Highly efficient siRNA delivery method by self-assembled RNA microsponges

Daehoon Han[1], and Jong Bum Lee[1]
[1]Department of Chemical Engineering, University of Seoul, Seoul, 130-743, South Korea

Statement of Purpose: RNA interference (RNAi) has great potential as an effective method to control gene expression. Although a variety of siRNA delivery methods have been developed, efficient intracellular delivery of siRNA is still difficult. To improve the delivery efficiency, a variety of methods have been developed using chemical modification, carrier particle and cationic material. However, many challenges still remain such as immune response and low loading efficiency. Our recent achievements, RNA microsponge[1], can overcome these limits. The RNA microsponge which is sponge-like particle includes numerous siRNA strands. Because microsponges are only composed of RNA, numerous siRNA strands can be delivered without toxicity. However, the structure of RNA microsponge is still needed to be improved in order to enhance the efficiency of cellular uptake. Therefore, researches on the control of size and electric surface charge of particles are required for highly efficient siRNA delivery.

Methods: RNA microsponge can be produced by rolling circle transcription (RCT) with T7 RNA polymerase (New England Biolabs, Ipswich, MA). Circularization of linear single stranded DNA (92-mer) which includes siRNA sequences can be performed by hybridization with primer DNA (22-mer). Hybridized circular DNA can be chemically closed by T4 DNA ligase (Promega, Madison, WI). With ribonucleotides (New England Biolabs, Ipswich, MA), closed circular DNA elongates predetermined long linear ssRNA which includes numerous siRNA sequences by T7 RNA polymerase. Through the RCT process, circular DNA produces 2 μm size of RNA microsponge. To improve the delivery efficiency of RNA microsponge, the conditions of self-assembly of RNA microsponges were changed with various concentrations of circular DNA and RNA polymerase. Through the control of RNA polymerase and circular DNA concentration, the size and shape of RNA microsponge can be changed to effective form which can increases efficiency of siRNA delivery.

Results: The RNA microsponge which can deliver numerous siRNA is produced by RCT process (Figure 1).

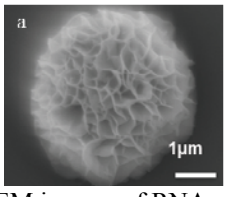

Figure 1. SEM images of RNA microsponge.

Also, concentration of circular DNA and T7 RNA polymerase controlled for improving the efficiency of siRNA delivery. A variety of particles can be produced by control of circular DNA concentration. Low concentration of circular DNA produced branch-like structure particle and rod-like structure particle respectively as shown in Figure 2a and 2b. Through the various structures of RNA particle, more appropriate siRNA delivery can be achieved. And the size of RNA microsponge can be decided by control of T7 RNA polymerase concentration. Increased concentration of RNA polymerase decreases the size of RNA microsponge to 600 nm and decreased concentration of RNA polymerase increases the size to about 5 μm (Figure 2c and 2d).

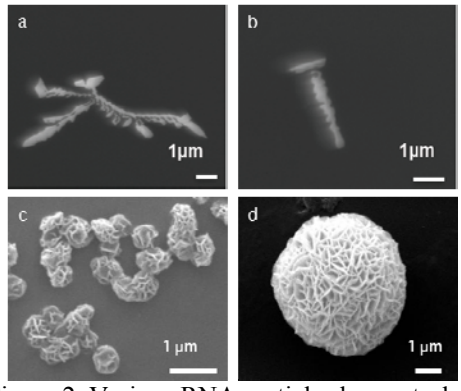

Figure 2. Various RNA particles by control of concentration. 30 nM of circular DNA produces branch-like particle (a) and 3 nM of circular DNA produces rod-like particle (b). Change of concentration of RNA polymerase results in the change of the size of RNA microsponge (c) and (d).

Conclusions: The RNA microsponge is produced by RCT. Because the RNA microsponge which is composed of only RNA strands includes numerous siRNA sequences, effective siRNA delivery can be achieved. To improve efficiency of siRNA delivery, the size and structure can be controlled by two different methods. The first method is condensation with cationic material. By condensation, the size of RNA microsponge can be decreased effectively. The other method is control of concentration of circular DNA and RNA polymerase. By control of RNA polymerase and circular DNA concentration, the size and structure can be changed to more appropriate shape for highly efficient siRNA delivery.

Reference:

1. Lee, J. B. Nature Materials. 2012;11:316–322.