AMERICAN JOURNAL OF MEDICAL AND CLINICAL SCIENCES, 2023
VOL 9, NO. 2, PAGE 1 – 3

RESEARCH ARTICLE

Open Access

Double Cell-Like Objects Created from Synthetic DNA (Human Placenta) Crown Cells Using Monolaurin

Shoshi Inooka*

Japan Association of Science Specialists, Japan.

ABSTRACT

Synthetic DNA crown cells (*artificial cells*) can be prepared *in vitro* using sphingosine (Sph)-DNA-adenosine-monolaurin compounds. These DNA crown cells can proliferate within egg whites *in vivo*. Synthetic DNA (*E. coli*) crown cells formed assemblies and cells proliferate after the addition of monolaurin and egg white and could be cultured on agar plates. In this study, it was examined whether such phenomena were observed in synthetic DNA (*Human placenta*) crown cells. In synthetic DNA (*Human placenta*) crown cells using monolaurin, several kinds of objects that may differ from original cells, were observed on the agar plates. Here, the microscopic characteristics of double cell-like objects are described.

ARTICLE HISTORY

Received 04 Mar 2024
Accepted 10 Apr 2024
Published 18 Apr 2024

KEYWORDS

Synthetic DNA (*Human placenta*) crown cells, Agar plate cultures, Sphingosine-DNA, Double cells proliferation, Monolaurin, Egg white.

Introduction

Artificial cells that are covered with DNA are referred to as DNA crown cells [1-3]. Synthetic DNA crown cells can be changed to DNA crown cells within egg white and can be prepared using sphingosine (Sph)-DNA and adenosine-monolaurin (A-M) compounds. Such DNA crown cells form assemblies or cells that proliferated in cultures in the presence of monolaurin and could be cultivated in test tubes [4-6]. In previous experiments using synthetic DNA (*E. coli*) crown cells with monolaurin or egg white, the formation of assemblies or cell proliferation in populations and during cultivation could be also observed when cultured on agar plates [7-9]. The present study examined whether such phenomena could also be observed when synthetic DNA (*Human placenta*) crown cells were cultured after the addition of monolaurin on agar plates.

In addition to cell proliferation, various types of objects were observed when synthetic DNA (*Human placenta*) crown cells were cultivated with monolaurin on agar plates. Here, one type of these objects; double cells like objects, are described and characterized based on microscopic observations.

Materials and Methods

Materials

The materials used were the same as those employed in a previous study [10,11]: Sph (Tokyo Kasei, Japan), DNA (Sigma-Aldrich), adenosine (Sigma-Aldrich; Wako, Japan), and monolaurin (Tokyo Kasei), adenosine-monolaurin (A-M) (a compound synthesized from a mixture of adenosine and monolaurin) [10,11]. Monolaurin solutions were prepared to a final concentration of 0.1 M in distilled water. Agar plates were prepared using standard agar medium (SAM) (AS ONE Japan).

Methods

Preparation of synthetic DNA (Human placenta) crown cells

Synthetic DNA (Human placenta) crown cells were prepared as described previously [10,11]. Briefly, 180 µL of Sph (10 mM) and 90 µL of DNA (1.6µg/µL) were combined, and the mixture was heated and cooled twice. Then, 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 (Human placenta) crown cells.

Culture of monolaurin with DNA (Human placenta) crown cells.

1. A total of 50.0 µL of sample was plated on the agar plate with a bacteria spreader.
2. Immediately, 1.5 mL of twice-diluted 0.1 M monolaurin was poured onto the agar plate.
3. After the excess monolaurin was removed, the plates were inverted and incubated for 7 days at 37°C.
4. Then, the plates were kept at 4°C and observed.

Microscopic Observations

Objects on the plate were directly observed by eye and under a light microscope.

Contact: Shoshi Inooka
Japan Association of Science Specialists, Japan.

Results and Discussion

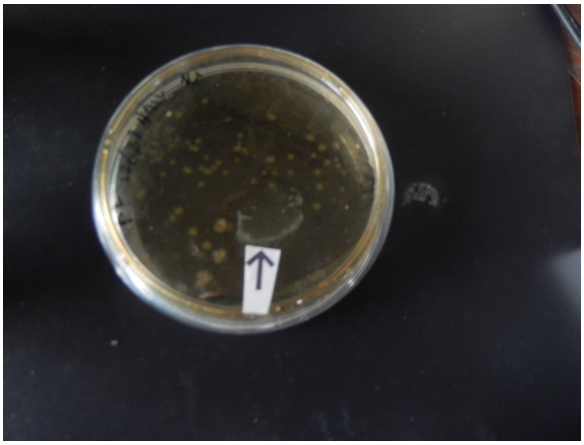

Figure 1: Photograph of the plates at 7 days after monolaurin addition. A white layer-like feature (arrow) was observed. The diameter of the Petri dish was 8.0 cm.

Features that could observed by eye appeared at 4 days after incubation. At 7 days of incubation, a white layer-like feature (arrow) was observed. The diameter of the Petri dish was 8.0 cm.

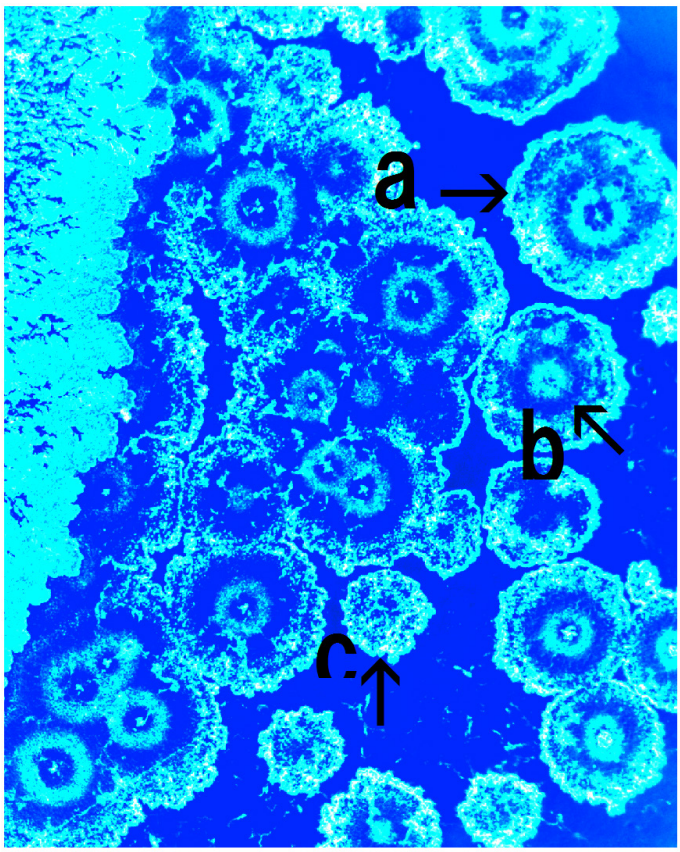

Figure 2: Microscopic appearance of objects shown in Figure 1 (arrow) at 7 days after monolaurin addition. Numerous round cells of various sizes were observed (arrows a, b, and c). The size of the cell (arrow c) was approximately 100–120 μm.

Numerous round cell-like objects of various sizes were observed (arrows a, b and c). The size (arrow c) is approximately 100–120 μm.

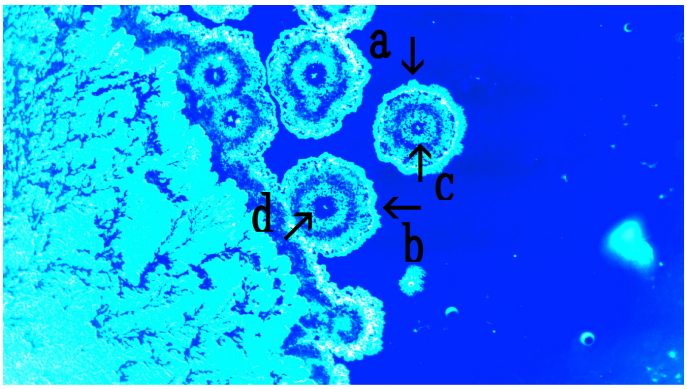

Figure 3: Microscopic appearance of objects shown in Figure 1 (arrow). Round objects (arrows a and b) enclosed other round objects (arrows c and d). The round object (arrow a) measured approximately 170–190μm.

Round objects (arrows a and b), which are enclosed other round objects (arrows c and d) were observed. The object (arrow a) had a size of 170–190 μm.

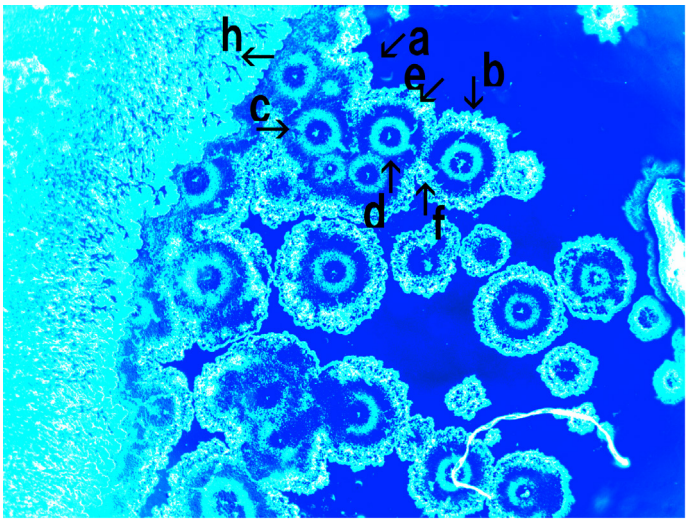

Figure 4: Microscopic appearance of objects shown in Figure 1 (arrow) at 7 days after monolaurin addition. An object (arrow a) enclosed five round objects (arrows c and d). An object (arrow d) appeared to be enclosed by a membrane (arrows e and f). An object enclosing a cell-like object was also observed (arrow b). Large spaces were observed (arrow h). The approximate size (arrow b) was 150–170 μm.

An object (arrow a) that enclosed five round objects (arrows c and d) was observed. An object (arrow d), possibly enclosed by a membrane (arrows e and f), was observed. An object enclosing a cell-like object was also observed (arrow b). Large spaces were observed (arrow h). The approximate size (arrow b) was 150–170 μm. To date, many kinds of synthetic DNA crown cells have been reported to form assemblies and cells that proliferate in the presence of monolaurin in test tubes [4-6]. In previous studies using synthetic DNA (*E. coli*) crown cells, the assemblies and/or proliferating cells could be observed on agar plates [7-9]. Interestingly, various types of objects in these assemblies or proliferating cells were observed when synthetic DNA (*E. coli*) crown cells were added to monolaurin or egg white. On the other hand, it was not clear whether other synthetic DNA crown cells, i.e., cells other than synthetic DNA (*E. coli*) crown cells,

formed such objects. It was therefore examined whether such objects were formed when monolaurin was added to synthetic DNA (Human placenta) crown cells. The results showed that various objects were formed and these are described here (Figures. 2, 3 and 4). The predominant objects included large round objects (Figure. 3, arrows a and b) that enclosed smaller round objects.

If the objects were living cells, then it could be said that cells containing smaller cells were formed; however, the precise mechanisms of object formation are unclear (Figures 2, 3 and 4). Interestingly, space h (Figure 4, arrow h) may be an assembly that consists of Sph-DNA adenosine-monolaurin, because the original constituents of objects were previously shown to comprise Sph-DNA-adenosine-monolaurin and not egg white and small round objects (Figures 4 arrows c and d) that may form in the observed spaces (Figure 4, arrow h). Moreover, membrane-like objects (Figure 4, arrows e and b) may also form in these spaces (arrow h). Then, these membrane-like objects (arrows b, e and f) enclosed round objects (arrows c and d) to form new objects (Figure 3, arrows a and b). In this case, if small objects (Figure 3, arrows c and d) were DNA crown cells, then cells that enclosed DNA crown cells were formed.

It was not clear whether these objects were living or not; however, since these objects (Figure 1 arrow) could be observed by eye after 4 days of culture, it was clear that they grow and it is suggested that they were living. As described previously [9], numerous objects may be formed when the various kinds of synthetic DNA crown cells, DNA crown cells, and cultured cells [12-15] were cultured with monolaurin or egg white on agar plates. Therefore, it is considered important to identify these objects. In previous experiments on synthetic DNA (*E. coli*) crown cells [9], the objects were named using the proposed convention Bacteria (*E. coli*) SDCCME (Thalli). Here, these cells are named Human (placenta) SDCCM (DC), where human placenta is the source of the DNA, SDCC is synthetic DNA crown cells, M is monolaurin, and DC is double cells. Although it is very important to clarify whether these objects are living or not, before starting such studies, the author first intends to clarify whether similar objects can also be observed in other DNA crown cells, or whether these structures are unique to only synthetic DNA (*E. coli*, human placenta) crown cells.

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).
3. Inooka S. Aggregation of sphingosine-DNA and cell construction using components from egg white. Integrative Molecular Medicine. 2016; 3(6): 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(2): 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(1): 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; 5(2): 493-498.
7. Inooka S. 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.
8. Inooka S. Microscopic Apperaarance of Monolaurin-Treated Syntheticc DNA (*E. coli*) Crown Cells in Agar Medium Supplemented with Egg White. Scientic Journal Biology & Life Science. 2024; 3(2): 1-5.
9. Inooka S. Synthesis and Microscopic Appearance of Thalus-like Objects tin Synthetic DNA (*E. coli*) Crown Cells Cretaed Using Monolaurin and Egg white. Open Acc J Repro & Sexual Disord. 2023; 3(2): 307-311.
10. Inooka S. Biotechnical and Systematic Preparation of Artificial Cells. The Global Journal of Research in Engineering. 2017; 17(1): 1-10.
11. Inooka S. Systenmatic Preparation of Artificail Cells (DNA Crown Cells). J Chem Eng Process Technol. 2017; 8(2): 1-4.
12. Inooka S. Preparation of a DNA (*E. coli*) Crown Cell line in Vitro-Microscopic Appearance of Cells. Annals of Reviews and Research. 2022.8(1).
13. Inooka S. Preparation and Microscopic Appearance of a DNA (Human Placenta) Crown Cell Line. Journal of Biotechnology & Bioresearch. 2023; 5(1): 655-658.
14. Inooka S. Preparation of a DNA (Akoya pearl oyster) crown cell line. Applied Cell Biology Japan. 2023.
15. Inooka S. Preparation of a DNA (HepG2 is a hepatoblastorma-derived cell line) Crown Cell Line. Journal of Tumor Medicine & Prevention. 2023; 4(2): 1-6.