Research Article ISSN 2689-1050

Chemical & Pharmaceutical Research

Microscopic Appearance of Cell Assembly and Proliferation in Agar Cultures of Synthetic DNA (*E. COLI*) Crown Cells with Monolaurin

Shoshi Inooka*

Japan Association of Science Specialists.

***Correspondence:**
Shoshi Inooka, Japan Association of Science Specialists, Japan.

Received: 09 Dec 2023; **Accepted:** 12 Jan 2024; **Published:** 18 Jan 2024

Citation: Shoshi Inooka. Microscopic Appearance of Cell Assembly and Proliferation in Agar Cultures of Synthetic DNA (*E. COLI*) Crown Cells with Monolaurin. Chem Pharm Res. 2024; 6(1): 1-4.

ABSTRACT

DNA crown cells (artificial cells), in which the outside of the membrane is covered with DNA, can be synthesized in vitro using sphingosine (Sph)-DNA-adenosine-monolaurin compounds. These cells can proliferate within egg whites in vivo. In a previous report on the culture and synthesis of synthetic DNA crown cells, it was shown that different lines of synthetic DNA crown cells (i.e., E. coli, Human placenta, Akoya pearl oyster, Hepato-blastoma-derived cell line; HepG2) could be prepared using egg white as a medium.

The present study examined whether the cell assembly and proliferation were observed when synthetic DNA (E. coli) crown cells were cultured on agar plates with monolaurin. In addition, the microscopic characteristics of the assemblies and the proliferated cells are described.

Keywords

Synthetic DNA (*E. coli*) crown cells, Agar plate cultures, Sphingosine-DNA, Cell proliferation.

Introduction

Artificial cells, referred to as DNA crown cells, are cells that are covered with DNA [1-3]. Synthetic DNA crown cells can be prepared and cultured using sphingosine (Sph)-DNA and adenosine-monolaurin (A-M) compounds, followed by incubation in egg white.

The cell assembly and proliferation were observed when synthetic DNA crown cells were cultivated with monolaurin in test tubes [4-6]. In addition, synthetic DNA (*E. coli*, human placenta, Acoya pearl oyster and HepG2) crown cells could be incubated in egg white and used to produce strains [7-10].

These phenomena were observed in liquid medium and it was not clear whether such phenomena could be observed after culture on solid agar media. Hence, in the present study, it was examined whether the cell assembly and proliferation could be observed in agar culture of synthetic DNA (*E. coli*) crown cells with monolaurin. The resulting findings were characterized microscopically.

Materials and Methods

Materials

The materials used were the same as those employed in a previous study [11,12]: Sph (Tokyo Kasei, Japan), DNA (Sigma-Aldrich, USA), adenosine (Sigma-Aldrich, USA. Wako, Japan), and monolaurin (Tokyo Kasei, Japan), adenosine-monolaurin (A-M) (a compound synthesized from a mixture of adenosine and monolaurin) [11,12]. Monolaurin solutions were prepared to a final concentration of 0.1 M in distilled water. Agar plates were prepared using standard agar medium (SMA; AS ONE, Japan)

Methods

Preparation of synthetic DNA (*E. coli*) crown cells

Synthetic DNA (*E. coli*) crown cells were prepared as described previously [11,12]. Briefly, 180 µL of Sph (10 mM) and 90 µL of

DNA (1.7 µg/µL) were combined, and the mixture was heated and cooled twice. A-M solution (100 µL) was added and the mixture was incubated at 37°C for 15 min. Next, 30 µL of monolaurin solution was added and the mixture was incubated at 37°C for another 5 min. The resulting suspension was used as the synthetic DNA (*E. coli*) crown cell solution.

Culture of monolaurin with DNA (*E. coli*) crown cells and incubation on agar plates

1. A total of 50 µL of sample was plated on an agar plate with a bacteria spreader.
2. Immediately, 1.5 mL of 0.1 M monolaurin which was diluted twice with distilled was poured on the agar plate.
3. After removing excess monolaurin on the upper surface of the plates, the plates were inverted and incubated for 1 h, 2 h and 2 days at 37°C.

Microscopic observations

Objects on the plates were directly observed using a light microscope.

Results and Discussion

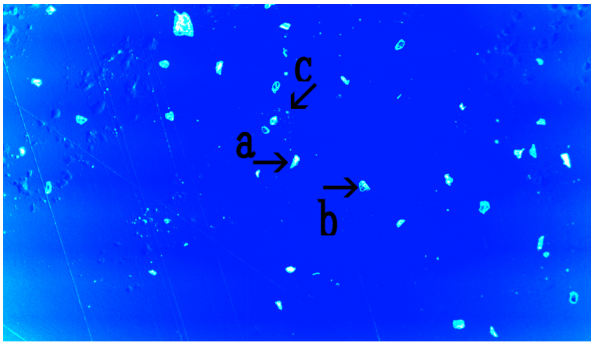

Figure 1: Shows objects on the plates before the addition of monolaurin. Numerous cell-like objects of various shapes and sizes were observed (arrows a, b, and c). The object indicated by arrow c measured approximately 5–7 µm.

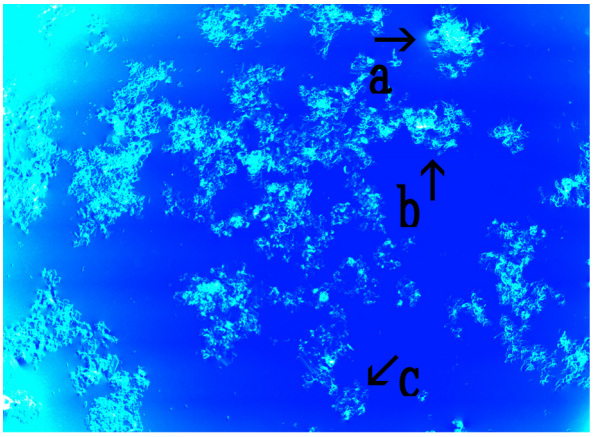

Figure 2: Shows the microscopic appearance of synthetic DNA (*E. coli*) crown cells immediately after monolaurin addition. Numerous clusters of cell-like objects were observed (arrows a, b, and c), with the diameter of each cluster (arrow c) measuring approximately 35–40 µm.

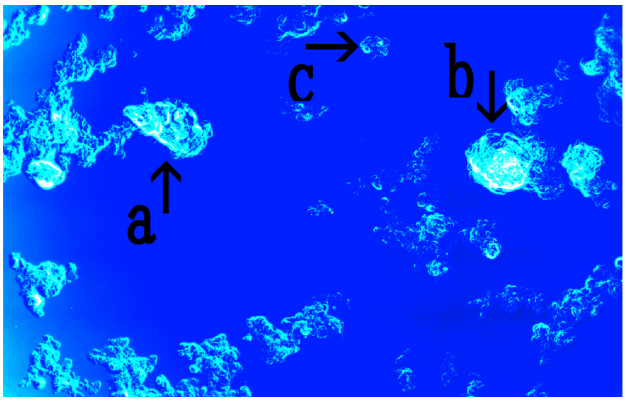

Figure 3: Shows the microscopic appearance of synthetic DNA (*E. coli*) crown cells after 1 h of monolaurin addition. Two objects like cell assemblies were observed (arrows a and b). The size of these objects (arrow c) was approximately 25–30µm.

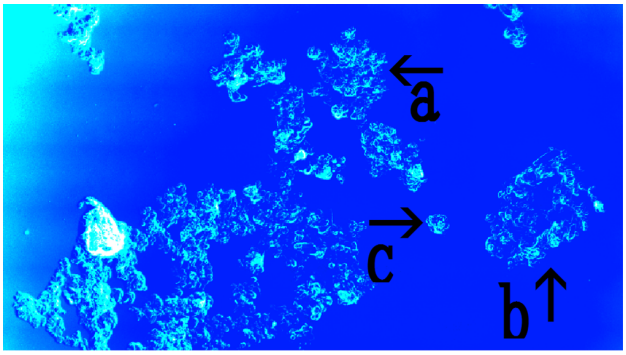

Figure 4: Shows the microscopic appearance of synthetic DNA (*E. coli*) crown cells at 2 h after monolaurin addition. The large two objects indicated by arrows a and b were considered to consist of several cell-like objects. The object indicated by arrow c measured approximately 15–20µm.

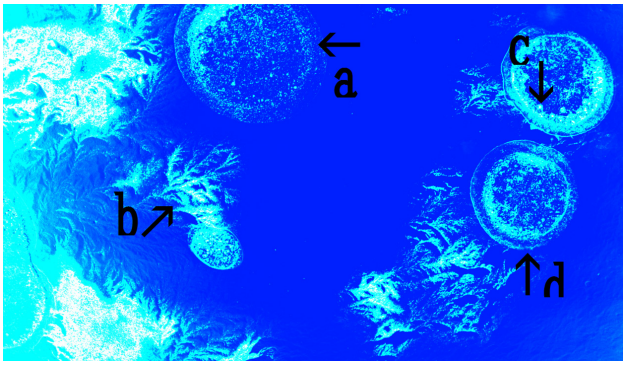

Figure 5: Shows the microscopic appearance of synthetic DNA (*E. coli*) crown cells at 2 days after monolaurin addition. Objects with numerous branch-like structures were observed (arrow b). Two large round objects containing numerous cell-like structures (arrow c) were observed (arrows a and d). The approximate size of cell-like structure was 8–10 µm (arrow c).

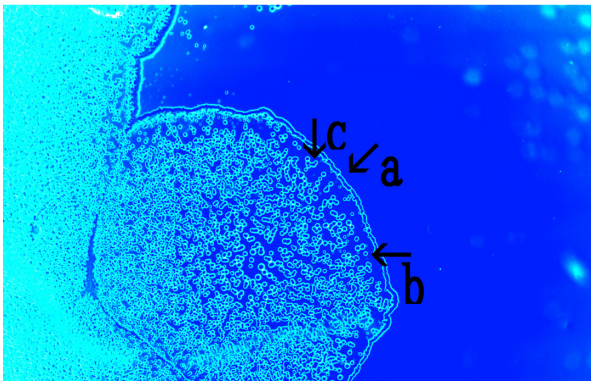

Figure 6: Shows the microscopic appearance of synthetic DNA (*E. coli*) crown cells cultured in the presence of monolaurin for 2 days. A large round object containing numerous cell-like objects was observed (arrow a). A single cell-like object (arrow b) and chain of cells (arrow c) was observed. The approximate size of a single cell-like object (arrow b) was 6–8 μm.

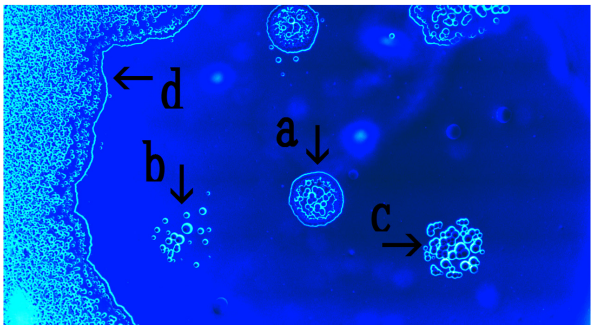

Figure 7: Shows the microscopic appearance of synthetic DNA (*E. coli*) crown cells cultured in the presence of monolaurin for 2 days. Several types of round objects were observed (arrows a, b, and c). One of these objects (arrow a) consisted of an outer-membrane with numerous cells, whereas the other objects (arrows b and c) did not possess a membrane and contained numerous, dispersed, cell-like objects (arrow b) and chains of cells (arrow c). A large space containing numerous cell-like objects was observed (arrow d). The approximate size of a dispersed, cell-like object (arrow b) was 15–20 μm.

In test tubes, synthetic DNA crown cells formed assemblies, crystal-like objects, and newly proliferated cells in the cultures with monolaurin [4-6]. However, it was not clear whether such structures would be formed on agar plates. The findings of this study showed that assemblies and cell proliferation both occurred on the agar plates. Specifically, assemblies were observed as soon as monolaurin solution was added to synthetic DNA (*E. coli*) crown cells. These assemblies may have been formed by loose binding between cells or cell components (Sph-DNA and A-M compounds) that were not used in cell synthesis. These assemblies may change to solid objects following the coalescence of several assemblies. The process, i.e., soft assembly formation, followed by the formation of solid objects, were similar to the process observed in test tubes [4-6].

Numerous different types of cell proliferation were observed after 2 days of monolaurin addition. Of these different types, the dominant type appeared to be cell proliferation within round objects (Figure 5), which likely indicated that cells developed within the round assemblies. However, it was not clear whether the cell-like objects within the round objects were DNA crown cells. Since the present experiments were conducted using only Sph-DNA, adenosine and monolaurin, the proliferated cells were composed of these compounds.

In addition, firework- or branch-like objects were observed [13]. These objects also consist of Sph-DNA, as demonstrated previously [3]. Thus, it was demonstrated that the relationships between synthetic DNA crown cells and monolaurin in test tubes were comparable to those on agar plates. Since synthetic DNA crown cells, including monolaurin-treated cells, could be cultivated in test tubes using egg white, future experiments will be conducted to clarify whether monolaurin-treated synthetic DNA (*E. coli*) crown cells can be cultivated in egg white on agar plates.

References

1. Inooka S. Preparation and cultivation of artificial cells. App. Cell Biol. 2012; 25: 13-18.
2. Inooka S. Preparation of Artificial Cells Using Eggs with Sphingosine-DNA. J. Chem. Eng. Process. Technol. 2016; 7: 1-5.
3. Inooka S. Aggregation of sphingosine-DNA and cell construction using components from egg white. Integr Mol Med. 2016; 3: 1-5.
4. Inooka S. Microscopic observation of assemblies formed in mixtures of DNA (Human placenta) crown cells with Bacilus subtilius. Arc Org Inorg Chem Scis. 2021; 5: 658-664.
5. Inooka S. Assembly Formation of Synthetic DNA (*E. coli*) crown cells with Salt "Reconstruction and Regeneration of Synthetic DNA Crown Cells". Am J Biomed Sci & Res. 2020; 16: 111-120.
6. Inooka S. Cell Proliferation from the Assembly of Synthetic DNA (*E. coli*) Crown Cells with Stimulation by Monolaurin Twice. Curr Tr Biotech & Microbio. 2022; 2: 493-498.
7. Inooka S. Preparation of a DNA (*E. coli*) Crown Cell line in Vitro-Microscopic Appearance of Cells. Ann Rev Resear. 2022; 8: 1-7.
8. Inooka S. Preparation and Microscopic Appearance of a DNA (Human Placenta) Crown Cell Line. Journal of Biotechnology & Bioresearch. 2023; 5: 655-658.
9. Inooka S. Preparation of a DNA (Akoya pearl oyster) crown cell line. Applied Cell Biology Japan. 36.2023. ISSN 2033-5800.
10. Inooka S. Preparation of a DNA (HepG2 is a hepatoblastoma-derived cell line) Crown Cell Line. J Tumor Med Prev. 2023; 4: 1-6.
11. Inooka S. Biotechnical and Systematic Preparation of Artificial Cells. The Global Journal of Researches in Engineering. 2017; 17: No1-C.

12. Inooka S. Systematic Preparation of Artificial Cells (DNA Crown Cells) J. Chem. Eng. Process Technol. 2017; 8: 327.

13. Inooka S. Formation and Microscopic Appearance of Fireworks-like Objects Created from Synthetic DNA (*E. coli*) Crown Cells with Adenosine-DNA (*E. coli*). Ann Rev Resear. 2023; 8: 555760.