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Preparation of Artificial Cells Using Eggs with Sphingosine-DNA

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Abstract

Artificial cells generated with defined compounds are useful for the study of the life science. I have developed a method for preparing artificial cells; this method involves chicken eggs and sphingosine-DNA. Artificial cells were prepared by incubating seeds within egg white; these seeds comprised sphingosine, DNA, and factors that bind sphingosine-DNA particles. A method that can be used to easily prepare artificial cells is described here.

Keywords: Artificial cells; Sphingosine; DNA; Adenosine

Introduction

The study of the artificial cells began in the 1960s and has continued ever since [1]. At present, artificial cells include nanoparticles, liposomes, polymersomes, microcapsules, and other such particles that are capable of one or more cellular functions [2]. Artificial cells can possess structures similar to those in regular cells including polymeric membranes, which allows for the metabolism or exchange of small molecules [3]. Additionally, artificial cells that enclose biologically active materials such as drugs, hormones, enzymes, or transgenes have been prepared and used for clinical applications [4,5].

In the earliest stages of artificial cell study, cellular compartments corresponding to the cell membrane were constructed as parts of the first artificial cells [6]. However, there has been no success to date in the preparation of artificial cells that can replicate a complex function of any biological cell organelle, such as protein synthesis by ribosomes.

To generate artificial cells with such functions, most studies focused on the generation of artificial cells have been conducted in the field of synthetic biology [7-9] and engineered cells have been successfully developed [10]. Such attempts to generate artificial cells have resulted in vesicle-like cells that contain only a few cellular components, such as synthetic or man-made genomes and enzymes [11,12]. Cells or vesicles with multiple functionalities have a variety of applications in a number of fields [13].

Recent work on artificial cells has focused on cell division or replication. Artificial cells that can replicate can be generated using techniques from synthetic biology, and these cells may acquire the machinery for transcription and translation.

In fact, many published findings on the division of artificial cells have been reported. Artificial cells that incorporate micelles can divide after mild agitation and the addition of phospholipids [14,15]. Moreover, many factors associated with the division of cells can be synthesized and function in liposomes, which are themselves a kind of artificial cell. Polymerase chain reaction could be carried out within a liposome containing a synthetic gene [16]. Notably, green fluorescent protein (GFP) has been produced within a liposome [17]. RNA was synthesized in phospholipids that were provided with adenosine-diphosphate [18].

Such evidence suggests that it is theoretically possible to prepare artificial cells that can be generated through the use of defined organic compounds or defined cell materials with the ability to replicate. I have studied the preparation of such artificial cells with sphingosine (Sph)-DNA and demonstrated that such artificial cells could be prepared using egg white.

First, artificial cell seeds that comprised Sph, DNA, and the components that bound Sph-DNA were prepared. Next, the artificial cells were prepared by incubating these seeds in egg white. This method for easily preparing artificial cells is described here.

Methods Summarized and Results

A depiction of the methods used in the study can be found in Figure 1.

Preparation of Sph-DNA particles

Materials: Sph (Sigma, USA)

DNA (*Eschericia coli, strain* B, Sigma, USA)

DNA (human placenta, Sigma, USA)

Mixing Sph with DNA led to the formation of the assembly of particles: these particles were then designated "a heated assembly" (Figure 1; Step 1).

Preparation of seeds of artificial cells

Preparation of seeds: Seeds were generated by adding the binding factors (BFs) of artificial cell seeds to Sph-DNA (Figure 1; Step 2).

Preparation of BF

The BFs used in the experiments were H-extract, F-fraction, or adenosine.

Preparation of H-extract: H-extract was prepared from the meat of an ascidian, the edible sea squirt (*Halocynthia orezi*), which was purchased from a local market. Ascidian meat and NaCl were mixed at a mass ratio of 95%:5% to generate H-extract. In each experiment, prepared H-extract stock was diluted 10 fold with distilled water.

Preparation of F fraction: F-fraction was prepared from H-extract that lacked proteins and nucleic acid. One such case is described below.

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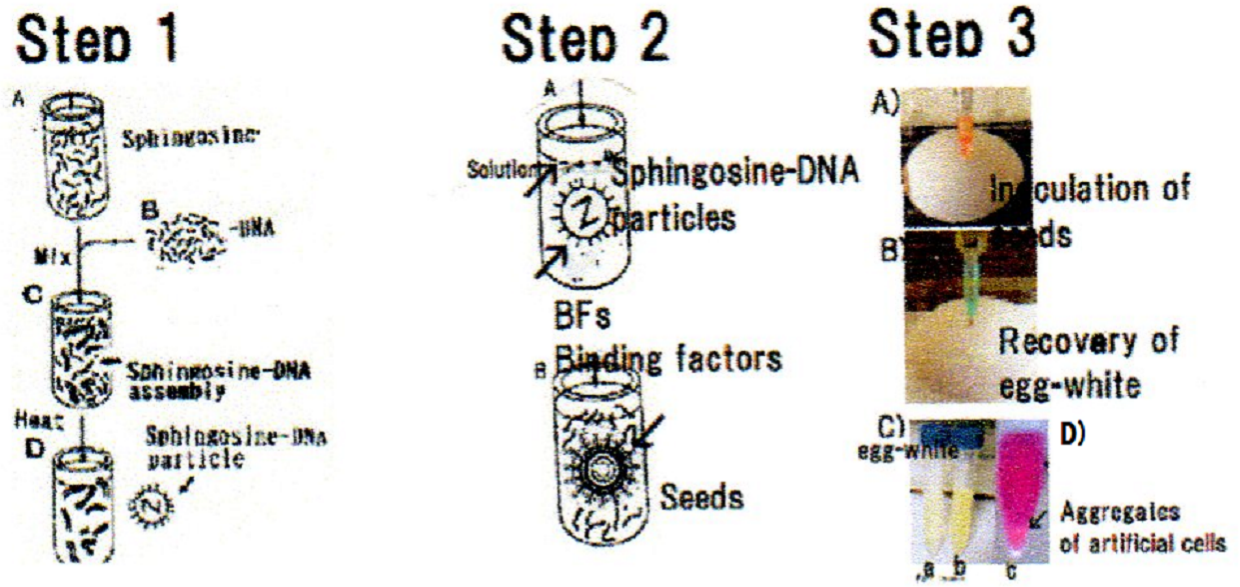

Figure 1: Summary of methods for preparing artificial cells.
Step 1: Preparation of the sphingosine (Sph)-DNA particles. When Sph solution (A) was added to the DNA solution (B), the Sph-DNA assembly (C) formed. Heating the assembly prepared the particles (D).
Step 2: Preparation of the seeds.
a) BF solution (H-extract, F-fraction, or adenosine) was added to Sph-DNA particles (A), and seeds were prepared (B)
Step 3: Preparation of artificial cells
A) Solution containing seeds were injected into egg white.
B) Eggs were incubated for 7 days at 37°C, and egg whites were collected.
C) Egg whites were removed from the injected eggs (C-b).
D) Egg whites were incubated with Dulbecco's modified Eagles medium containing 10% bovine serum (D-MEM). The aggregates (C-c, arrow) of artificial cells were observed in the bottom.

Undiluted H-extract (83 mg: wet weight) was incubated at 65°C for 30 minutes in a microfuge tube. 400 µl of F-solution (from the DNA extraction, DNAs-ici!F, Rizo Inc, Japan) was then added to the tube, and the tube contents were mixed. Then, equal volume (400 µl each) of phenol and chloroform were added to the tube. The contents of the tube were mixed until an emulsion formed. The tube was centrifuged for 10 minutes at 6,714 × g.

The aqueous phase was separated from the organic phase and an equal volume of isopropanol was mixed with the aqueous phase; this mixture was separated by centrifugation for 10 minutes at 15,107 × g. The upper fluid was collected and dried. The fraction was used as F-fraction. In subsequent experiments, this F-fraction was dissolved in distilled water (1 ml).

Adenosine (0.1M, Sigma, USA) was also prepared in distilled water

Thus, seeds were generated by addition of H-extract, F-fraction, or adenosine to Sph-DNA particles. Though artificial cells could be prepared with each type of BF used here, the best DNA-BFs combination for preparation of artificial cells (including undetectable BF) may not yet have been tried.

Generation of artificial cells: Seeds comprising mixtures of Sph-DNA and BFs were injected into egg white (Step 3A) and incubated for 7 days at 37°C; the inoculated egg white was then removed from the shell (Step 3B) and kept in a test tube (Step 3C).

The cells were then cultivated in Dulbecco's modified Eagles' medium containing 10% bovine serum (D-MEM). Artificial cells aggregated at the bottom of the culture tubes (Step 3C, arrow).

An example for illustration

Described here are examples of the preparation of artificial cells involving prokaryotic DNA or eukaryotic DNA.

Case 1: Preparation of artificial cells that comprised Sph, prokaryotic DNA, and adenosine.

Preparation of seeds (Sph-DNA: adenosine particles)

A mixture of 90 µl of Sph (10 mM) and 40 µl of DNA (1.7 µg/µl) (*E. coli*, strain B) was prepared. The mixture was heated for a few minutes, and 300 µl of adenosine solution was added into the Sph-DNA mixtures.

Preparation of artificial cells

A hole was drilled in the shell of each white Leghorn egg, which had been purchased from a local market, and 100~200 µl of Sph-DNA-adenosine mixtures (seeds) was injected into the white (albumin) of each egg. Each egg was then incubated for 7 days at 37°C. The albumin of each egg was collected and 0.5~1.0 ml of each albumin collection was added to 10 ml of D-MEM, and each such mixture was cultured for 2 days at 37°C. Aggregates (artificial cells) were then collected.

Figure 2 shows a phase contrast micrograph of seeds that were prepared with Sph, *E. coli* DNA, and adenosine. These seeds were homogenous, small, spherical, particles of approximately ~50 nm.

Figure 3 shows a fluorescence micrograph of the same field of view

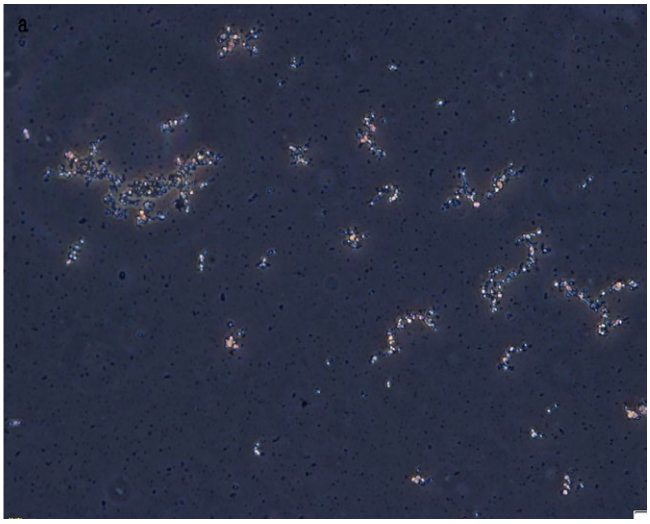

Figure 2: Phase contrast microscopic integrity of artificial cells seeds that were prepared with Sph-DNA from *E. coli* and adenosine. Sph solution was added to *E. coli* DNA. After heating, adenosine solution was then added to Sph-DNA mixtures (seeds). Each seed was observed as a dot. Scale bar is 20 μm.

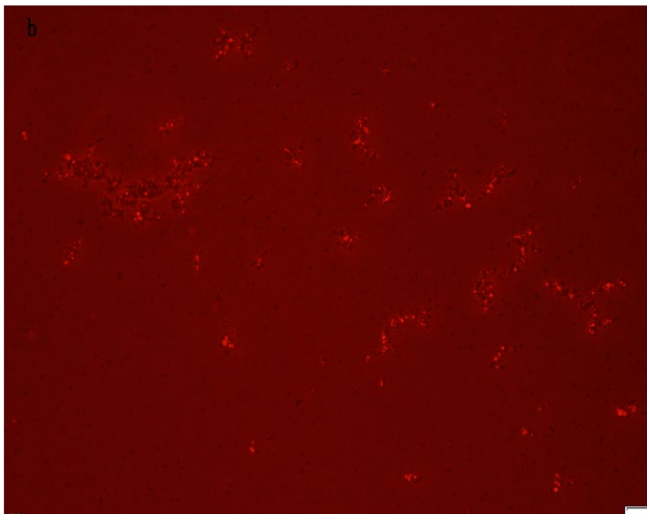

Figure 3: Fluorescence microscopic integrity of artificial cells seeds that were prepared with Sph-DNA from *E. coli* -adenosine. The seeds that were prepared in Figure 2 are shown under a fluorescence microscope. Fluorescence was observed on the seeds. The image is the same field of view as shown in Figure 2, this image shows that the seeds possess DNA. Scale bar is 20 μm.

shown in Figure 2. Brilliant light was observed, indicating that the artificial cells seeds contained DNA.

Figure 4 shows a phase contrast micrograph of artificial cells that were prepared with Sph-DNA-adenosine mixtures that contained seeds.

The cells appeared as individual dots or aggregates of dots.

Case 2 Preparation of artificial cells that contained Sph, eukaryotic DNA, and F-fraction.

Preparation of seeds that each comprised a Sph-DNA particle and an F-fraction

A mixture containing 90 μl of Sph (10mM) and 40 μl of the heat treated DNA (see below) (1.7 μg/μl) was prepared; the mixture was then boiled. To prepare heat-treated human DNA, 40 μl of DNA from human placenta was boiled and immediately cooled. This procedure was carried out three times. F-fraction (300 μl) was added to the Sph-DNA mixture.

Figure 5 shows a phase contrast micrograph of seeds that were prepared with Sph-DNA (human placenta) and F-fraction. The seeds were shaped like clusters of wads. Also, Figure 5 shows that the seeds consisted of a wad or several wads.

Figure 6 shows a fluorescence micrograph of the seeds shown in Figure 5. Brilliant light was observed in each single wad and separately in each cluster of wads, indicating the wads contained DNA.

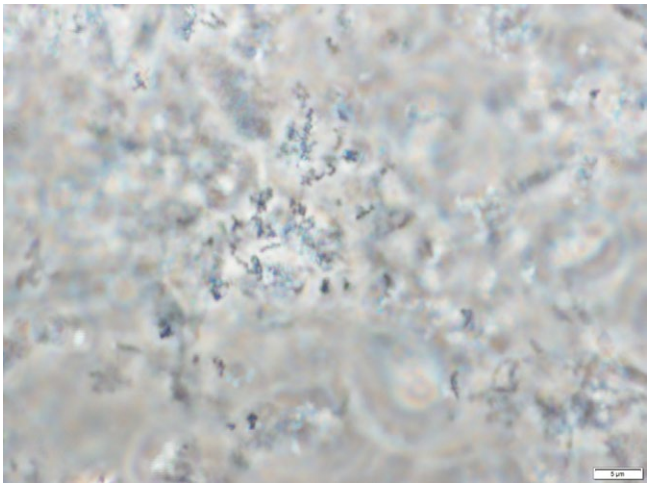

Figure 4: Phase contrast microscopic integrity of cells that were formed with Sph-DNA from *E. coli* and adenosine. Sph- *E. coli* DNA-adenosine mixtures (seeds) were incubated within egg-whites. Then, egg whites were cultivated in D-MEM. Aggregates (artificial cells) were observed. The artificial cells were observed as individual dots or an aggregate of dots. Scale bar is 5 μm.

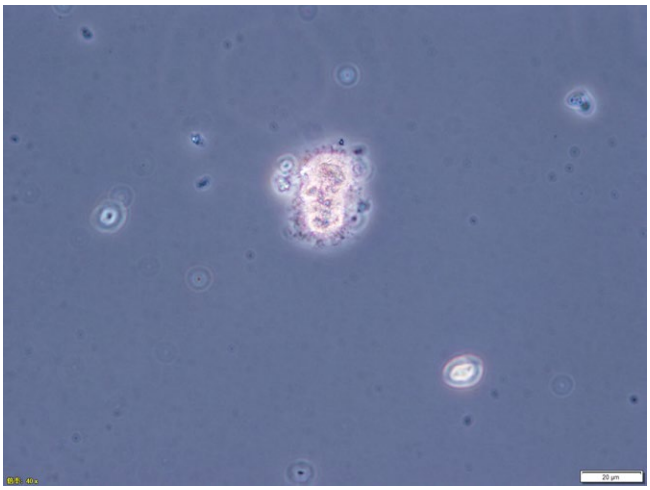

Figure 5: Phase contrast microscopic integrity of artificial cells seeds that were prepared with Sph-DNA from human placenta and F-fraction. Sph solution was added to the heated human placenta DNA solution. Then, F-fraction was added to the mixture. Seeds were examined under a phase contrast microscope. A single wad or aggregates, each aggregate like a clump of wads, were observed. Scale bar is 20 μm.

Preparation of artificial cells

Preparation of artificial cells was carried out as described in Case 1.

Figure 7 shows an image of artificial cells that were prepared from seeds comprising Sph-DNA from human placenta and F-fraction. The images show cells with a round or ellipse-like shape. Individual, single cells or clusters of three cells were also observed. Thus, artificial cells corresponding to their respective seeds could be easily prepared using egg white.

Other characteristics of artificial cell seeds or cells

Expression of a plasmid (pGLO) that contains GFP gene, which encodes GFP, within artificial cells. These artificial cells could produce GFP.

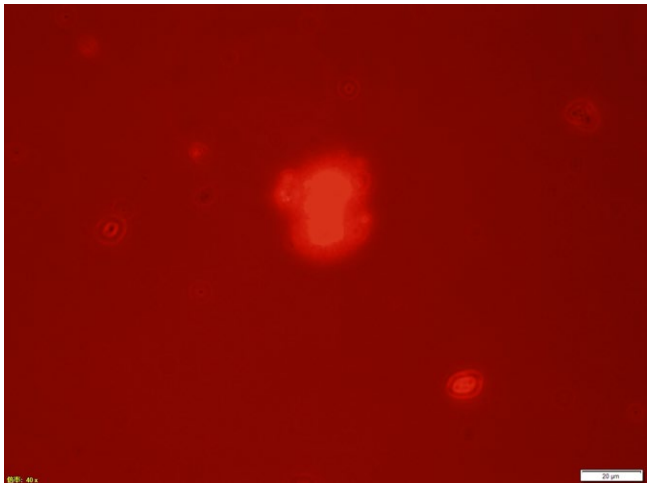

Figure 6: Fluorescent microscopic image of seeds that were prepared with Sph-DNA from human placenta and F-fraction. Russet light is observed in each cell or a part of a cell aggregate, indicating that DNA is present in the seeds. The image is the same field of view shown in Figure 5. Scale bar is 20 μm.

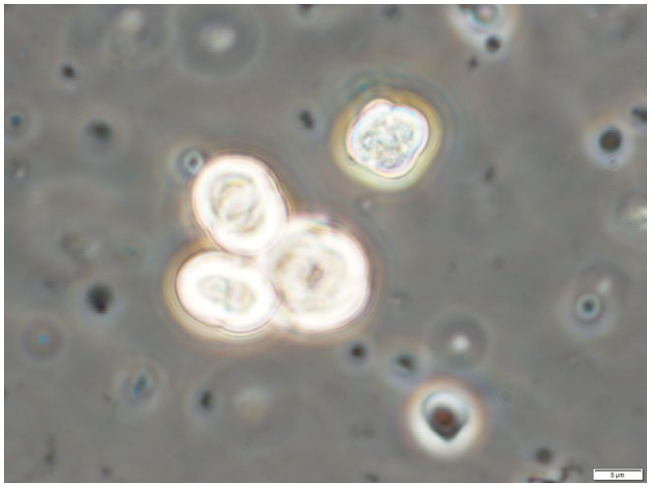

Figure 7: Phase contrast microscopic image of artificial cells that were prepared with seeds (Sph-DNA from human placenta and F-fraction). Seeds were incubated in egg white for 7 days; after which, 1 ml of egg white was cultivated in D-MEM for 2 days. The aggregates were viewed under a phase contrast microscope. Each artificial cell had a roughly round or ellipse-like shape. Individual artificial cells and clusters of three cells were observed. Scale bar is 20 μm.

A mixture containing 90 μl of Sph, 40 μl of DNA (*E. coli* 1.7 μg/μl), and 20 μl of pGLO plasmid (12 ng/μl) was prepared, and the mixture was heated for a few minutes. H-extract (300 μl) was added into the Sph-DNA mixture. This new mixture was used as a seed preparation. These seeds were then incubated within egg white.

Artificial cells were stained with ethidium bromide solution

Figure 8b shows that green light was observed in part of the cell monolayer; this finding indicated that these cells contained GFP.

Figure 8c shows the russet light of artificial cells, suggesting that DNA was present in the artificial cells.

Figure 8a shows phase a contrast micrograph of artificial cells. The cells were observed as individual dots.

Electron microscopic image of artificial cells

Figure 9 shows an electron micrograph of the artificial cells that were prepared with Sph, DNA (*E. coli*) and H-extract. Cellular compartments that may have corresponded to the cell membrane were clearly observed in these artificial cells, indicating that the cells had built at least one identifiable cell structure.

Maintenance and storage of artificial cells

Artificial cells in egg white prepared using Sph-DNA (*E. coli*) and H-extract could be stored at 4°C for 52 weeks and could be maintained by inoculating a sample of egg white containing artificial cells into fresh egg white every 1-2 weeks.

Adenosine in seeds (Sph-DNA-adenosine)

High Performance Liquid Chromatography (HPLC) was used to examine whether adenosine bound to Sph-DNA. Sph and DNA were mixed and boiled; adenosine solution was added to the Sph-DNA solution. The precipitate was then recovered and dissolved in 1 ml

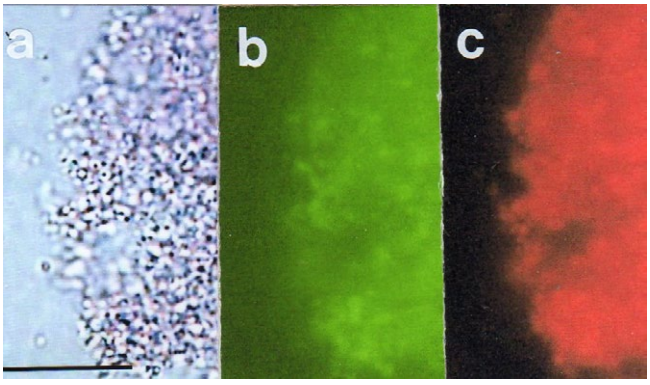

Figure 8: Fluorescent microscopy of artificial cells that were prepared with seeds (Sph-DNA (*E. coli* plasmid) and H-extract) Sph solution was added to *E. coli* DNA (plasmid). Then H-extract was added to the mixture. The mixture (containing seeds) was incubated in egg white for 7 days. Egg white that contained artificial cells was cultivated in D-MEM medium, and the aggregates that formed at the bottom of each tube were stained with ethidium bromide solution. The cells were smeared on a slide glass to form a cell monolayer. Images a though c all show the same field of view.

a) Phase contrast microscope image without filters. Scale bar 20 μm.
b) Green light is observed in cell monolayer with the B: U-MNIBA2 filter, indicating that GFP (green fluorescent protein) was present. Scale bar is 20 μm.
c) Russet light is observed in the cell monolayer with the G-U-MWIG2 filter, indicating that DNA is present. Scale bar is 20 μm.

of buffer. The HPLC chromatogram from analysis of this precipitate is shown in Figure 10a. Only one main peak was evident on this chromatogram. Additionally, adenosine was tentatively identified based on retention times (6.71 in adenosine) and comparisons with standard compounds (Figure 10b). The findings indicated that adenosine bound to Sph-DNA particles (namely seeds) that consisted of Sph-DNA-adenosine.

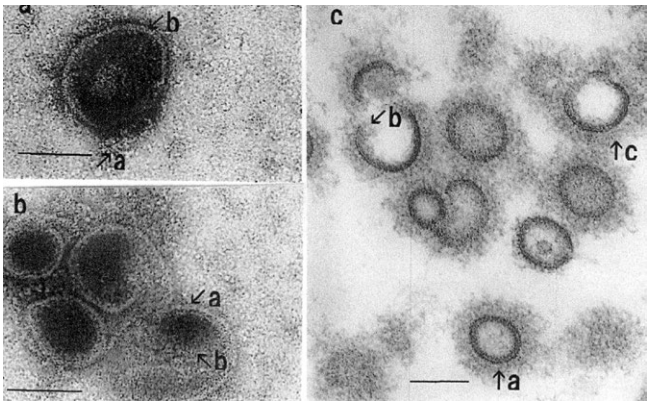

Figure 9: Electron microscopic image of artificial cells that were prepared with Sph-DNA (*E. coli*) and H-extract [19].

a) Negative scanning electron micrograph of an artificial cell. The cell is spherical (arrow a), and appears to be in an envelope (arrow b) Scale bar is 100 nm.

b) Negative scanning electron micrograph of artificial cell cluster. Artificial cells formed a cluster comprising four cells. One cell (arrow a) has an irregular shape with narrowing in the middle (arrow b). Scale bar is 100 nm.

c) Scanning electron micrograph of thin section of artificial cells. Artificial cells appear as rings (arrow a). One cell (arrow b) with a cut ring shape is also observed. These cells are coated with structures of irregular lengths (arrow c). Scale bar is 100 nm.

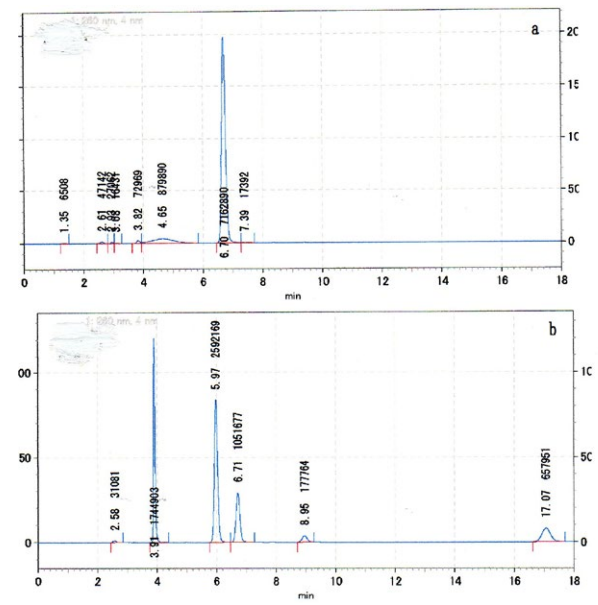

Figure 10: High performance liquid chromatography (HPLC) of adenosine that bound to Sph-DNA (*E. coli* DNA). Sph and *E. coli* DNA were mixed. After boiling, adenosine was added to the mixture. The precipitates were recovered and resolved in buffer.

a) The chart of the adsorbed adenosine. Vertical scale is absorbance at 260 nm, and the chapter is retention time (minutes). One component is represented in the vertical scale.

The chart of commercial adenosine is evident with the retention time of 6.71 minutes.

Previously, I have demonstrated the methods to prepare artificial cells using cultured cells [19]. The current methods to prepare artificial cells involving the use of egg white can be performed easily because it does not require instruments for culturing cells. As such, this method is readily managed by anyone interested in studying the life sciences.

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