LUPINE PUBLISHERS

Current Trends on Biotechnology & Microbiology

DOI: 10.32474/CTBM.2023.03.000161

ISSN: 2641-6875

Research Article

Cell Proliferation in Assemblies of Synthetic DNA (E. Coli) Crown Cells in the Presence of $Mgcl_2$

Shoshi Inooka*

Japan Association of Science Specialists, Japan

***Corresponding author:** Shoshi Inooka, Japan Association of Science Specialists, Japan

Received: December 24, 2022 **Published:** January 9, 2023

Abstract

DNA crown cells are cells that have outer membranes that are covered with DNA. They are synthesized using sphingosine (Sph)-DNA-adenosine mixtures in vitro and can proliferate within egg whites (DNA crown cells). Previous studies have shown that assemblies of synthetic DNA (E. coli) crown cells were formed spontaneously, and that the assemblies changed to crystal-like substances with the addition of monolaurin. Moreover, cells proliferated from crystal-like substances after being treated with monolaurin twice. On the other hand, the assembly of synthetic DNA crown cells has been shown to form in the presence of various agents, such as salts, inorganic materials, and some bacteria and change into crystal-like substances after treatment with monolaurin. In the present study, whether cell proliferation, which was observed to occur spontaneously in assemblies, also occurred in assemblies formed using inorganic materials was examined. The present experiments showed that cell proliferation occurred in assemblies to which MgCl2 was added. Cell proliferation in the presence of MgCl2 was performed by

a) incubating synthetic DNA (E. coli) crown cells in MgCl2 followed by

b) incubation for approximately 2 days with monolaurin.

Keywords: synthetic DNA (E. coli) crown cells; assembly; sphingosine-DNA; cell proliferation

Introduction

Artificial cells, or DNA crown cells, are covered with DNA [1-3]. Such cells are generated by incubating synthetic DNA crown cells in egg white. Synthetic DNA crown cells can be prepared with sphingosine (Sph)-DNA and A-M compounds [4,5]. It has previously been shown that the assemblies of synthetic DNA (E. coli) crown cells can form spontaneously, and also after the addition of commercial salts [6], NaCl, KCl, MgCl2, and FeCl3 solutions, [7], Bacillus subtilis [8,9], and yeast [10]. Such assemblies transform into crystal-like substances after treatment with monolaurin. In spontaneously formed assemblies, cells proliferated after being treated twice with monolaurin [11].The present study clarified whether cell proliferation was observed in assemblies that were formed with inorganic materials.

Materials and Methods

Materials

The following materials were used: Sph (Sigma, USA and Tokyo Kasei, Japan), DNA (E. coli, B1 strain; Sigma-Aldrich, USA), adenosine (Sigma, USA and Wako, Japan), monolaurin (Tokyo Kasei, Japan), and MgCl2 (Hayashi Junyaku Co. Ltd., Japan). In addition, a compound synthesized from a mixture of adenosine and monolaurin (A-M) [5] was used. Monolaurin solutions were

prepared to a concentration of 0.1M in distilled water. MgCl2 solutions were prepared to a concentration of 20% in distilled water.

Methods

Preparation of DNA crown cells

The generation of artificial cells using Sph-DNA-A-M was performed as described previously [4,5]. Briefly, l80 µg of Sph (10 mM) and 90 µL of DNA (1.7µg/µL) were combined, and the mixture was heated and cooled, twice. A-M compound (100 µL) was added, and the mixture then was incubated at 37 ºC for 15 min. Next, 30 µL of monolaurin was added, and the mixture was incubated at 37 ºC for another 5 min. The resulting suspension was used as synthetic DNA (E. coli) crown cells.

Test of cell proliferation

a) A total of 25 µL of synthetic DNA (E. coli) crown cells was incubated with 25 µL of MgCl2 solution for 18 h at 37°C.

b) Then, 25 µL of monolaurin was added to the synthetic DNA (E. coli) crown cells.

c) The mixture was incubated for approximately 1 and 2 days at 37 ºC,

d) Then, 25 µL of monolaurin was added to the mixtures and incubated for 20–40 min at 37°C.

e) Then, samples (procedures 3 and 4) were observed under a light microscope.

Microscopic observations

A drop of each sample was placed on a slide glass and covered with a cover glass. The resulting slide was then observed under a light microscope.

Results and Discussion

Microscopic appearance of synthetic DNA crown cells treated for approximately 1 day with monolaurin

Synthetic DNA crown cells were incubated with MgCl2 for 18 h, then with monolaurin for approximately 1 day (Figure 1). Numerous crystal-like substances were observed (Figure 1, arrows a and b). Cell-like objects measuring approximately 15–20 µ m in size were also observed (Figure 1, arrow c). No cell proliferation was observed. As reported previously [7], assemblies of synthetic DNA (E. coli) crown cells were formed in the presence of MgCl2 and crystal-like substances were formed after incubation with monolaurin. In the present study, as shown in Figure 1, it was confirmed that the assembly of synthetic DNA (E. coli) crown cells occurred after incubation with MgCl2 and that crystal-like substances were formed after incubation with monolaurin.

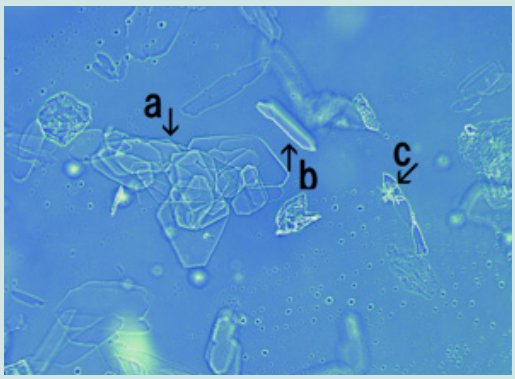

Figure 1: Microscopic appearance of proliferated synthetic DNA (E. coli) crown cells that were incubated with $MgCl_2$ for 18 h and treated with monolaurin for approximately 1 day. Many crystal like substances were observed (arrow a). Clear crystal-like substances were observed (arrow b). Cell-like objects (arrow c) were approximate 15–20 µm in size.

Cell proliferation

Assemblies of synthetic DNA (E. coli) crown cells together with MgCl2 were incubated with monolaurin for approximately 2 days and cell proliferation was observed (Figures 2, 3 & Figure 4). Many cell-like objects formed a population of cells (Figure 2, arrow c). Tube-like objects were observed (Figure 2, arrows a and b). Various objects of different size and shape were observed along the tube (Figure 2, arrow d). One of these objects was approximately 15–20 µm in size (Figure 2, arrow e). Most objects in Figure 2 may have had the same origin, which was likely the tube-like object shown in (Figure 2, arrow a) Tube-like objects were observed (Figure 3, arrows a and b). In addition, a population of cell-like objects of various sizes was observed at the top of the tube (Figure 3, arrow c). Such cell populations (Figure 3, arrow c) may be derived from the tube (Figure 3, arrow a). A free cell (approximately 15–20 µm in size) was observed (Figure 3, arrow d). Thin tube-like objects were observed (Figure 4, arrows a and b). Cell-like objects of various sizes and shapes were observed (Figure 4, arrows c, d and e). Cells were approximately 5–10 µm in size (Figure 4, arrow e).

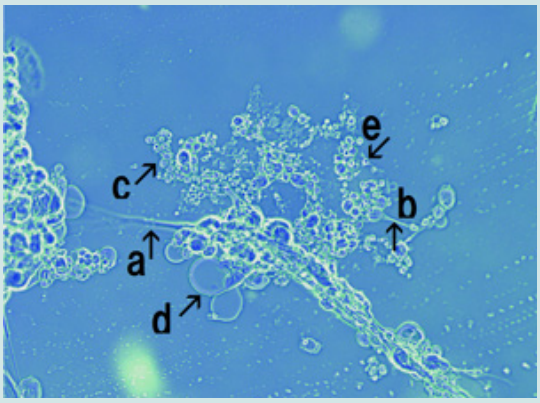

Figure 2: Microscopic appearance of proliferated synthetic DNA (E. coli) crown cells that were incubated with $MgCl_2$ for 18 h and treated with monolaurin for approximately 2 days. Numerous cell-like objects which formed a population of cells were observed (arrow c). Tube-like objects were observed (arrows a and b). Various objects of different size and shape were observed along the tube (arrow d). One of these objects measured approximately 15–20 µm (arrow e).

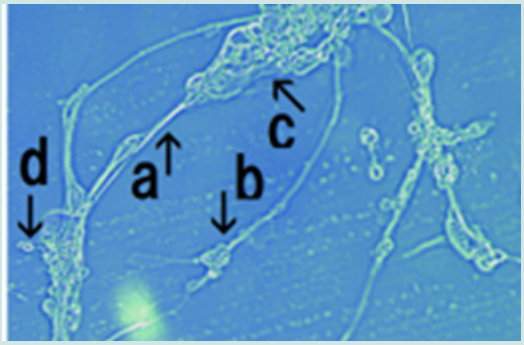

Figure 3: Tube-like objects were observed (arrow a). A population of cell-like objects of different size were observed along the tube (arrows b and c). This cell population (arrow c) may be derived from the tube (arrow a). A free cell (approximately 15–20 µm in size) was observed (arrow d).

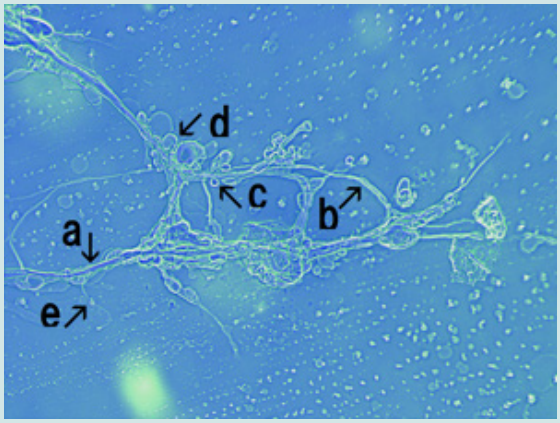

Figure 4: Thin tube-like objects were observed (arrows a and b). Cell-like objects of different size were observed along the tube (arrows c, d and e). The cell-like object was approximately 5–10 µm in size (arrow e).

Transformation of proliferated cells into a crystal-like substance

Synthetic DNA (E. coli) crown cells were incubated with MgCl2 for 18 h and were treated with monolaurin for approximately 2 days. They were then incubated with monolaurin for a further 15–40 min. Crystal-like assemblies were observed (Figure 5, arrow a) and cell-like objects were observed (Figure 5, arrow b). The cell-like objects (approximately 10–15 µm in size) were

observed within the assembly (Figure 5, arrow c). Parts of cell-like objects were observed (Figure 5, arrow b). Most proliferated cells transformed into crystal-like substances. No characteristic cell proliferation was observed. In the present study, it was examined whether cell proliferation which was previously observed to arise spontaneously in assemblies [11] was observed in the assembly incubated with inorganic materials, such as NaCl, KCl, $MgCl_2$ and FeCl3 [7]. Cell proliferation was observed with $MgCl_2$, but not with NaCl and KCl in the present study. Further, cell proliferation may have occurred after addition of $FeCl_3$, but the findings were unclear. However, cell proliferation may occur in the presence of NaCl or KCl under other experimental conditions. On the other hand, cell proliferation occurred spontaneously in the twice-treated monolaurin experiment after incubation with monolaurin for approximately 2 days. In the $MgCl_2$ experiment, cell proliferation occurred after incubation with monolaurin for approximately 2 days, without being stimulated twice. The findings showed that there were differences in the response to monolaurin between the assemblies that formed spontaneously and the assemblies after MgCl2 addition. Moreover, many tubes of various sizes and shapes were observed when cells proliferated after incubation with $MgCl_2$, and many cells were observed at the tops of tubes. The findings indicated that cells proliferated from the tubes. However, it was unclear whether the phenomenon was characteristic to $MgCl_2$.

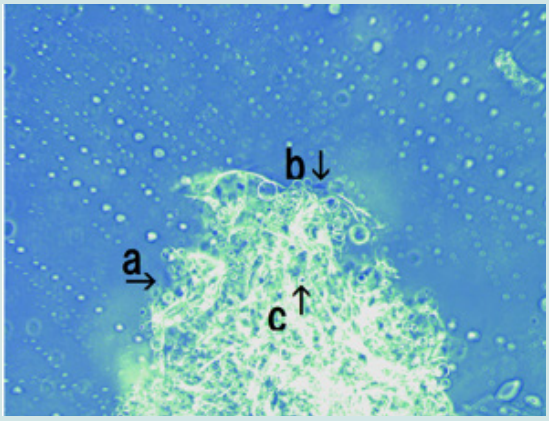

Figure 5: Microscopic appearance of proliferated synthetic DNA (E. coli) crown cells that were incubated with $MgCl_2$ for 18h then with monolaurin for approximately for 2 days and treated with monolaurin for approximately 15–40 min. Crystal-like assemblies were observed (arrow a). Cell-like objects was observed (arrow b). A free cell (approximately 10–15 μm) was observed within the assembly (arrow c).

Several tubes were also observed in the cells that proliferated spontaneously and cells were observed in the tops of the tubes. Both results suggested that cells proliferated from the tubes. However, it was clear that there was a difference in the appearances of the cells that arose spontaneously and those that arose after incubation with MgCl2. One reason for these findings was that these tubes were derived from the assemblies comprising cells that proliferated spontaneously [11] however, the origin of tubes was not clear in the experiments where cells proliferated in the presence of MgCl2. In addition, the cells that proliferated in the presence of MgCl2 changed to crystal-like substances after further treatment with monolaurin, whereas cell proliferation was spontaneous when cells were treated twice with monolaurin [11]. Though the mechanism of how monolaurin causes cells to proliferate is unclear, monolaurin may have two functions: proliferation and crystallization. Since crystal-like substances were Sph-DNA associated substances [3], monolaurin may function in Sph-DNA associated substances. Thus, in the present experiments using synthetic DNA (E. coli) crown cells, it was suggested that there were differences in cell formation between cells that proliferated spontaneously and cells that proliferated in the presence of $MgCl_2$. On the other hand, there are many ways in which cell proliferation can be achieved. The present experiments were carried out by incubating synthetic DNA (E. coli) crown cells and $MgCl_2$. Many kinds of synthetic DNA crown cells can be prepared [12,13], and thus, there are many ways in which cell proliferation can be achieved. However, it has been reported in a series of synthetic DNA crown cells studies that it is important to clarify whether synthetic DNA crown cells, including proliferated cells, are living or not [14-16]. Continued experiments on cell proliferation and synthetic DNA crown cell cultures will be required to clarify whether synthetic DNA crown cells in solution are living or non-living, say, synthetic DNA crown cells change to life in vitro (DNA crown cells).

References

1. Inooka S (2012) Preparation and cultivation of artificial cells. App Cell Biol 25: 13-18.
2. Inooka S (2016) Preparation of Artificial Cells Using Eggs with Sphingosine-DNA. J Chem Eng Process Technol 7(1): 277.
3. Inooka S (2016) Aggregation of sphingosine-DNA and cell construction using components from egg white. Integrative Molecular Medicine 3(6): 1-5.
4. Inooka S (2017) Biotechnical and Systematic Preparation of Artificial Cells. The Global Journal of Researches in Engineering: 17.

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

6. Inooka S (2022) Assemblies Formation of Synthetic DNA (E.coli) Crown Cells with Salt. "Reconstruction and Regeneration of Synthetic DNA Crown Cells" American Journal of Biomedical Science & Research 16(1).

7. Inooka S (2022) The assembly in synthetic DNA crown cells with inorganic salts and the transformation of DNA crown cells to crystal like substance in the presence of monolaurin. Novel Research in Science: 11(2).

8. Inooka S (2021) Assembly and Bacterial Growth Suppression in the Co-cultures of Synthetic DNA (Akoya Pearl Oyster) Crown Cells with Bacillus subtilis. Applied Cell Biology Japan 34: 67-84.

9. Inooka S (2022) Assemblies in Synthetic DNA (Nannochloropsis Species) Crown Cells with Bacillus subtilis. Acta Scientific Microbiology 5(1).

10. Inooka S (2021) Synthetic DNA Crown Cells Kill Yeast: Relationship with Assembly Formation of Yeast. Civil Engineering Research Journal 12(2): 555831.

11. Inooka S (2022) Cell Proliferation from the Assembly of Synthetic DNA (E. Coli) Crown Cells with Stimulation by Monolaurin Twice. Current Trends on Biotechnology Microbiology 2(5).

12. Inooka S (2018) Systematic Preparation of DNA (Akoya pearl oyster) Crown Cells. App. Cell Biol. Japan 31: 21-34.

13. Inooka S (2017) Systematic Preparation of Bovine Meat DNA Crown Cells App. Cell Biol. Japan 30. 13-16.

14. Inooka S (2022) Microscopic appearance of synthetic DNA (E.coli) crown cells in primary culture. App. Cell Biol Japan 35: 71-98.

15. Inooka S (2022) Microscopic Appearance of Synthetic DNA (E.coli) Crown Cells in Secondary Cultures. Novel Research in Science 12(3).

16. Inooka S (2022) Preparation of a DNA (E. coli) Crown Cell line in Vitro-Microscopic Appearance of Cells-Annals of Reviews and Research: 8(1).

DOI: 10.32474/CTBM.2023.03.000161