Graphene Oxide Mediated Delivery of DNAzyme for Simultaneous Detection and Knockdown of Viral Gene

Seongchan Kim¹, Soo-Ryoon Ryoo¹, Dong-Eun Kim² and Dal-Hee Min¹

¹Department of Chemistry, Seoul National University, Seoul, 151-747, Korea; ²Department of Bioscience and Biotechnology, Konkuk University, Seoul, 143-701, Korea
dalheemin@snu.ac.kr

ABSTRACT SUMMARY
Effective DNAzyme (Dz) delivery system was developed based on nano-sized graphene oxide (nGO) for simultaneous detection and silencing of target mRNA expression.

The fluorescence signal of FAM-labeled Dz (FAM-Dz) was quenched in the presence of nGO by binding of FAM-Dz with nGO surface through pi-pi stacking interaction and recovered by duplex formation with complementary mRNA target sequence. The Dz/nGO complex system allowed convenient monitoring of target mRNA in living cells and down-regulation of the target mRNA expression by catalytic degradation through Dz at the same time.

The present nGO based Dz delivery system can be harnessed as a powerful theragnostic tool for the treatment of viral diseases such as hepatitis C.

INTRODUCTION
DNAzyme (deoxyribozyme, Dz) is a promising therapeutic candidate for down-regulation of viral genes. Dz is relatively stable, less degradable and cost-effective compared to siRNA.

Hepatitis C is a serious viral disease which affects about 180 million people around world. Although hepatitis C virus (HCV) infection is usually asymptomatic, the virus develops chronic symptom up to 85% of the infected people. As there is no vaccine and standard therapy except pegylated interferon-alpha with ribavirin, development of effective therapeutics is urgently required.

Here, the intracellular delivery of Dz that targets mRNA of HCV NS3 gene was developed by utilizing nGO. The N-terminal one-third of the NS3 proteins plays a role for serine protease and the remainder has RNA helicase activity, which are essential for viral replication in human liver cells. Therefore, HCV infection can be treated by efficient knockdown of HCV NS3 gene.

EXPERIMENTAL METHODS
GO was synthesized according to a modified Hummers method and the GO was sonicated to prepare nGO. The nGO was characterized by atomic force microscopy (Park system, Korea), elemental analyzer (Thermo Scientific, U.S.A.), Raman (Horiba Jobin Yvon, France) and UV-vis spectroscopy (Shimadzu, Japan) which provided the physical/chemical characteristics of nGO such as width, height, average size, and element contents.

The loading capacity of Dz to nGO was determined by adding nGO to FAM-Dz stock solution until the fluorescence of FAM-Dz was completely quenched at 492 nm emission wavelength. Cytotoxicity of nGO to replicon Huh7 (Rep-Huh7) cells was estimated by MTT cell viability assay.

FAM-Dz/nGO complex was transfected to Rep-Huh7 cells that have been seeded in a 12-well plate. For the cellular uptake study, the nucleus of the cell were stained by Hoechst 33342 and the cell images were obtained using a Ti inverted fluorescence microscope (Olympus, Japan).

Total RNA isolation was carried out by using Trizol reagent (Invitrogen) and quantifying the RNA by Nanodrop Take3 (BioTek, U.S.A.). Then, Superscript II reverse transcriptase (Invitrogen) was used to cDNA synthesis, followed by PCR amplification (BioRad, U.S.A.).

Western blot was carried out using Immobilon-P membrane (Millipore) with anti-HCV (Virostat), anti-GAPDH (ABFrontier), and horseradish-peroxidase-conjugated secondary
antibody (Sigma) and then the membrane was visualized by a Luminescent Image analyzer (GE Healthcare, Sweden).

Dz derivatives and siRNA were purchased from Genotech Corp. and Bioneer Corp., Korea.

RESULTS AND DISCUSSION

We investigated the Dz delivery based on nGO in cellular experiment using Rep-Huh7 cells. Fluorescence corresponding to FAM was observed using an inverted fluorescence microscope to estimate the degree of cellular uptake of Dz/nGO complex as well as to detect the presence of the complementary sequence of mRNA (Figure 1). The images showed green fluorescence corresponding to FAM-Dz at perinuclear region when FAM-Dz/nGO was treated to the cells. However, green fluorescence was barely observed in the FAM-scDz/nGO treated cells (scDz: scrambled sequence Dz). The data suggested that Dz/nGO could pass through the cell membrane while maintaining quenched fluorescence in the absence of target RNA in cytoplasm but showed increased fluorescence in the presence of target RNA, indicating that nGO based Dz delivery system can be applied as a biosensor to monitor target mRNA with low background signal in living cells.

To investigate the silencing of NS3 gene by Dz/nGO complex, Rep-Huh7 cells were incubated with Dz/nGO complex and Dz-loaded Lipofectamin2000 (Lipo) as a control. As shown in Figure 2, NS3 mRNA expression of the Dz/nGO complex treated cells more substantially decreased than that of the Dz/Lipo treated cells.

![Figure 1. Fluorescence images of Rep-Huh7 cell incubated for 12 h after transfected with FAM-Dz/nGO and FAM-scDz/nGO. Hoechst 33342 stained nucleus blue and FAM appeared green at perinuclear region. Scale bar is 100 μM.](image1.png)

![Figure 2. Expression level of GAPDH and NS3 of Rep-Huh7 cell incubated for 48 h after transfected with Dz and scDz loaded nGO is estimated by semi-quantitative RT-PCR.](image2.png)

CONCLUSION

We developed a Dz delivery system based on nGO and demonstrated its utility in Rep-Huh7 liver cells carrying HCV NS3 gene. The system served as mRNA sensing platform which showed strong fluorescence signal when the FAM-Dz formed duplex with complementary mRNA sequence. At the same time, the target gene silencing was successfully achieved by Dz mediated degradation of the target mRNA, HCV NS3 in the present study. This system was relatively convenient and simple to apply by just mixing FAM-Dz with nGO in an aqueous solution.

We think that the present nGO based Dz delivery system will be readily utilized both for monitoring and treating HCV infection in the future.

REFERENCES