Novel Solution of Nucleic Acid Delivery for *in-vivo* Diagnosis: Self-assembled Nanopieces

Hongchuan Yu[1,3], Yupeng Chen[1, 2], Hicham Fenniri[4], Larry W. McLaughlin[3], Eric M. Darling[1], Thomas J. Webster[2] and Qian Chen[1]
[1]Department of Orthopaedics, Warren Alpert Medical School and [2]School of Engineering, Brown University, Providence, RI, 02912, USA. [3]Department of Chemistry, Boston College, Chestnut Hill, MA, 02467, USA. [4]Department of Chemistry, University of Alberta, Edmonton, ABT6GZV4, CANADA.

Statement of Purpose:

Modern clinic treatment requires fast and reliable detecting methods designed to achieve precise diagnosis. Therefore, many have recognized that the real-time, *in-situ* gene diagnosis is a great approach to meet those requirements due to its high selectivity and accuracy. For such purpose, different types of nucleic acid probes have been developed, for example, molecular beacons emit fluorescence after binding the target mRNA in cells. One key for success of using nucleic acid probes is to ensure the efficient delivery into living cells. Previously, various methods were developed for nucleic acid delivery *in-vitro*. However feasibility, effectivity and safety of those approaches are three main limitations to their *in-vivo* applications.

To solve problems in nucleic acid delivery *in-vivo*, a novel type of nanomaterial, self-assembled Nanopieces, was investigated in this study. Nanopieces were generated from incorporation of rosette nanotubes (RNTs) and nucleic acids, such as small RNAs or molecular beacons. Under physiological conditions, RNTs and nucleic acids undergo a self-assembly process through electrostatic interactions and base stacking to form non-covalent, yet stable super molecular nanostructures, which we termed Nanopieces. In this study, formation and properties of novel nucleic acid delivery vector, Nanopieces, were investigated, and a successful, effective *in-vivo* gene diagnosis using this novel technique was achieved.

Methods:

For Nanopieces formation, UV-vis and CD spectroscopy was used to observe the different absorbance curves between 10μg/mL rosette nanotubes (RNTs), 100nM siRNA (scrambled sequence, used as a nucleic acid control) and Nanopieces (incorporated RNT/siRNA). Moreover, electrophoresis was used to confirm the incorporation and measure the electric charge of formed Nanopieces.

To study the disassembly ability of Nanopieces to release functional nucleic acids after delivery, the high resolution thermo analysis was used to determine the melting temperature of Nanopieces. And several pH conditions were detected to show the pH-dependence of disassemble.

Electron microscopy characterization of Nanopiece self-assembly, morphology and size distribution was analyzed via transmission electron microscopy (TEM) and atomic force microscopy (AFM).

In-vivo diagnosis of Nanopieces was determined via the intracellular delivery of molecular beacons in mice. Nanopieces were generated via the incorporation of rosette nanotubes (RNTs) and fluorescent molecular beacons targeting a house-keeping gene, GAPDH. After injection of the RNT/molecular beacon nanopieces into mouse femur, fluorescence molecular tomography (FMT) was used to test the intracellular delivery over 9 days.

Results:

UV result indicated the formation of Nanopieces composites involving interaction between RNTs and nucleotides. CD spectra showed that Nanopieces resembled RNTs' structure. And Tm experiment proved that Nanopiece composites stabilities were dependent to environment pH condition. Successful self-assembly of RNT and nucleic acids resulted in a morphological transformation from nanotubular structures to Nanopieces (**Fig. 1**). TEM and AFM studies showed the median length of the nanopieces is 141.4 nm; width 23.6nm and height 21.8 nm. *In-vivo* imaging via FMT showed strong positive fluorescence signals one hour after the RNT/fluorescent molecular beacon nanopieces were injected in the mouse femur. In contrast, minimal signals were seen by injection of molecular beacon alone, nanotubes (RNTs) alone or saline (**Fig. 2**). Quantitative FMT demonstrated that the molecular beacon fluorescence signals decreased over time, but significant signal intensity persisted for 5 days *in-vivo* after injection.

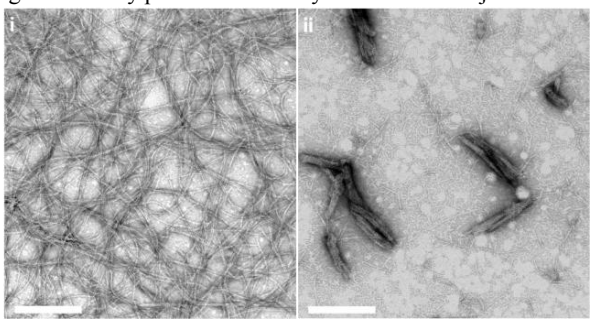

Figure 1. TEM scans of RNT (i) and self-assembled Nanopieces (ii). (White scale bar is 100nm.)

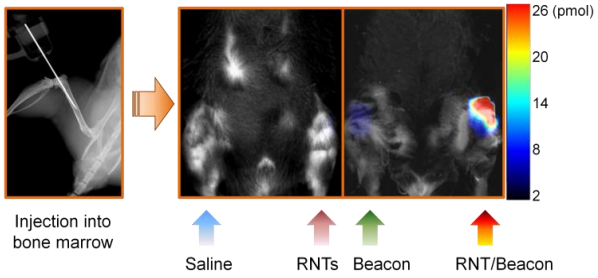

Figure 2. *In-vivo* delivery. Significant increase of fluorescence in mice femur by local injection of GAPDH molecular beacon delivered using Nanopieces compared with saline only, RNT only and molecular beacons only.

Conclusions:

In this study, nucleic acids were simply incorporated with RNTs through a self-assembly process. After a morphological transformation, Nanopieces were successfully generated. Especially, such Nanopieces achieved successful intracellular delivery and highly effective fluorescence signal diagnosis *in vivo*. Therefore, we have developed a novel and promising nanotechique to deliver nucleic acids intracellularly for in vivo gene diagnosis.