is a cellular process that plays a significant role in the internalization of nanoparticles. This mechanism is characterized by the involvement of specialized plasma membrane invaginations called caveolae, which are coated with caveolin proteins. These invaginations facilitate the uptake of specific molecules from the extracellular environment into the cell.

Caveolae are distinctive vesicle structures with dimensions ranging from 50 to 100 nanometers, and they owe their stability to the presence of caveolin proteins in their coats. When triggered by certain signals, caveolin-coated vesicles are internalized and transported within the cell through a complex signaling cascade. These vesicles are commonly directed to cellular organelles such as the Golgi apparatus and the endoplasmic reticulum.

Clathrin- and caveolin-independent endocytosis

Virus-like particles and various types of nanoparticles possess the ability to enter the cell plasma membrane and access the interior of cells through mechanisms different from clathrin and caveolin-dependent pathways. One scientist proposed avenue for such cellular entry, independent of clathrin and caveolin, involves lipid rafts present in cell membrane. These rafts are specialized microdomains enriched with cholesterol and sphingolipids within the plasma membrane, and they perform endocytosis upon stimulation.

Phagocytosis

Phagocytosis is a cellular uptake process performed by immune cells, including macrophages, neutrophils, and B lymphocytes. A primary role of phagocytosis is to eliminate foreign elements like pathogens, diseased cells, and synthetic or biological materials that do not belong to the body. When it comes to nanoparticles, their uptake via phagocytosis usually begins with a physical attachment to receptors on the surface of phagocytic cells. With this array of plasma membrane receptors, phagocytes are adept at identifying and efficiently clearing nanoparticles from circulation in body.

Phagocytes recognize and clear nanoparticles by opsonization, which involves the attachment of immunoglobulins, complement proteins, and other serum proteins to the nanoparticle surface. Following engulfment by phagocytes, nanoparticles become enclosed within phagosome vesicles that subsequently fuse with lysosomes, forming structures called phagolysosomes. These phagolysosomes possess the ability to degrade foreign substances enzymatically and biochemically, including nanoparticles. As phagocytosis serves as a highly efficient mechanism for clearing opsonized nanoparticles, it presents a notable challenge in the development of effective nanomedicines.

Macropinocytosis

Macropinocytosis constitutes a category of indiscriminate cellular uptake mechanisms. These mechanisms involve the engulfing of extracellular fluids and dissolved substances through extensions of the plasma membrane that are supported by actin. Macropinocytosis happens through actin-based signaling and reshaping of the membrane. Via this route, nanoparticles and other assimilated elements become enclosed within sac-like structures known as macropinosomes. These sacs can vary in size, ranging from approximately 0.5 to 1.5 μm.

EFFECT OF VARIOUS PROPERTIES OF NANOPARTICLE ON CELLULAR UPTAKE

Study of the effect of various properties of NPs such as size, shape, surface charge, surface hydrophobicity/hydrophilicity on cellular uptake is crucial as these parameters directly affect the uptake level.

Effect of Size

The size of nanoparticles is a critical factor that affects how efficiently they are taken up by cells and their toxicity to living cells. Additionally, NP size plays a significant role in determining the specific pathway through which they are internalized. Smaller NPs, ranging from a few nanometers to several hundred nanometers, enter cells either through pinocytosis or macropinocytosis.

NPs within the size range of 250 nm to 3 μm exhibit optimal phagocytosis in vitro. Meanwhile, NPs in the size range of 120–150 nm are internalized through clathrin- or caveolin-mediated endocytosis, with the maximum size for this pathway being around 200 nm. In the caveolae-mediated pathway, larger NPs face difficulties in uptake due to the size of caveolae. The specific endocytic pathway a particular NP utilizes can vary depending on its size.

Studies have shown that there is an ideal size of approximately 50 nm for efficient NP internalization, with reduced uptake observed for smaller particles (around 15–30 nm) or larger NPs (approximately 70–240 nm). Additionally, NPs ranging in size from 30–50 nm interact effectively with cell membrane receptors and are subsequently internalized through receptor-mediated endocytosis.

In the context of drug delivery using NPs, a major concern is to prevent their elimination by the immune system and to prolong their circulation in the bloodstream, enhancing their availability at the target site. Increasing NP size can lead to a higher clearance rate, making it essential to understand the role of NP size in cellular uptake to design effective and safe NPs for medical applications.

However, studies exploring the relationship between NP size and uptake pathways have produced inconsistent results.

Effect of Shape

In addition to size, the shape of nanoparticles plays a crucial role in determining both their uptake pathways and their movement within cells.

In one study it was shown that spherical AuNPs were taken up five times more efficiently than rod-shaped AuNPs in HeLa cells. In another study by the same researchers, they investigated the uptake of spherical and rod-shaped transferrin-coated AuNPs in three different cell lines (STO cells, HeLa cells, and SNB19 cells) and found that spherical AuNPs were internalized at a higher rate by all cell lines compared to rod-shaped AuNPs.

Effect of Surface Charge

Another crucial factor influencing the cellular uptake of nanoparticles is their surface charge. In recent years, researchers have employed nano-surface modifications to engineer the surface charge of NPs, making them either positively charged (cationic) or negatively charged (anionic).

Negatively charged cell membranes tend to enhance the uptake of positively charged NPs, leading to higher internalization of positively charged NPs compared to neutral or negatively charged ones. However, it is important to note that the uptake of positively charged NPs can disrupt the integrity of cell membrane and increase toxicity, often leading to cell death. Conversely, neutrally charged NPs are typically internalized at a lower rate compared to negatively charged NPs.

Additionally, surface charges influence the mechanisms of NP uptake. Positively charged NPs are mainly internalized by cells through macropinocytosis, while negatively charged NPs are taken up through clathrin-/caveolae-independent endocytosis.

Manipulating the surface charge densities of NPs can control their interactions with cells, optimizing uptake while minimizing cytotoxicity, essential characteristics for NPs considered for biomedical applications.

Effect of Hydrophobicity

The hydrophobicity of nanoparticles is a significant factor that influences their interaction with cell membranes. Several studies have explored the impact of NP hydrophobicity on these interactions. One studies results showed that hydrophobic NPs were incorporated into the cell membrane, creating inclusions, while hydrophilic NPs were found to adsorb onto the cell membrane.

Effect of Elasticity

The elasticity (stiffness, hardness, and rigidity) of nanoparticles is an inherent factor that significantly influences their uptake by cells. NP elasticity refers to their ability to resist changes when subjected to forces. Young's modulus, is a commonly used index to gauge NP elasticity. A higher Young's modulus value indicates greater NP elasticity, and vice versa. Various analytical devices or instruments, such as atomic force microscopes, rheometers, are used to measure this value on NPs.
NPs with higher elastic values are referred to as hard NPs, examples of which include gold NPs, quantum dots, and magnetic NPs. Conversely, NPs with lower elastic values are termed soft NPs, and examples include hydrogels, liposomes, and biodegradable polymers.

Numerous studies have focused on how NP elasticity impacts cellular uptake and have reported that cells tend to preferentially internalize stiffer NPs more efficiently compared to softer NPs.

INTRACELLULAR TRAFFICKING OF NPS

Following internalization, the subsequent important aspect is the intracellular trafficking of NPs, which determines their destination within cellular compartments, their cytotoxicity, and their therapeutic effectiveness.

Once NPs are taken up by cells, they initially encounter membrane-bound intracellular vesicles known as early endosomes. Early endosomes play a crucial role in transporting cargo to specific cellular destinations. Some of the cargo is recycled to the plasma membrane via recycling endosomes. Early endosomes undergo maturation and differentiation processes, transforming into late endosomes. Late endosomes eventually merge with lysosomes, forming endolysosomal vesicles. Within these vesicles, hydrolytic enzymes degrade the trapped NPs. However, certain NPs can evade this pathway and are released into the cytoplasm, bypassing lysosomal degradation.

Another significant intracellular degradation pathway is autophagy, where cytoplasmic contents are enclosed within autophagosomes and delivered to lysosomes for breakdown and recycling. Autophagy also plays a role in degrading aggregated proteins and dysfunctional organelles to maintain cellular homeostasis.

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