PAPER PRESENTATION
BY
KAMALESHE
Gold, Poly(β-amino ester) Nanoparticles for Small Interfering RNA Delivery

Nano Lett., Vol. 9, No. 6, 2009, 2402-2406

Jae-Seung Lee,† Jordan J. Green,‡ Kevin T. Love,§ Joel Sunshine,‡ Robert Langer,§ and Daniel G. Anderson*,§
† Department of Materials Science and Engineering, Korea UniVersity, Seoul, 136-713, Republic of Korea,
‡ Department of Biomedical Engineering, Johns Hopkins UniVersity School of Medicine, Baltimore, Maryland 21205,
§ The DaVid H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
RNA interference -

- RNA interference is a system within living cells that helps to control which genes are active and how active they are.
- It is an endogenous process whereby double-stranded RNA (dsRNA) can mediate the catalytic destruction of its homologous mRNA target.
- RISC – RNA induced silencing complex
Need for work-

- The safe and effective intracellular delivery of siRNA remains the most challenging barrier to the broad application of siRNA in the clinic.
- To prove use of PBAEs as a delivery enhancer, and gold nanoparticles (AuNPs) as a scaffold to assemble siRNA strands.
Plan -

- Step 1: Preparation of NH2-PEG-AuNPs
- Step 2: Conjugation of HS-siRNA to NH2-PEG-AuNPs by biodegradable disulfide linkage
- Step 3: Quantitative analysis of #(siRNA)/AuNP
- Step 4: Optimization of reaction conditions
- Step 5: Preparation of PBAE
- Step 6: Preparation of Au-siRNA-PBAE nanoparticles:
- Step 7: Cellular transfection and gene knockdown
Preparation of NH2-PEG-AuNPs

- The synthesis of the siRNA-AuNP begins with modifying AuNPs with HS-(CH2CH2O)n-NH2, (M.W. ~1000Da)

\[
\text{AuNP (colloid 15nm, ~2.5nM)} + \text{HS-PEG-NH2 (20mg)} \quad \text{12hr incubation at } 25^\circ \text{C}
\]

(washing and redispersion in PBS containing 0.01 % polysorbate surfactant Tween20 for further use)

Tween 20 is a polysorbate surfactant whose stability and relative non-toxicity allows it to be used as a detergent and emulsifier in scientific, and pharmacological applications.
Conjugation of HS-siRNA to NH2-PEG-AuNPs

- Conjugation of HS-siRNA to NH2-PEG-AuNPs
  - conjugation of SPDP to NH2-PEG-AuNPs
  - conjugation of HS-siRNA to SPDP-PEG-AuNPs

i) SPDP - \( N\)-succinimidyl 3-(2-pyridyldithio) propionate

\[
\text{NH2-PEG-AuNPs} + \text{SPDP} \xrightarrow{25^\circ C} \text{40 min. vigorous vortexing} \\
\text{(400\,\mu L, 30 nM)} \quad \text{(400 \,\mu L, 3 mM in PBS, 10 \%DMSO)} \\
\text{(washing with PBS containing 0.01 \% Tween20 to get pallete)}
\]
Conjugation of HS-siRNA to NH2-PEG-AuNPs

**ii) siRNA - antifirefly luciferase**

A = HS-siRNA + NaCl + Borate buffer + Tween 20
(15 μM) (2.5 M) (400 μL, pH 8.5, 30 mM) (0.01 %)

B = SPDP-PEG-AuNPs pallele

25°C, 40 hrs

A + B → siRNA-AuNPs
(400μL) (pallele) vigorous vortexing

Concentration was adjusted to 15nM

washing with PBS
0.01 % Tween 20
Quantitative analysis of #(siRNA)/AuNP:

- siRNA-AuNPs were incubated in 0.05 M dithiothreitol solution in PBS (pH 7.4, 0.01 % Tween 20) for 30 min at 30 °C to cleave the disulfide bonds.
- Number of released siRNA strands were quantitatively analyzed by RiboGreen™ RNA reagent.

Protocol is available at,
Optimization of reaction conditions

- Optimization of reaction conditions to increase loading of siRNA on AuNP,
- High salt stability (up to 2.5M NaCl) of NH2-PEG-AuNPs was important to facilitate siRNA conjugation
Optimization of reaction conditions

- Increase in salt concentration helps to reduce repulsion between the negatively charged siRNA strands and hence increases loading.
- Loading of siRNA per AuNP without SPDP was low, indicates that HS-siRNA is conjugated primarily to SPDP, not to the AuNP surface by displacing NH2-PEG-SH from gold.
- Slight formation of AuNP aggregates takes place after 40 hrs, hence the optimum conjugation time for HS-siRNA to AuNPs was determined to be 40 hrs.
- Optimization results into ~30 strands of siRNA per particle.
Preparation of PBAE:

C + 32 → C32 - Ac

C32 - Ac + R-NH₂ → C32 - X
Preparation of PBAE

1,4-butane diol diacrylate
(3532 mg) + 5-amino-1-pentanol
(1533 mg)

Addition of 9.1 g THF and vortexing

A

A + End-capping amine solution
(40 mL, 0.25 M) → Stirring in dark (24 hr) → Amine terminated PBAE

Acrylate-terminated PBAE ~5 g
Preparation of PBAE

- Precipitation -
  End-modified polymers were precipitated by the addition of 10 volumes of diethyl ether and centrifugation at 2,500 rpm for 2 minutes.

- Washing -
  Polymers were washed twice and dried in a vacuum desiccator.
Preparation of Au-siRNA-PBAE nanoparticles
Preparation of Au-siRNA-PBAE nanoparticles

Preparation was done in following steps,

dilution to

\[
PBAE \quad \rightarrow \quad 0.36 \, \mu g/\mu L
\]

(100 \, \mu g/\mu L in DMSO)  \quad \text{(In 25 mM acetate buffer, pH 5)},

Rapid addition of
siRNA-AuNPs (15 nM)
to make,

10.8 \, \mu g of PBAE and 300 fmol of AuNPs in 100 \, \mu L
( incubated for 10 min )
## Preparation of Au-siRNA-PBAE nanoparticles

- Library of PBAEs was used to synthesize siRNA-AuNPs and then to screen for their ability to facilitate functional siRNA delivery, in vitro.
- ζ-potential measurements,
  - siRNA-AuNPs = ca. -34 mV
  - PBAE-siRNA-AuNPs = ca. +13 mV

\[
\begin{array}{c|c|c}
103 & H_2N & H_2N \\
116 & H_2N & H_2N \\
117 & H_2N & H_2N \\
118 & H_2N & H_2N \\
122 & H_2N & H_2N \\
208 & H_2N & H_2N \\
210 & H_2N & H_2N \\
212 & H_2N & H_2N \\
213 & H_2N & H_2N \\
221 & H_2N & H_2N \\
225 & H_2N & H_2N \\
228 & H_2N & H_2N \\
\end{array}
\]
Preparation of Au-siRNA-PBAE nanoparticles

- UV-vis spectra taken during various synthetic steps,
Cellular transfection and gene knockdown

- HeLa cells were genetically engineered to express both firefly luciferase and Renilla luciferase.
- Then cells were allowed to adhere at 37 °C, 5 % CO2 overnight in growth medium (10 % FBS and 90 % phenol red-free DMEM).

A + HeLa cells  4hr incubation replaced by fresh growth (in 100 µL well)  24hr incubation
37 °C, 5 % CO2

Dual-GloTM Luciferase Assay

Protocol available at,
http://www.promega.com/tbs/tm058/tm058.html
Cellular transfection and gene knockdown

- **Experimental Conditions for Lipofectamine 2000**
  (commercially available liposome-based delivery agent)

  A = Lipo2000 (17.8 µL in 513 µL OptiMEM) and wait 5 min.

  B = siRNA(0.28 mg/mL) diluted to 1.79 µL in 3.33 mL OptiMEM

  C = Add 530 µL of B to A and wait 20 min.

  D = Add 20 µL C to 130 µL of media of the positive control
  (The final [siRNA] = ~ 1 nM).
Results:

Control = Untreated cells
Results:

- Unassembled siRNA combined with PBAEs did not exhibit any silencing effect.
Results:

- Cell viability test was performed by Cell Titer96® AQueous One Solution Cell Proliferation Assay (Invitrogen)

Results:

TEM images of the HeLa cells exposed to 3 nM (A) PBAE-siRNA-AuNPs
PBAE is C32-221,
(B) siRNA-AuNPs without PBAEs,
(C) unmodified AuNPs,
(D) no nanoparticles (control).

- The images were taken with a Tecnai G2 Spirit at HV 80 kV.
Conclusions:

- PBAEs (C32-228 and 221) exhibit significantly better efficiency than the commercially available liposome based delivery agent (Lipofectamine2000, Invitrogen).
- No significant toxicity was observed when PBAE is used as siRNA delivery agent.
- PBAEs can be used as a delivery enhancer in gene/Drug delivery.
- Gold nanoparticles are essential as scaffolds for the development of some complex drug delivery vehicles.
Thank you
- $\text{C}_{2n+2}\text{H}_{4n+6}\text{O}_{n+2}$
- \(\text{N-succinimidyl 3-}\text{(2-pyridyldithio) propionate}\)

- DMSO is an important polar aprotic solvent. It is less toxic than other members of this class like HMPA. Because of its excellent solvating power, DMSO is frequently used as a solvent for chemical reactions involving salts.
The sulfur center in DMSO is nucleophilic toward soft electrophiles and the oxygen is nucleophilic toward hard electrophiles.

Opti-MEM® I Reduced Serum Media is ideal for use during cationic lipid transfections using our available list of transfection reagents.