University of Saskatchewan
College of Pharmacy and Nutrition

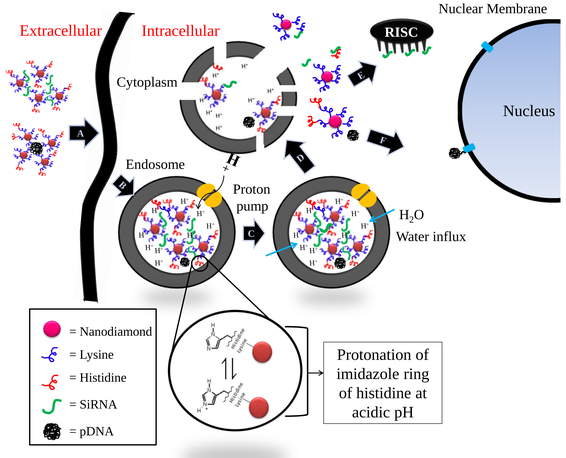
Smart nanodiamonds as carriers for gene therapy: Introducing pH sensitivity in carrier design to modulate intracellular gene transfer by diamoplexes

Saniya S. Alwani, Raj Rai, Deborah Michel and Ildiko Badea

INTRODUCTION

As gene therapy advances as a new arm in modern medicine, there is need to develop sophisticated vectors for its successful application. We utilized nanodiamonds, the most biocompatible carbon nanomaterials to design smart carriers. Our prototype lysyl-NDs **(K-NDs)** comprised a primary-amine rich cationic surface, capable of complexing anionic genetic materials to form "diamoplexes". K-NDs achieved major physicochemical and biological milestones as a delivery vector, however, the overall transfection efficiency was modest.

In order to overcome intracellular barriers and improve ND-mediated gene transfection, histidine a pH modulating moiety was introduced in the functionalization forming histidyl-lysyl-NDs **(HK-NDs)** and lysyl/histidyl-lysyl-NDs **(H_{50}-K_{50}-NDs)** where histidine was attached either 100% or 50% of lysine moieties on the surface.



Schematic illustration of histidine-mediated endosomal escape

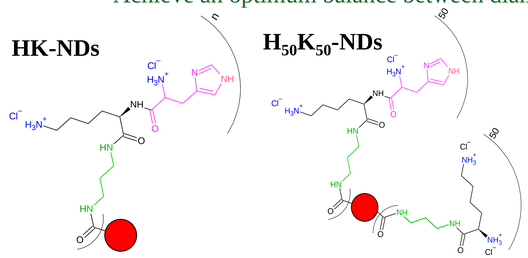
METHODS

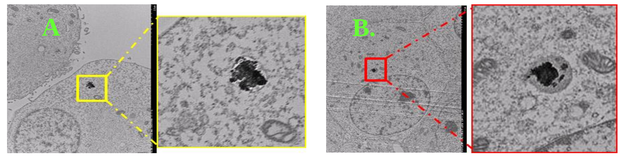
Thermogravimetry was used to calculate surface loading of functionalized NDs. Cell Viability was compared post-treatment to evaluate biocompatibility profiles. Formation of diamoplexes was assessed via gel retardation assay. Flow cytometry was used to evaluate cellular interactions and siRNA delivery via functionalized NDs.

OBJECTIVE

Introducing histidine, a pH modulating moiety on prototype lysyl-NDs (K-NDs) to:

- Overcome intracellular barriers for gene transfer i.e., endo/lysosomal entrapment of diamoplexes.
- Achieve an optimum balance between diamoplex formation and transfection efficiency of the carrier.





Rate-limiting intracellular barrier

Entrapment in (A) endosomes upon entry which later transform in (B) lysosomes leading to enzymatic degradation of loaded siRNA.

RESULTS

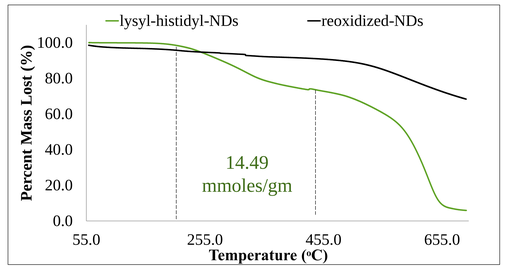


Figure 1. Thermograms of lysyl-histidyl-NDs in comparison with carboxylated reoxidized NDs shows two-point decline confirming the presence lysine and histidine dissociating from the surface at different temperatures.

Sample	Volume particle size distribution		Zeta Potential (mV) ± S.D.
	Size Range (nm)	% Distribution	
HK-NDs	30-90	56	30 ± 3
	100 – 200	31	
	210 – 300	9	
H_{50}-K_{50}-NDs	< 200	50.5	23 ± 6
	210 - 500	30.8	
	510 -1000	10.7	
	> 1000	8.2	

Table 1. Particle size distribution and zeta potential of HK-NDs and H_{50}-K_{50}-NDs. Particle size is represented as % volume; mV = millivolts (mV)

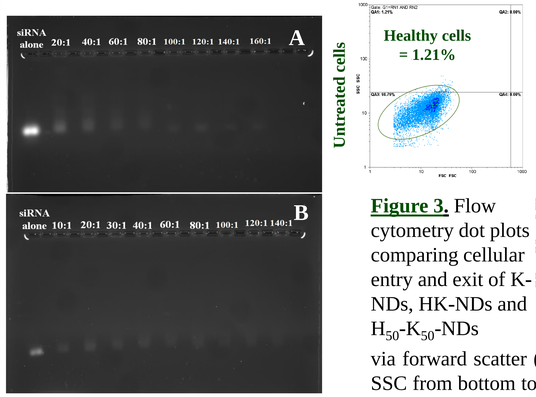


Figure 2. Binding of (A) HK-NDs and (B) H_{50}-K_{50}-NDs with siRNA.

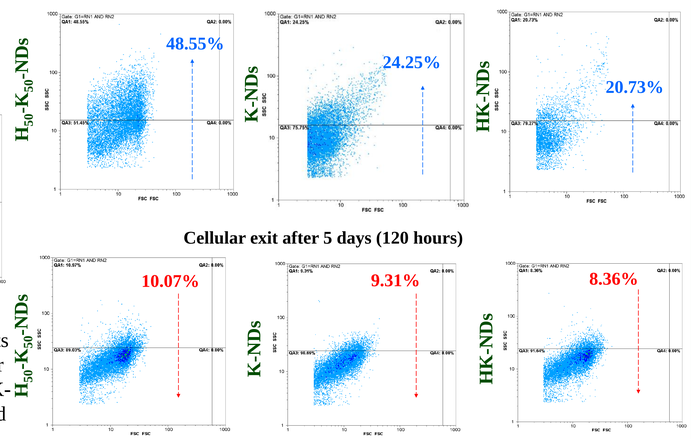


Figure 3. Flow cytometry dot plots comparing cellular entry and exit of K-NDs, HK-NDs and H_{50}-K_{50}-NDs via forward scatter (FSC) as the x-axis and side scatter (SSC) as y-axis. **Horizontal panel**: higher SSC from bottom to top i.e., from QA3 to QA1 (blue arrows) corresponds to increase in granularity and internal complexity of the cells secondary to ND uptake. **Vertical panel:** Back shift from QA1 to QA3 (red arrows) corresponds decrease in SSC due to exit of internalized diamonds.

RESULTS (CONT.)

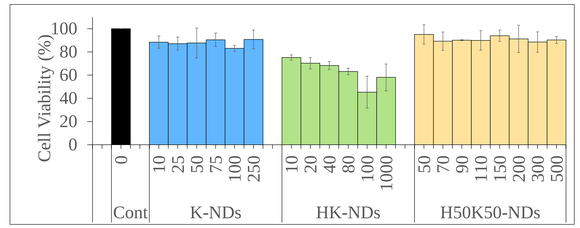


Figure 4. Cell viability after functionalized NDs exposure. Unlike K-NDs, HK-NDs reduced cell viability to as low as 45%. Reducing the histidine on ND-surface to 50% in H_{50}-K_{50}-NDs significantly improved cell-viability to > 90% at highest concentrations.

Percent GFP knockdown		
Increase in low GFP producing and GFP-free cells		
L/S	**K-NDs/CS**	**K-NDs/S**
52 ± 1.7	37	38 ± 1.6
L/S	**HK-NDs/CS**	**HK-NDs/S**
52 ± 1.7	38	* **45 ± 2.6**
L/S	**$H_{50}K_{50}$-NDs/CS**	**$H_{50}K_{50}$-NDs/S**
31 ± 0.9	5	* **18 ± 3.2**

Table 2. Percent GFP knockdown induced by diamoplexes of histidine-modified NDs in comparison to the K-NDs. Although lower than the commercial control lipofectamine, diamoplexes of histidine-modified NDs shows significantly higher siRNA-mediated GFP knockdown. Abbreviations: CS=negative-control siRNA; L=lipofectamine; S= anti-GFP siRNA

CONCLUSION & ACKNOWLEDGEMENT

This study is first in the field of ND research to demonstrate that amino acid functionalized diamond NPs form novel biocompatible gene carriers. Also, stringently controlled histidine-mediated pH modulation renders high efficiency to diamoplexes. Organelle-level effects will direct specific design pre-requisites to enable ND-based gene therapeutics.

S. Alwani is a recipient of Alexander Graham Bell Canada Graduate Scholarship from NSERC Canada. Financial support was provided by NSERC and USASK College of Pharmacy and Nutrition.