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Systematic Preparation of Artificial Cells (DNA Crown Cells)

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Abstract

Artificial cells, which are engineered particles that mimic one or many functions of biological cells, have attracted much attention in recent decades as a new technology. In a previous study, artificial cells were prepared using chicken eggs and sphingosine-DNA. The resulting DNA crown cells were surrounded by a membrane containing lipid and DNA, which was formed with egg white components. The study describes a novel method that allows the easy preparation of DNA crown cells using adenosine and monolaurin.

Keywords: Adenosine; Artificial cells; DNA crown cells; Sphingosine DNA

Introduction

There has been significant progress in the generation of artificial cells [1-4], however, till date, no artificial cells have been constructed that can autonomously replicate. In previously established method [5,6] in which artificial cells can be cultivated in egg whites by combining adenosine with sphingosine (Sph) and DNA. In studying the mechanism underlying the formation of artificial cells, It was demonstrated that Sph and DNA aggregate with egg white components, and artificial cells are formed from these Sph-DNA aggregates [6]. The surface of these artificial cells is covered with DNA, which were named DNA crown cells. Previous experiments [6] have also suggested that DNA crown cells can be formed from adenosine and lipids, as well as the proto-cells of artificial cells generated within egg white. To determine if this could indeed be done, preparation of DNA crown cells was performed using adenosine and monolaurin compounds, and was successful in this task.

As shown in this study and others, DNA crown cells can be prepared by several methods from Sphingosine (Sph) and DNA fibers, which are formed from a mixture of Sph and DNA (Figure 1) [7-9].

Methods and Results

Materials

Sph was purchased from Sigma (St. Louis, MO, USA), DNA was obtained from *Escherichia coli* strain B (Sigma), adenosine was purchased from Sigma and Wako (Osaka, Japan), and monolaurin was obtained from Tokyo Kasei University (Tokyo, Japan).

Procedure

Step 1: Preparation of the adenosine-monolaurin compound

- The adenosine-monolaurin compound was prepared by adding 0.4 mL (0.1 M) monolaurin to 0.4 mL (0.1 M) adenosine solution.
- After mixing, 0.15 mL ethanol was added to the solution, after which the precipitate was collected and dried.
- Then the adenosine-monolaurin precipitate was dissolved in 1.0 mL distilled water and used in subsequent experiments.

Step 2: Preparation of Sph-DNA and adenosine-monolaurin aggregates

- Sph (90 µL, 10 mM) was added to 40 µL DNA (1.7 µg/µL).
- After the mixture was heated, adenosine-monolaurin solution (50 µL) was added.
- Then, one drop of ethidium bromide solution was added to the Sph-DNA/adenosine-monolaurin mixture, which was placed on a glass slide
- Observation was done with the help of phase contrast and fluorescence microscopy.

In general, two types of aggregates, mucoid (Figures 2a and 2b) and crystal types (Figure 2c) were prepared. Fluorescence was observed in the inside of the crystal type aggregates (Figure 2d), demonstrating that Sph-DNA aggregates were successfully and easily prepared.

Step 3: Synthesis of DNA crown cells

- To synthesize DNA crown cells, Sph (90 µL, 10 mM) was added to 40 µL DNA (1.7 µg/µL).
- After the mixture was heated, adenosine-monolaurin solution (50 µL) was added to Sph-DNA mixture.
- Then, monolaurin solutions (50 µL, 0.1M) were added to the Sph-DNA/ adenosine-monolaurin mixture.
- After mixing, they were observed as described above in Step 2.
- The cells of various sizes were observed (Figures 3a, 3b and 3c).

Cellular Russell bodies were observed on the outermost surface of the cells, indicating that the surface comprised DNA crown cells (Figures 3d, 3e and 3f). DNA crown cells were formed from the aggregates of both mucoid and crystal types. Thus, these cells could be easily prepared using purified Sph, DNA, and the adenosine-monolaurin compound. Specifically, DNA crown cells were formed from Sph-DNA aggregated with adenosine-monolaurin, which led to the formation of branches of Sph-DNA fibers that can spontaneously seal. Large cells can shrink by several nanometers, resulting in the formation of cells of appropriate sizes.

Discussion

In this study, preparation of DNA crown cells was done by using

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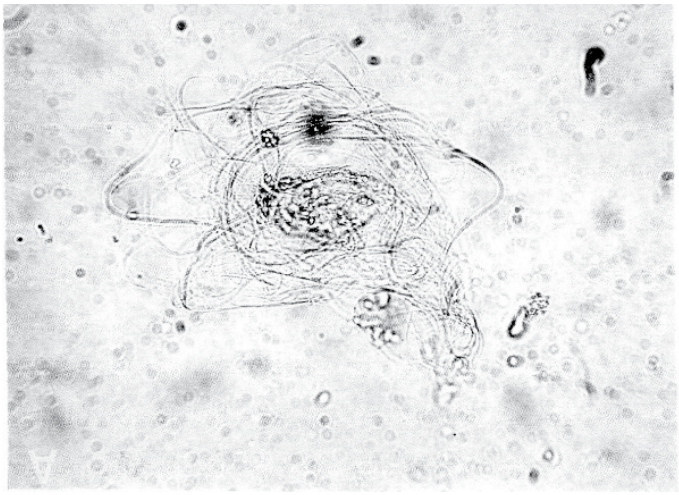

Figure 1: Fibrous assembly of sphingosine (Sph)-DNA.

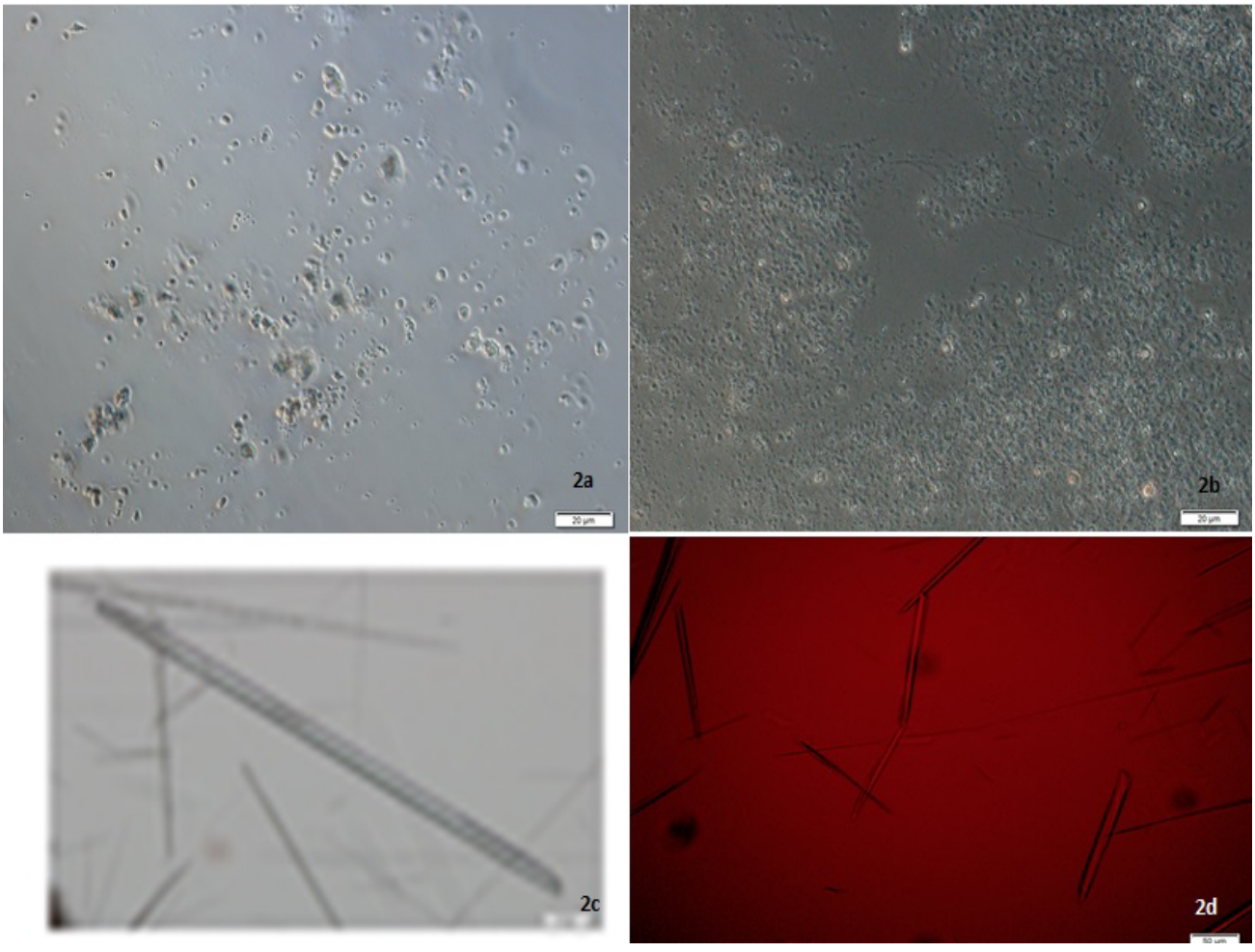

Figure 2: Aggregates of Sph-DNA with the compound formed by mixing adenosine and monolaurin. Sph was added to DNA, and then adenosine-monolaurin solution was added to the Sph-DNA mixture. Two types of aggregates formed: mucoid-type (Figures 2a and 2b) and crystal-type (Figure 2c). The sample was stained with ethidium bromide. Russell bodies were observed in the inside of the crystal aggregates by fluorescence microscopy (Figure 2d), suggesting that the inside contains DNA. Scale bar is 20 μm in Figure 2a and 2b and 50 μm in Figure 2c and 2d.

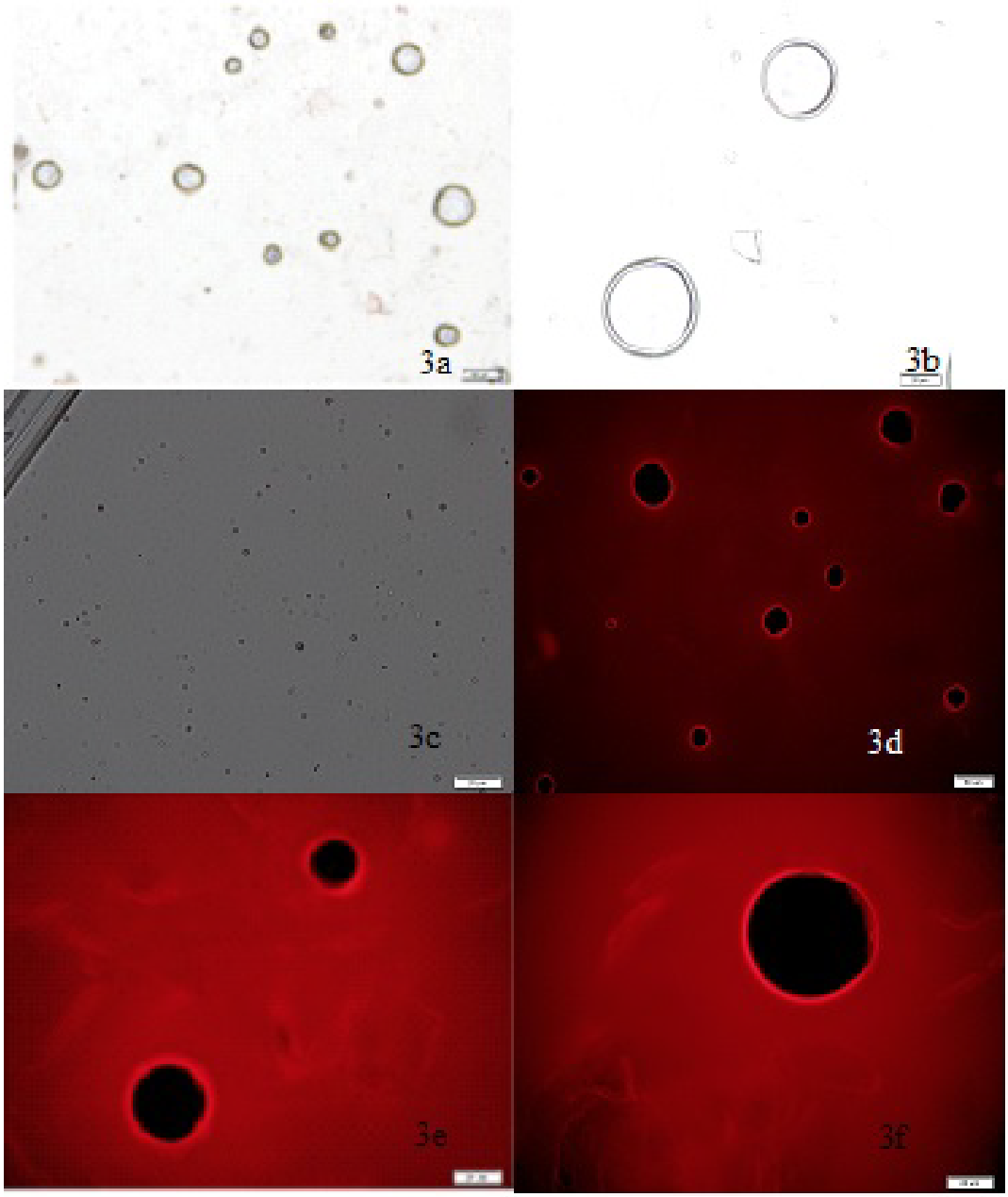

Figure 3: Preparation of DNA crown cells. Sph was added to DNA. After mixing, adenosine-monolaurin was added to the Sph-DNA mixture, and then monolaurin solution was added to the Sph-DNA/adenosine-monolaurin mixture. Cells of various sizes were observed by phase contrast microscopy, which led to the observation of DNA crown cells (Figures 3a and 3c). These cells were stained with ethidium bromide. Russell bodies were observed on the surfaces of the cells by fluorescence microscopy, indicating the presence of DNA (DNA crown cells) on the cell surface (Figures 3d-3f). Scale bar is 50 μm in Figures 3a and 3d, and is 20 μm. In Figure 3b, 3c, 3e and 3f & Figure 3a, 3b, 3d and 3e are the same field of view.

Sph, D NA, adenosine, and monolaurin. In a previous study, artificial cells [10] were generated using Sph, DNA, and nucleosides instead of adenosine, showing that compounds prepared by mixing nucleosides and monolaurin can also form Sph-DNA aggregates [11]. In addition, lipids contained within egg white bind to nucleosides to also form Sph-DNA aggregates. Moreover, DNA crown cells can be prepared using DNA from other sources. Thus, various types of DNA crown cells consisting of different components can be prepared with the method presented here.

To date, the obvious characteristics of DNA crown cells were their structure, a large loop of DNA similar in structure to plasmids, and their ability to replicate [12]. It is common knowledge that cells consist of a membrane made of lipid-polymer complexes comprising proteins and carbohydrates complexed with lipids. However, cells with membranes consisting of DNA and lipid have not been reported. Even if DNA crown cells prove that artificial cells can be generated from egg white components, it is very important to determine if DNA crown cells can contribute to scientific fields, and specifically what fields. As

such, studies using DNA crown cells may provide a new road towards uncharted fields in the life sciences.

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