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The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Development of efficient gene carriers based on self-assembled DNA nanostructures and understanding their interactions with the cell

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Choi, Jonathan Chung-hang	Prof. Zhang, Chuan
Post	Associate Professor	Associate Professor
Unit / Department / Institution	Department of Biomedical Engineering, The Chinese University of Hong Kong	School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University
Contact Information	jchchoi@cuhk.edu.hk	chuanzhang@sjtu.edu.cn
Co-investigator(s) *(with title)*	N/A	N/A

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	1/1/2017		
Project Completion date	31/12/2020		
Duration *(in month)*	48		
Deadline for Submission of Completion Report	31/12/2021		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Evaluate the ability of DNA nanostructures to enter the cell as a function of size and shape, and also investigate their biological mechanism for cellular uptake.
2. Evaluate the intracellular trafficking and degradation events of DNA nanostructures as a function of size and shape.
3. Prepare novel DNA nanostructures with enhanced intracellular stability.

4. Assess the potential of employing our size-optimized, shape-optimized, and stabilized DNA nanostructures for delivering functional nucleic acids to the cell.

5.2 Revised Objectives

Date of approval from the RGC: N/A

Reasons for the change: N/A

1.
2.
3. ....

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

Objectives 1 & 2: In 2019, we reported that oligonucleotides appended with a polythymidine [poly(T)] segment accumulate inside the nucleus when the cells are under gentle compression

imposed by the weight of a glass coverslip, without inducing severe cytotoxicity or oxidative stress (Chen Z *et al.*, *ACS Appl. Mater. Interfaces*, 2019; US Patent No.: 10,533,189). Such delivery of poly(T) is mediated by importin β and nucleoporin 62. Our method enhanced the intranuclear delivery of antisense oligonucleotides (ASO) to cells and inhibition of a reporter gene and an intranuclear oncogene. Next, we achieved intranuclear delivery of "spherical nucleic acids (SNAs)" when cells were under compression by a glass coverslip for several hours, leading to up to ~50% nuclear accumulation without predominant endosomal entrapment or severe cytotoxicity (Li H's PhD Thesis, 2020). Gentle compression upregulates genes linked to focal adhesion and Rho/ROCK signaling, and further incubation with poly(T) strands upregulates genes linked to myosin and nuclear import; ultimately, poly(T) SNAs enter the nucleus *via* importin-β. Finally, we reviewed how DNA nanostructures enter cells in terms of pathways and receptors involved, and how DNA nanostructures traffic to organelles inside the cell, degrade, or leave the cell (Chiu YTE and Li H *et al*., *Small*, 2019).

Objective 3: In 2019, the Hong Kong team jointly disclosed a strategy to deliver functional nucleic acids to cells by using DNA-polydopamine (PDA) hybrid nanostructures (US Patent No.: 10,240,190). By leveraging this technology, the Hong Kong team jointly reported PDA-coated gold nanorods immobilized with aptamer-linked distyryl boron dipyrromethene (DSBDP) for detecting miR-21 in cancer cells (Dai G *et al.*, *Nanoscale*, 2021). By using this oncogenic stimulus, the photodynamic effect of the DSBDP-based photosensitizer was activated due to the dissociation of the conjugate from the nanorods upon hybridization. After i.v. injection of the nanorods into tumour-bearing mice, the fluorescence intensity of the tumour was increased. Upon irradiation, the nanorods reduced tumor size without causing severe tissue damage. In 2021, the Hong Kong team reported if coating NPs with PDA influences their interactions with cells (Liu Y *et al.*, *ACS Nano*, 2021). We employed gene knockdown and overexpression to prove the role of dopamine receptor D2 (D2DR) in the cellular uptake of PDA-coated NPs. By i.v. injecting PDA-coated NPs to mice, we revealed their binding to D2DR in the liver by competitive inhibition and immunohistochemistry and their preferential association to D2DR-rich resident Kupffer cells by flow cytometry.

Objective 4: In 2018, the Mainland team reported DNA nanogels for encapsulating siRNA and delivery to tumors (Ding F *et al.*, *Angew. Chem. Int. Ed.*, 2018). These nanogels are stable against degradation and support knockdown of a target cancer gene in cancer-bearing mice. The Hong Kong PI served as a co-author and provided comments on the cellular uptake of DNA nanogels. In 2019, the Hong Kong team reported that coating NPs with DNA oligonucleotides allow for elevated and faster delivery to atherosclerotic plaques (Zhang L *et al.*, *ACS Appl. Mater.*, 2019; US Patent No.: 10,973,927). This is the first demonstration of using DNA nanostructures for targeting atherosclerotic plaques. Our Mainland PI served as a co-author and commented on the design of DNA nanostructures. In 2020, the Mainland team reported the grafting of maleimides onto a phosphorothioate ASO to generate the construct of maleimide-grafted ASO (Guo Y *et al.*, *Chem. Commun.*, 2020). By interacting with the cell membrane thiols that trigger cellular internalization, this maleimide-grafted ASO exhibited enhanced cellular uptake and gene silencing. The Hong Kong PI served as a co-author and commented on the cellular uptake of the ASO. In 2021, the Hong Kong team filed a patent on using folic acid-based NPs to deliver nucleic acids for managing chronic kidney disease (CKD) (Chan CKW's PhD Thesis, 2019; US Patent Application No.: 63/104,2622). We reported the effects of NP size, ligand loading, and disease stage on the i.v. delivery of NPs and nucleic acids to kidney tubules, where CKD occurs. Folic acid NP-mediated delivery of ASO against miR-21, a promoter of fibrosis, reduced renal fibrosis and tissue degeneration.

Potential for further development of the research and the proposed course of action *(maximum half a page)*

We have exploited our findings on the elevated and faster delivery of DNA-coated NPs to atherosclerotic plaques (Zhang L *et al., ACS Appl. Mater.,* 2019; US Patent No.: 10,973,927) to kickoff another project on using microRNA-coated iron oxide NPs for treating atherosclerosis in mice. Upon completion of efficacy studies in mice, we plan to test the microRNA-based NPs in higher order animals (*e.g.*, pigs and humans), eventually aiming for clinical translation. Moreover, we plan to liaise with biotechnology or pharmaceutical companies to commercialize our transfection technique on the intranuclear delivery of poly(T)-containing oligonucleotides using gentle compression with a glass coverslip (Chen Z *et al., ACS Appl. Mater. Interfaces,* 2019; US Patent No.: 10,533,189).

For the studies on the intranuclear delivery of SNA using gentle compression by a glass coverslip (Li H's PhD Thesis, 2020) and the use of folic acid and ASO-based NPs to deliver nucleic acids for managing CKD (Chan CKW's PhD Thesis, 2019; US Patent Application No.: 63/104,2622), the manuscripts of both works will be submitted in several weeks. Upon publishing and patenting the work on CKD which reveals the translational potential of our folic acid and ASO-based NPs for treating renal fibrosis, we will plan for the pharmacological development of the nucleic acid nanoplatform for treating CKD in clinical trials.

At last, both the Hong Kong and Mainland teams wish to continue our existing collaboration by leveraging our expertise in bio-nano interaction and DNA nanotechnology, respectively. In the future, we plan to take advantage of different funding opportunities available to support research between Hong Kong and mainland. We wish to go beyond our existing studies (mainly on cancer) and investigate how DNA nanostructures benefit gene delivery to other challenging disease destinations, say kidney and brain. The COVID-19 pandemic made our previously scheduled laboratory visits and face-to-face research discussions more challenging during the second half of this funding period, but we managed to maintain our intellectual ties remotely and produce research outputs. When COVID-19 subsides, we hope to resume our physical research interactions and plan more synergistic research experiments together.

7. The Layman's Summary

(describe <u>in layman's language</u> the nature, significance and value of the research project, in no more than 200 words)

The research project has expanded our understanding in how DNA nanostructures interact with the cell. Such are valuable data because DNA nanostructures are an important class of bionanomaterials with attractive properties for gene therapies, such as biocompatibility and abundant entry to cells. On the materials front, we have learned how to make structures of defined sizes and DNA sequences and shapes and understood how these parameters govern the cellular entry of DNA oligonucleotides and DNA nanostructures. Moreover, we have learned how to enhance the stability of DNA nanostructures by using polydopamine (PDA) and how PDA-coated nanoparticles interact with cells and receptors. On the biology front, we have learned that polythymidine [poly(T)] DNA sequences and poly(T) 3D nanostructures can abundantly enter the cell nucleus when the cells are under extracellular compression *in vitro*, due to the involvement of cytoskeleton and mechanosensing pathways. For animal studies, we have demonstrated the *in vivo* delivery of DNA nanostructures to different disease

locations, including atherosclerotic plaques, tumors, and fibrotic kidneys. These data will benefit the design of DNA nanostructures as nanomedicines or gene therapies.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s)	Title and Journal/ Book	Submitted to RGC	Attached to this report	Acknowledged the support of this Joint Research Scheme	Accessible from the institutional repository
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*	***bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	*(with the volume, pages and other necessary publishing details specified)*	*(indicate the year ending of the relevant progress report)*	*(Yes or No)*	*(Yes or No)*	*(Yes or No)*
2018				**Ding F**, Mou Q, Ma Y, Pan G, Guo Y, Tong G, **Choi CHJ**, Zhu X, **Zhang C***.	A crosslinked nucleic acid nanogel for effective siRNA delivery and antitumor therapy. *Angew. Chem. Int. Ed.*, 57, 12, 3064-3068.	31-12-2018	Yes	Yes	Yes
2019				**Zhang L**, Tian XY, Chan CKW, **Bai Q**, Cheng CK, Chen FM, Cheung MSH, Yin B, Yang H, Yung W-Y, Chen Z, **Ding F**, Leung KCF, **Zhang C**, Huang Y, Lau JYW, **Choi CHJ***.	Promoting the delivery of nanoparticles to atherosclerotic plaques by DNA coating. *ACS Appl. Mater. Interfaces*, 11, 15, 13888-13904.	31-12-2018	Yes	Yes	Yes
2019				Chiu YTE†, **Li H**†, **Choi CHJ***.	Progress towards understanding the interactions between DNA nanostructures and the cell. *Small*, 15, 26, 1805416.	8-2-2019	Yes	Yes	Yes
2019				Chen Z†, **Li H**†, Zhang L, Lee CKY, Ho LWC, Chan CKW, Yang H, **Choi CHJ***.	Specific delivery of oligonucleotides to the cell nucleus via gentle compression and attachment of polythymidine. *ACS Appl. Mater. Interfaces*, 11, 31, 27624-27640.		Yes	Yes	Yes

2020				Guo Y, Zhang J, Pan G, **Choi CHJ**, Wang P, Li Y, Zhu X, **Zhang C**.	Grafting multi-maleimides on antisense oligonucleotide to enhance its cellular uptake and gene silencing capability. *Chem. Commun.*, 56, 54, 7439-7442.		Yes	Yes	Yes
2021				Chiu YTE, **Choi CHJ***.	Enabling transgenic plant cell-derived biomedicines with nanotechnology. *Adv. NanoBiomed Res.*, 1, 1, 2000028.		Yes	Yes	Yes
2021				Dai G†, Choi CKK†, Zhou Y, Bai Q, Xiao Y, Yang C, **Choi CHJ***, Ng DKP*.	Immobilizing a hairpin DNA-conjugated distyryl boron dipyrromethene on gold@polydopamine core-shell nanorods for microRNA detection and microRNA-mediated photodynamic therapy. *Nanoscale*, 13, 6499–6512.		Yes	Yes	Yes
2021				Liu Y, Choi CKK, Hong H, Xiao Y, Kwok ML, Liu H, Tian XY, **Choi CHJ***.	Dopamine receptor-mediated binding and cellular uptake of polydopamine-coated nanoparticles. *ACS Nano*, 15, 13871–13890.		Yes	Yes	Yes

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
August, 2017/ Beijing	DNA nanostructures for cardiovascular applications	6th International Conference on DNA Nanotechnology	31-12-2018	Yes	Yes	No
June, 2018/ Chongqing	Intranuclear delivery of DNA oligonucleotides	7th International Conference on DNA Nanotechnology	31-12-2018	Yes	Yes	No

August, 2018/ Hong Kong	DNA nanostructures for biomedical applications	EMedi Summit 2018	31-12-2018	Yes	Yes	No
April, 2019/ Snowbird, Utah	Promoting the delivery of nanoparticles to atherosclerotic plaques by DNA coating	16th Annual Conference on Foundations of Nanoscience (FNANO19)	No	Yes	Yes	No
June, 2019/ Beijing	Engineering DNA nanostructures for in vivo delivery to atherosclerotic plaques	2019 International Society for Heart Research (ISHR) World Congress XXIII	No	Yes	Yes	No
July, 2019/ Wuxi	Cell-nano interactions of non-cationic bionanomaterials	8th International Conference on DNA Nanotechnology	No	Yes	Yes	No

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
CHAN Ka Wing Cecilia	PhD in Surgery	August 2015	August 2019
LI Huize	PhD in Biomedical Engineering	August 2016	November 2020

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

Patents granted:

1. Bian L, **Choi CHJ**, Choi CKK. Nano-constructs for polynucleotide delivery. US Patent No.: 10,240,190. Date of patent: 26/3/2019.
2. **Choi CHJ**, Chen Z, Zhang L, **Li H**. Highly specific delivery of polynucleotides to the cell nucleus via compression. US Patent No.: 10,533,189. Date of patent: 14/1/2020.
3. **Choi CHJ**, Zhang L. Materials and methods for effective in vivo delivery of DNA nanostructures to atherosclerotic plaques. US Patent No.: 10,973,927. Date of patent: 13/4/2021.

Patents filed:

Choi CHJ, **Chan CKW,** Lau JYW. A dual targeting and therapeutic nanoparticle for treating renal fibrosis. US Patent Application No.: 63/104,2622.

12. Statistics on Research Outputs *(Please ensure the summary statistics below are consistent with the information presented in other parts of this report.)*

	Peer-reviewed journal publications	Conference papers	Scholarly books, monographs and chapters	Patents awarded	Other research outputs (Please specify)
No. of outputs arising directly from this research project [or conference]	8	6	0	3	N/A