Oligoethylene glycol mediates knockdown effect of small interfering RNAs conjugated goldnanoparticles.

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ABSTRACT

Small interfering RNAs (siRNAs) - have a huge potential in treatment of many diseases resulting from aberrant gene expression including cancer, diabetes and atherosclerosis. In order to realize the therapeutic potential of siRNAs, the most important hurdle i.e. crossing cell membrane and delivery to cytoplasm in a stable form, must be addressed by developing effective and safer delivery platforms. In the present study, we have taken a systematic approach to investigate the use of gold nanoparticles as delivery platforms for firefly siRNAs and the effect of chemical modifications: oligo ethylene glycol (OEG) and lipofectamine on gold nanoparticle-siRNA conjugates. Thiolated siRNAs were covalently conjugated to gold nanoparticles (20 nm) and these conjugates (*GNP-siRNA*) were either used alone, or further modified with OEG (*GNP-siRNA-OEG*) or lipofectamine (*GNP-siRNA-lipofectamine*) followed by incubation with 3T3-L1 cells expressing the firefly luciferase gene (FFL). The analyses revealed that FFL siRNAs transfected with lipofectamine or *GNP-siRNA-OEG or GNP-siRNA-lipofectamine* have shown knockdown. However, there was no knockdown with *AuNP-siRNA.* These results suggest that OEG has beneficial properties on *AuNP-siRNA* conjugates either affecting cell uptake or routing the conjugates to the cytoplasm or both and thus improving delivery of siRNAs to the cytoplasm in the effective knockdown of the FFL gene.

Keywords: gold nanoparticles, siRNA, ologoethylene glycol, gene knockdown

1 INTRODUCTION

Small interfering RNAs (siRNAs) -catalytic sequence-specific gene silencers have a huge potential in treatment of diseases resulting from aberrant gene expression. However, there is a clear need for developing effective delivery platforms for achieving targeted knockdown of genes. Recent research using gold nanoplatforms with siRNA molecules with Oligoethelene glycol spacer followed by passivation with Oligoethelene glycol (OEG) resulted in gene knock down [1]. However, the effect of chemical modifications such as OEG on gold nanoparticle- siRNA conjugates was not taken into consideration in interpreting the results and the authors concluded that the gold nanoparticles act as efficient transfection platforms for siRNA delivery to the cytoplasm. In the present study, we have taken a systematic approach to investigate the use of gold nanoparticles as delivery platforms for firefly luciferase (FFL) siRNAs and the effect of chemical modifications: oligo ethylene glycol (OEG) on gold nanoparticle-siRNA conjugates.

2 METHODOLOGY

2.1 Design of siRNAs for FFL: There are no available designed siRNAs for the FFL gene for pGL4 vectors (Promega). Hence the available sequence information of siRNAs for FFL was taken from PGL-3 vector (Promega). Sequence alignment of the coding sequence of FFL gene sequences was done using Clustal W. The target siRNA sequences for FFL in PGL4-vectors were selected based on the sequence location and similarities with siRNAs PGL3 series. FFL-

Sense -5'- CCUACGCCGAGUACUUCGAdUdU-3'

Antisense- 5'-UCGAAGUACUCGGCGUAGG dUdU-3

The design for siRNA for gold nanoparticle conjugation-

5'-Thio-C6linker(S-S)-AAAAAAAAAAAAACCUACGCCGAGUACUUCGAdUdU-3' (Sense)

5'-UCGAAGUACUCGGCGUAGGdUdU 3'(Antisense)

2.2 Reduction of thiol-modified siRNA: Thiolated siRNAs were reduced in 400 µl of 3% TCEP for 1 h followed by addition of 150 µl of 9.5 M ammonium acetate and 1.5 ml of 100% ethyl alcohol. The samples were frozen at -80˚C for 20 min and centrifuged at 13000 rpm for 15 min to collect the pellet of siRNAs. The pellet was allowed to dry for 20 min upon removal of the supernatant. The pellet was resuspended in 200 µl RNase free water.

2.3 Conjugation of thiol-modified siRNA with gold nanoparticles (GNP): The conjugation of siRNA to GNP (20 nm) is done according to procedure described by Giljohann et al. [1]with modifications. Briefly, 1 nmole of siRNA was added to 1 ml GNPs. The sample was treated

with gradient concentrations of NaCl (0.1M, 0.2M and 0.3M each for 20 min). After salt aging the sample was incubated with 30 µmol/ml of thiolated OEG (11-Mercaptoundecyl)tetra(ethylene glycol; Sigma

) or no OEG. The sample was centrifuged at 13000 rpm for 20 min at 4˚C and washed three times with sterile 1X PBS. The pellet was finally resuspended in 100 µl of RNAse free water . The conjugation was verified by gel electrophoresis.

2.4 Transfection assays:

For the validation of designed siRNAs, 3T3-L1 cells were trasfected with lipofectamine in 6-well plate format with 2 µg of [pGL4.10 (luc2)/SV40] along with 0.2 µg renilla luciferase plasmid vector [pGL4.74(hRluc/TK)]. 2 µg of FFL or scrambled siRNAs were transfected simultaneously with plasmid transfections (concurrent) or after 24 h of vector transfections (Post) or 24 h before vector transfections (Pre). The cells were harvested after 48h after siRNA trasfection in both concurrent and Pre-treatments, while after 24 h in the case of Post-treatment. The cells were harvested and assayed for FFL and renilla luciferase activity using a Dual luciferase assay system (Promega) following the manufacturer's recommendations.

In the case of testing gold nano-siRNA complexes (with OEG or no OEG), each well was incubated either with 1 picomole of GNP or 2 µg of FFL siRNA or scrambled siRNA or GNP-siRNA (with no OEG) treated with lipofectamine after 24 hrs of vector transfections. Cells were incubated for 24 hrs after the treatments and harvested for luciferase assays

3. RESULTS

3.1 Validation of designed siRNAs: To determine whether the designed siRNAs were able to knockdown the targeted FFL gene, the -

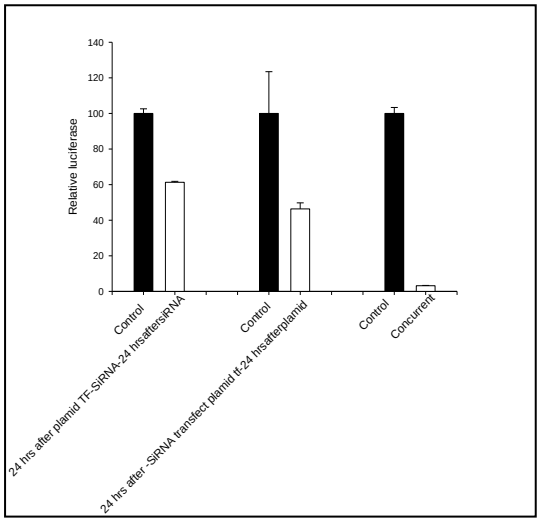

Fig. 1. The designed siRNAs were able knockdown in three different conditions the targeted firefly gene
as describein methodology and results.

transfection studies were conducted in three different conditions. First, the 3T3-L1 cells were transfected with FFL plasmid and incubated for 24 h before FFL siRNA transfections. This was followed by further incubation for 24 h before cells were assayed for FFL activity. Under this condition, siRNAs were able to knock down FFL gene by 40% (Fig.1 left). Second, the 3T3-L1 cells were transfected with FFL siRNAs incubated for 24 h before FFL plasmid transfections. The cells wre harvested after 24 h and assayed for FFL activity. FFL siRNA were able to knockdown gene by approximately 50% (Fig.1 centre). Third 3T3-L1 cells were transfected with FFL siRNAs and FFL plasmids at the same time and cells were assayed after 48 hrs after transfections. The analyses clearly suggest that FFL siRNA were able to knockdown FFL gene by 96% (Fig.1 right). These results clearly establish the functionality of siRNAs in knocking down the targeted FFL gene.

3.2 Design of the study: To determine the efficiency of knockdown of siRNAs conjugated to GNP , the following

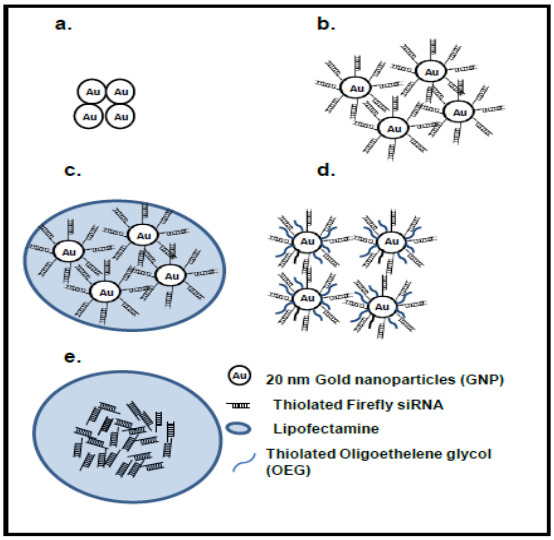

Fig. 2. Schematic representation of the preparations used to incubate with 3T3-L1 cells expressing Firefly gene were represented a. Gold nanoparticles (GNP) b.Gold nanoparticles conjugated with firefly siRNAs (GNP-siRNA) c. Gold nanoparticles conjugated with firefly siRNA and treated with lipofectamine d. Gold nanoparticles conjugated with firefly siRNAs and oligo ethelene glycol (G*NP-siRNA-OEG*) e. siRNAs treated with commercial transfection agent, lipofectamine (siRNA-lipofectamine)

design depicted in Fig. 2 was used. Cells were incubated with a. Gold nanoparticles (GNP) b. Gold nanoparticles conjugated with FFL siRNAs (GNP-siRNA) c. Gold

nanoparticles conjugated with FFL siRNA and treated with lipofectamine d. Gold nanoparticles
conjugated with firefly siRNAs and oligo ethylene glycol (G*NP-siRNA-OEG*) e. siRNAs treated with commercial transfection agent, lipofectamine (siRNA-lipofectamine)

3.3 Knockdown of GNP-siRNAs is dependent on OEG:

3T3-L1 cells expressing FFL gene, upon incubation with gold nanopartilces (GNP) alone or GNP-siRNA conjugates or transfected with

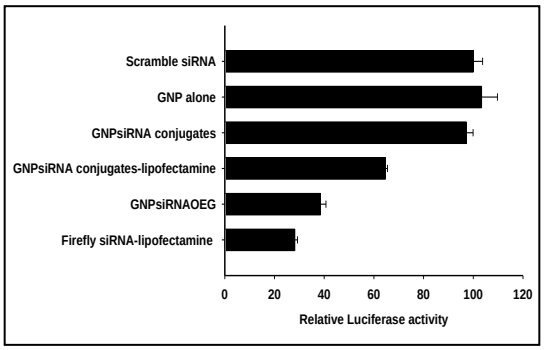

Fig. 3. 3T3-L1 cells were trasfected with lipofectamine in 6-well format with 2 µg of [pGL4.10 (luc2)/SV40] along with 0.2 µg [pGL4.74(hRluc/TK)]. After 24 h of vector transfections , the cells were treated either a. scrambled siRNA-lipofectamine b. gold nanopartilces (GNP) alone c. GNP-siRNA conjugates d. GNP-siRNA conjugates-lipofectamine e. Gold nanoparticles conjugated with firefly siRNAs and oligo ethelene glycol (G*NPsiRNAOEG*) f. firefly siRNAs [5'-Thio-C6 linker (S-S)-AAAAAAAAAAAAACCUACGCCGAGUACUUCGAdUdU-3' (Sense); 5'-UCGAAGUACUCGGCGUAGGdUdU -3'(Antisense)] transfceted with lipofectamine.

siRNA did not result in knockdown of FFL gene (Fig.3) Whereas, incubation of GNP-siRNA-OEG; tranfections with GNP-siRNA or FFL siRNAs resulted in the knockdown of the targeted gene as quantified by luciferase reporter assays. These results clearly suggest that siRNAs retained their functionality upon addition to gold surfaces and their delivery to the target cytoplasm seems to be facilitated by OEG or lipofectamine.

4. CONCLUSIONS

The studies conducted here in suggest that Oligoethelene glycol aids *AuNP-siRNA* conjugates to have biological activity in knocking down the targeted firefly gene. The future research will reveal if OEG affects the stability of siRNA or cellular uptake and trafficking of the conjugates.

5 . REFERENCES

1. Giljohann, D.A., et al., *Gene regulation with polyvalent siRNA-nanoparticle conjugates.* J Am Chem Soc, 2009. **131**(6): p. 2072-3.