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Gas-Associated Emergence of Living Cells from Non-Living DNA Crown Cell Seeds

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ABSTRACT

Self-replicating artificial cells, referred to as DNA crown cells, have previously been shown to generate antibiotic-producing cells when cultured with egg white powder and various partners. However, the mechanisms underlying the emergence of these antibiotic-producing cells remains unclear. The seeds of DNA crown cell are non-living, and are comprised of sphingosine, DNA, and adenosine, which then develop into DNA crown cells within egg white. In a previous study, it was demonstrated that gas production, designated as Bovine blueberry ex, occurred during the cultivation of egg white-powder-enclosed DNA (bovine meat) crown cells in conjunction with blueberry extract as a partner on agar. In this study, it was investigated whether exposure of non-living DNA crown cell seeds to gases generated under such culture conditions could induce the emergence of living cells. Gas was supplied to DNA crown cell seeds, prepared using E. coli, successfully produced living cells. The resulting cells were named Gas (Bovine blueberry ex) crown (E. coli) cells, reflecting both the origin of the inducing gas and the DNA used in the preparation of crown cell seeds. The present findings demonstrate that gas exposure can promote the conversion of non-living crown cell seeds to living cells, and also to establish a reproducible method for generating new living cells from non-living DNA-based structures.

Keywords

DNA (bovine) crown cells, Sphingosine-DNA, Crown cell seeds, Gas crown cells, Gas powder (Crown bovine meat-blueberry-ex-P), Monolaurin, Gas production.

Introduction

The original of living cells from non-living materials is one of the most fundamental and unresolved questions in biology. Numerous models have been proposed to explain how primitive molecular assemblies could acquire the structural and functional properties of living systems. Within this context, DNA-based artificial structures have attracted attention as potential models for studying the minimal requirements for cellular emergence.

In the field of DNA-based artificial structures, artificial cells capable of self-replicating within egg white were first reported in 2012 [1]. These cells were initially constructed using sphingosine, DNA and Ascidian (Sea squirt) extract. In 2016, it was demonstrated that the active components of the Ascidian extracts are the nucleosides uridine and thymidine [2]. Even if adenosine was not contained in Ascidian extracts, adenosine was found to be particularly effective in cell proliferation.

Moreover, additional characteristics of artificial cells were described, including artificial cell seeds (hereafter referred to as Crown cell seeds) (sphingosine-DNA-adenosine), and their general morphology [3]. Moreover, when adenosine was added to cultured egg white, adenosine-lipid conjugates that promote the production of artificial cells was identified, suggesting that the importance of nucleoside-lipids in the production of artificial cells [4].

In that study, the lipid moiety of this conjugate was found to correspond to monolaurin, and so adenosine-monolaurin (A-M) compounds were prepared. Based on these findings, artificial cells

could be produced [4] and the outer membrane of artificial cells was comprised of DNA, resulting in the designation of these cells as "DNA Crown Cells" in 2016 [4].

DNA crown cells, which have been shown to develop into fully self-replicating cells after incubation in egg white produced using DNA crown cell seeds that can be prepared with sphingosine, DNA, and adenosine, or synthetic DNA crown cells, which can be prepared with sphingosine, DNA, adenosine, and monolaurin, all of which are commercially available compounds. A comprehensive procedure for generating DNA crown cells with synthetic DNA crown cells was reported in 2017 [5] and numerous DNA crown cells and associated cell strains have been produced to date [6-14]. These cells have been prepared and stored stably at 4°C for nearly a decade.

Also, when cultured in agar with egg white, monolaurin, salt, or microorganisms, synthesized DNA crown cells formed assemblies of diverse morphological objects [15-20].

Antibiotic-producing cells were also isolated from beer produced using co-cultures of DNA crown cells and yeast [21]. Based on these findings, egg white powder enclosing different DNA crown cells in conjunction with different substances has been prepared, and numerous antibiotic-producing cells have been produced using agar cultures of these powders [22-27]. Such a culture system (i.e., using egg white powder) can be regarded as being equivalent to an egg-white-based culture system, which obviates the need to use fresh eggs for culture.

On the other hand, despise these extensive observations, the mechanism by which these cells self-replicate remains unknown.

In previous experiments, to better understand the emergence of self-replicating cells, it was examined whether gas production occurs when egg white powder enclosing DNA (bovine) crown cells combined with a blueberry extract is cultured. The findings showed that four kinds of gas were produced. This study examined whether the gas produced gas was associated with cell generation.

Environmental gases are known to influence the assembly and composition of biochemical processes. However, their potential role in inducing life-like behavior in new viable DNA structures has not been systematically examined. This study focused on the gasses produced during the culture of Bovine DNA crown cells supplemented with blueberry extract, a preparation referred to as gas (Bovine blueberry ex). Specifically, the role of these gasses as catalytic factors that induce the emergence of living cells from non-living cell seeds was examined. The findings showed that exposure to these gasses results in the formation of living cells, which have been designated here as Gas (Bovine blueberry ex) crown cells.

This gas-mediated transformation provides a new experimental model for studying the physicochemical process that potentially underlines the origin of living cells and establish a practical method for generating living cells from non-living DNA-based natter.

Materials and Methods

Materials

DNA (bovine meat) crown cells that had been prepared previously and stored in a refrigerator at approximately 4°C were used in this study [6,22]. However, the methods are described here again for clarity.

The materials used in the present study were the same as those employed in previous studies [5]: Sph (Tokyo Kasei, Japan), DNA (from beef), *E. coli* DNA (Sigma-Aldrich, USA), adenosine (Sigma-Aldrich; Wako, Japan), monolaurin (Tokyo Kasei), and adenosine-monolaurin (A-M), a compound synthesized from a mixture of adenosine and monolaurin [5]. Monolaurin solutions were prepared to a final concentration of 0.1 M in distilled water. Agar plates; agar medium (SMA) (AS ONE, Japan). Tissue culture plates (35 mm) (IWAKI, Japan), and blueberries from a local market.

Methods

Preparation of DNA (bovine meat) crown cells (6, 22).

Step 1. A total of 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.
Step 2. A-M solution (100 µL) was added and the mixture was incubated at 37°C for 15 min.
Step 3. A total of 30 µL of monolaurin solution was added, and the mixture was incubated at 37°C for another 5 min.
Step 4. Approximately 0.3 mL of the suspension was then injected into egg white and incubated for 7 days at 37°C. Egg white was subsequently recovered and used as DNA (Bovine meat) crown cells.

Preparation of blueberry extracts

Blueberry (about 50 fruit) were ground using a mortar and pestle and suspended in 3 mL of distilled water.
Preparation of gas powder

1. First, 3 mL of blueberry extract was mixed with 3 mL of egg white.
2. The mixture was incubated for 5 h at 37°C.
3. Then, approximately 25 mL of fresh egg white was added to the mixture.
4. The fluid was poured into two Petri dishes and dried for 1–2 days at 37°C.
5. Dried materials were collected and powder was prepared using a mortar and pestle.
6. The powder (Figure 1) was named Gas powder (Crown-Bovine-meat-blueberry-ex-P).

This powder was stored at room temperature until use.
Preparation of crown (*E. coli*) cell seeds

1. Sphingosine (180 µL) and *E. coli* DNA (90 µL) were combined and subjected to two cycles of heating and cooling.
2. After cooling, 100 µL of adenosine was added and the mixtures

were incubated at 37°C for 5 min.

Figure 1: Photograph of the powder used in the experiments.

In a previous report, this preparation was referred to as artificial cell seeds; however, in this study, they are referred to as crown (*E. coli*) cell seeds (DNA derived from *E. coli*).

Procedures and apparatus to generate gas-associated multiple living cells

1. A small amount (40–50 mg) of Gas powder (Crown Bovine-meat blueberry-ex. P) was added to an agar plate and incubated for 2 days at 37°C. Then, approximately 1.5 mL of 0.1 M monolaurin solution was dispensed onto a plate.
2. A dish was placed in the center of the plate which the powder was cultivated and the monolaurin solution was added.
3. About 100 µL of crown (*E. coli*) cell seeds were then dispensed onto the dish which was then placed in a bag. The plate was then incubated for 2 days at 37°C.
4. After incubation, the dish was removed, and objects that had grown on the surface were collected using approximately 1 mL of distilled water. Then, approximately 200 µL of the resulting suspension was spread on a fresh agar plate. Each plate was then incubated at 37°C for 1 and 2 days (primary culture)

   A portion of the objects that were grown in the primary culture were collected using 200 µL of distilled water, and approximately 50 µL of the resulting suspension was then poured onto fresh agar medium.

   The plate was then incubated at 37°C for 1 day to obtain the secondary culture.

   The various resulting structures were designated as Gas (Bovine-blue berry) Crown (E. coli) cells, with the designation reflecting that these structures were generated though the interaction of Gas Powder (Bovine-blueberry ex.P) and crown (*E. coli*) cell seeds derived using *E. coli* DNA.
5. Cultures of cells in the gas-generation system.

   After 2 days of incubation following monolaurin addition, the dish was removed and representative samples of the objects that had grown on the agar plate were collected. The collect materials were then poured onto agar plates and incubated at 37°C for 1 days.

The gas generating system mentioned here refers to the process in which cells are generated through the interaction between the gas produced by the gas powder and the crown cell seeds. Therefore, various systems can be established by altering either the crown cell seeds or the gas powder.

General observations

Objects on the plates could be observed directly with the naked eye and under a light microscope.

Results and Discussion

Figure 2 shows a photograph of an agar plate immediately after spreading the Gas powder (Crown-Bovine-meat-blueberry-ex-P), suggesting that the powders was comprised of particles of various sizes.

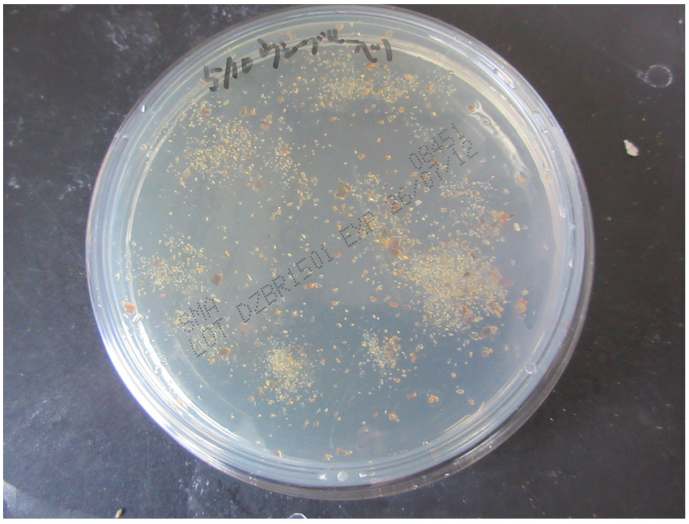

Figure 2: Photograph taken immediately after spreading to gas powder (Crown-Bovine-meat-blueberry-ex-P) onto the agar medium. Particles of various sizes and shapes were observed to cover the entire Petri dish.

As shown in Figure 3, large objects of various sizes and shapes appeared on the agar plate after 2 days of the powder being spread, suggesting that the powder was dissolved and diffused within the agar.

Figure 4 shows a photograph of a dish with the lid removed that had been placed on an agar plate at 2 days after spreading the powder.

Figure 5 shows a photograph of a dish with cultures at 2 day after the addition of monolaurin. Brownish objects in the gas generated system were observed, suggesting that the cultured gas powder interacted in some way with the monolaurin.

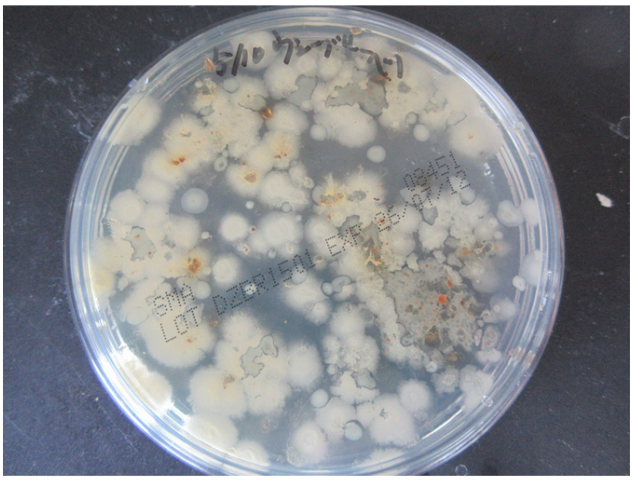

Figure 3: Photograph of the agar medium at 1 day after spreading the gas powder. Large objects of various sizes and shapes were observed on the Petri dish.

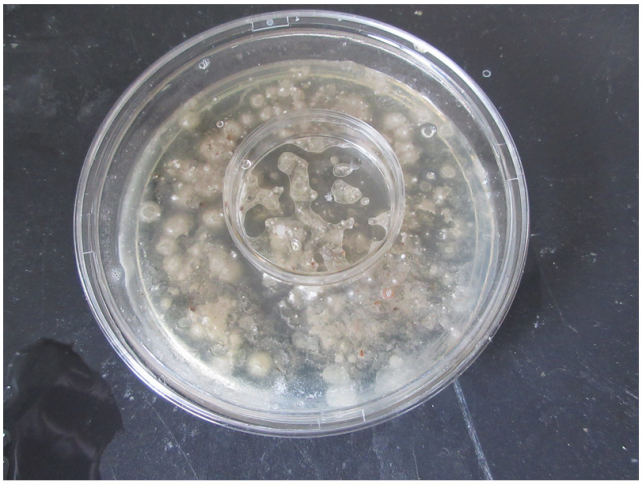

Figure 4: Photograph of a dish placed on the agar medium before incubation.

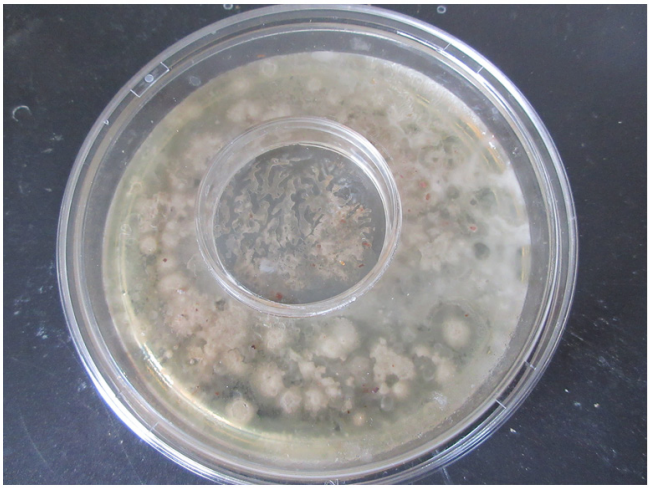

Figure 5: Photograph of a dish with cultures after two days of incubation with the gas powder and monolaurin. Brownish objects were observed.

As shown in Figure 6, particle-like objects of various sizes were observed in the crown cell seeds that had been poured onto a dish. These particles were similar in shape and size to crown cell seeds.

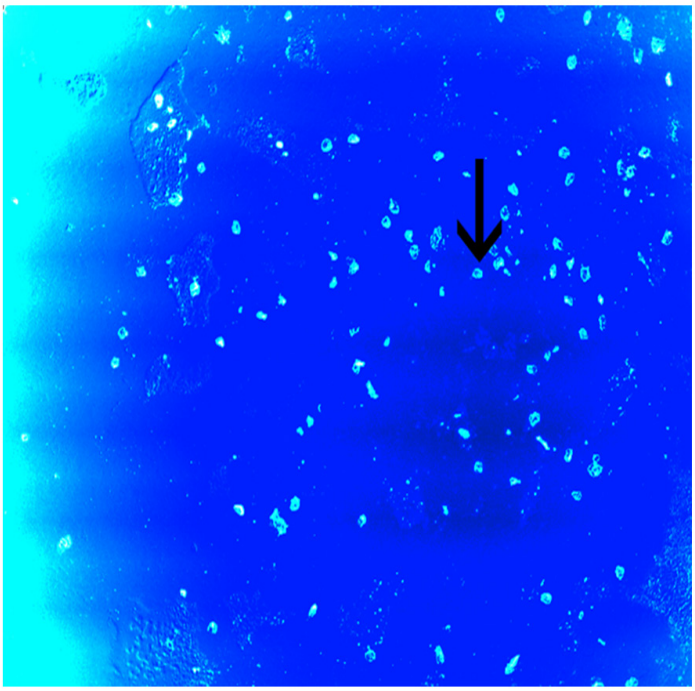

Figure 6: Microscopic appearance of the crown cell seeds placed in the dish before setting the dish. Numerous particle-like objects of various sizes and shapes were observed.
Approximate size of the object indicated by the arrow was 20 µm.

Figure 7 shows the microscopic appearance of objects (No. 1) after 2 days of culture of the dish shown in Figure 6. Numerous particles of various sizes and shapes were observed.

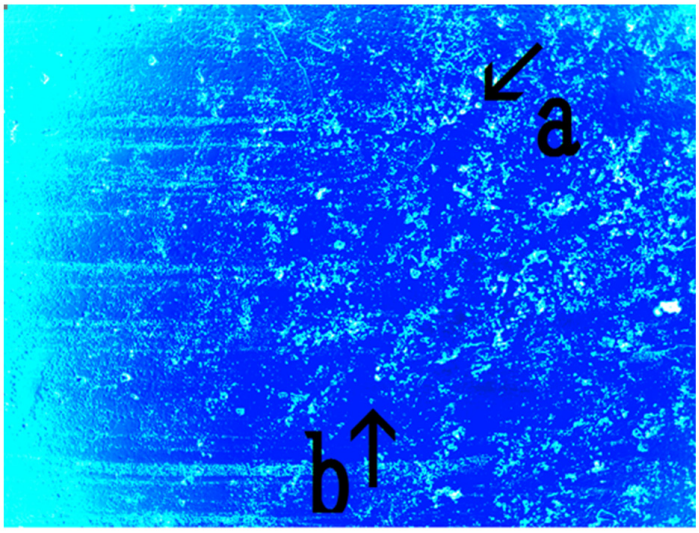

Figure 7: Microscopic appearance of objects (No. 1) shown in Figure 6 after two days of incubation with added monolaurin. Numerous particles of various sizes and shapes were observed. Colorless particles were observed (Figure 6a). Approximate size of the objects shown in Figure 7b is 5 µm.

Figure 8 shows a microscopic image (No. 2) of the dish co-cultured with a monolaurin for 2 days, corresponding to the sample shown in Figure 7.

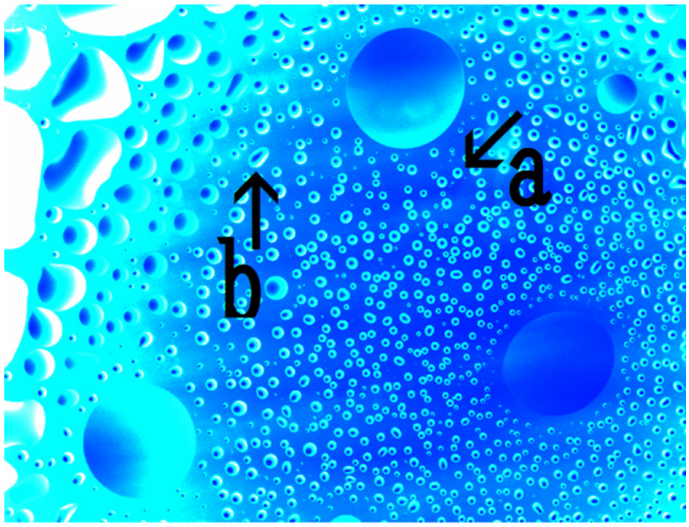

Figure 8: Microscopic image (No. 2) of the dish at 2 days, corresponding to the sample shown in Figure 7. Numerous spherical objects with various sizes were observed. Colorless objects were observed (Figure 7b). The approximate size of objects shown in Figure 7a was 20 µm.

Numerous spherical-like objects were observed over the entire plate, suggesting that the gas made the crown cell seeds spherical in shape.

Figure 9 shows several relatively large colony-like objects on the agar medium after collecting the materials from the dishes in which the crown cell seeds shown in Figures 6 and 7 were cultured. The image shows that the materials within dish were living, i.e., that multiple living cells were generated from non-living crown cell seeds spread onto a dish and co-cultivated in the gas generation system.

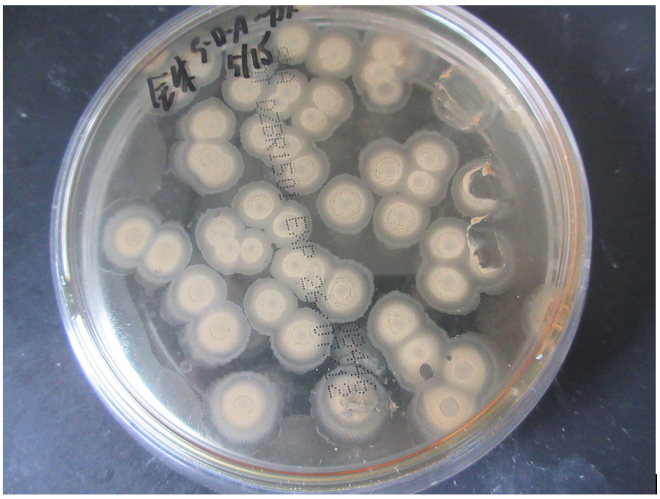

Figure 9: Photograph of the agar medium after culturing the materials from the objects within the dish shown in Figures 7 and 8. Several relatively large colony-like objects were observed (primary culture).

Figure 10 shows a microscopic image (No. 1) after 1 day of primary culture.

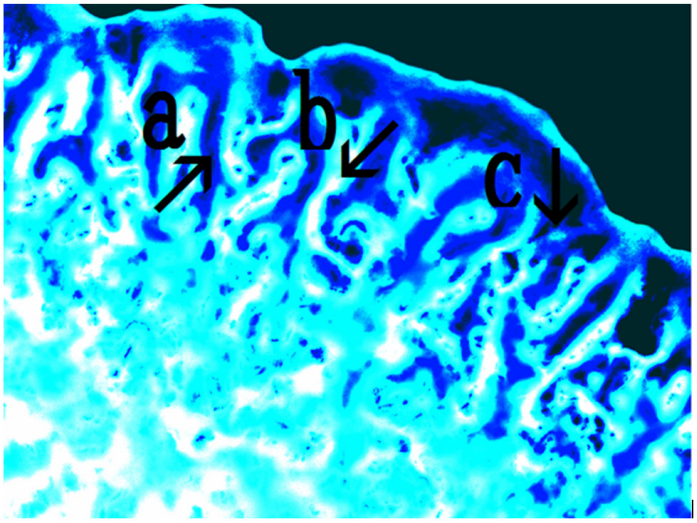

Figure 10: Microscopic image (No.1) at 1 day of primary culture. Branched objects were observed (Figure 10a). Colorless objects were observed (Figure 10b). The approximate size of the objects shown in Figure 10c was 40 µm.

Figure 11 shows a microscopic image (No. 2) after 1 day of primary culture.

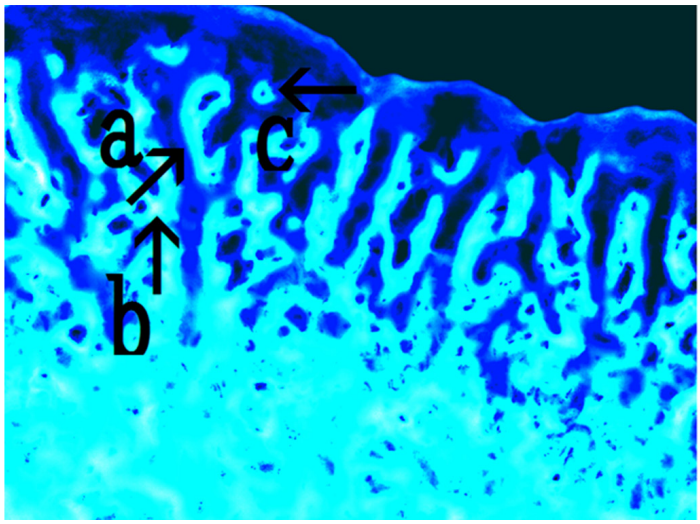

Figure 11: Microscopic image (No. 2) corresponding to the primary culture shown in Figure 9.

Many irregularly shaped objects were observed (Figure 11a). Colorless objects were observed (Figure 11b). The approximate size of the objects shown in Figure. 11c was 60 µm.

Figure 12 shows the microscopic appearance of objects (No.1) after 2 days of culture.

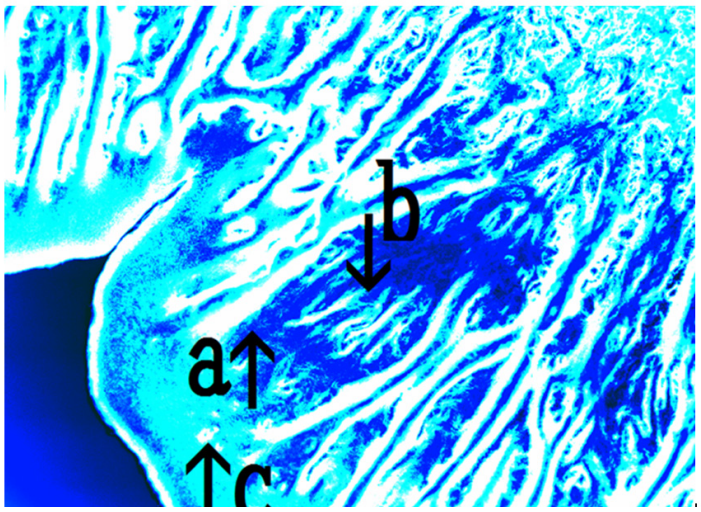

Figure 12: Microscopic image (No. 1) of cultures after 2 days of primary culture. Elongated objects of various size were observed (Figures 12a and b). The approximate size of the objects shown in Figure 12c was 60 µm.

Figure 13 shows the microscopic appearance of objects (No. 2) after 2 days of culture.

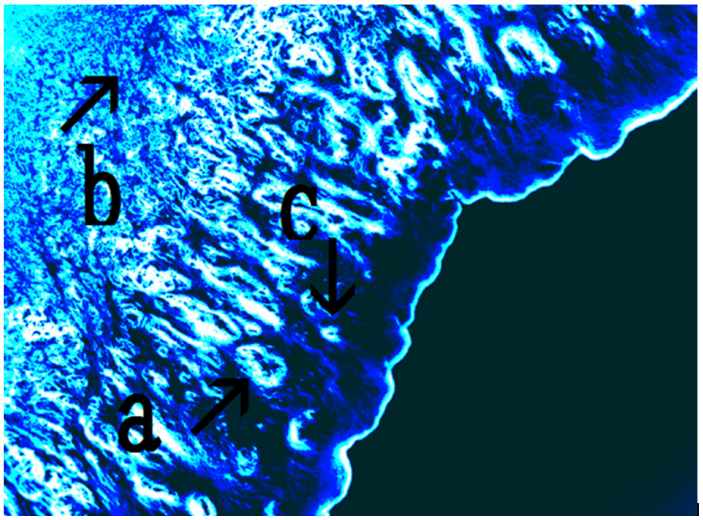

Figure 13: Microscopic image (No .2) of the primary culture after 2 days of culture. Circular objects of various sizes were observed (Figures 13a and 13c). An object similar to an individual cell was observed (Figure 13b). The approximate size of the objects shown in Figure 13c was 60 µm.

Figure 14 Photograph of second-generation culture obtained by collecting and culturing a portion of material shown in Figure 9.

Figure 15 shows the microscopic appearance of objects (No. 1) after 1 day of secondary culture.

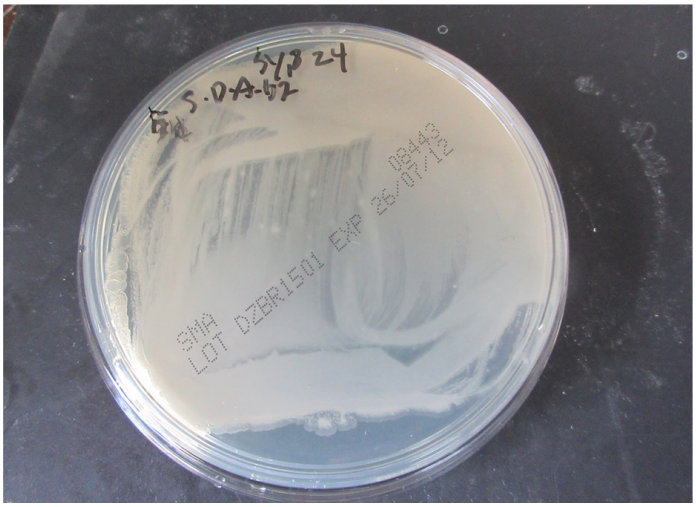

Figure 14: Photograph of second-generation culture obtained by collecting and culturing a portion of the material shown in Figure 9.

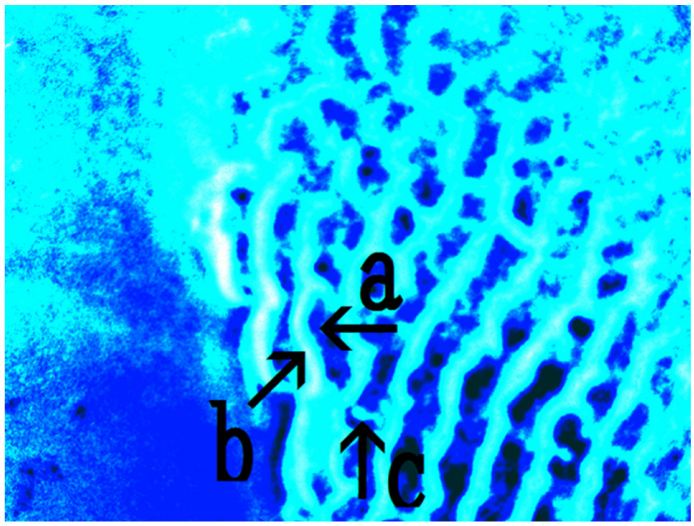

Figure 15: Microscopic image (No. 1) of second-generation culture at day 1 of culture. Elongated or connected objects were observed (Figure 15a). Colorless objects were observed (Figure 15b). The approximate size of the object shown in Figure. 15c was 70 µm.

Figure 16 shows the microscopic appearance of objects (No.2) after 1 day of secondary culture.

Figure 17 shows a photograph of brownish cells in the gas generation system.

Figure 18 shows a photograph of a plate on which a portion of cells from the gas generated system were cultured. Microorganism-like objects were observed, suggesting that the cell-like objects in the gas generated system were viable.

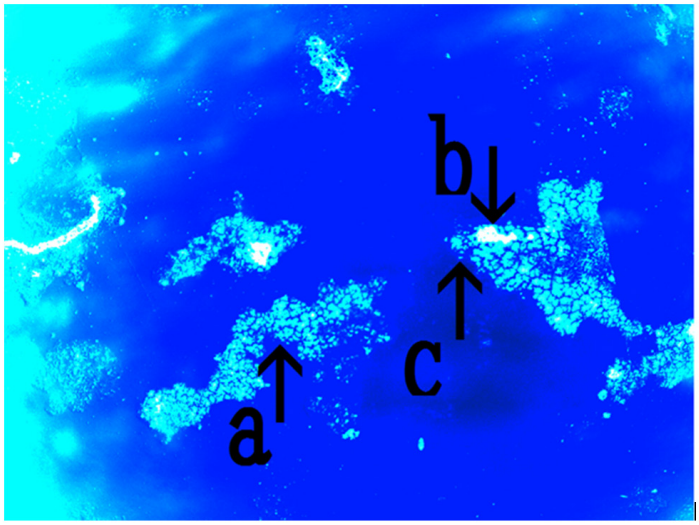

Figure 16: Microscopic image (No. 2) of second-generation cultures at day 1 of culture. Assemblies of gas crown cells (Figure 16a) and colorless assemblies (Figure 16b) were observed. The approximate sizes of the object shown in Figure 16c was 3 μm.

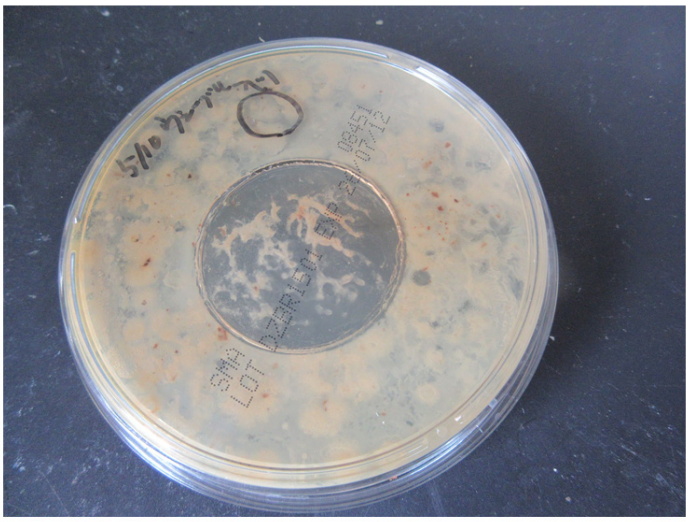

Figure 17: Appearance of the cells on the agar medium in the gas assay system. Several brownish objects were observed.

In previous studies, it was demonstrated that antibiotic-producing cells were generated through the combination of DNA crown cells and partners [2-27]. Importantly, the generation process was found to be common across all combinations tested.

Therefore, the use of powder prepared from the combination of DNA crown cells (bovine meat) and blueberry extract in this study is not particularly unique.

As noted earlier, numerous antibiotic-producing cells were produced during the culture period following the addition of monolaurin. However, the underlying reason for such a phenomenon and the processes involved during the course of cultivation remain unclear.

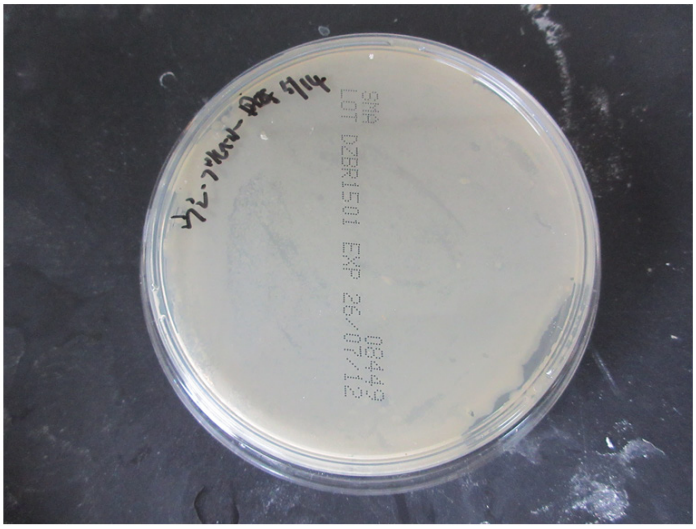

Figure 18: Appearance of the agar medium after culturing a portion (see inset) of the material shown in Figure 17. Several microorganism-like objects are observed on the medium surface.

To better clarify this point, gas production was investigated during cultivation and four kinds of gas were produced [28]: dimethyl sulfide (DMS), 5-(2-methylpropyl) nonane, 1-3-dichlorobenzene, and dodecane.

However, whether the generation of these gasses have any relationship to the proliferation of antibiotic-producing cells remains unknown.

The present experiments examined whether gas production was associated with cell generation. To this end, non-living crown (*E. coli*) cell seeds were cultivated within the gas production system (Figure 4).

The microscopic appearance of crown cell seeds before culture showed particle-like objects (Figure 6). At 2 days after monolaurin addition, numerous particles or spherical objects were observed (Figures 7 and 8). The objects were collected and cultured on agar medium. Several relatively large colony-like objects were observed (Figure 9).

The findings imply that living cells were produced from non-living materials (Crown cell seeds). The observed cells were named gas crown cells, specifically, Gas (bovine blueberry ex) crown (*E. coli*) cells.

The microscopic appearance of the objects grown in the collected samples is shown in Figures 10–13, 15 and 16). Most of these objects exhibited diverse morphologies, including elongated, connected and branched structures. In addition, most of these objects contained colorless structures, possibly representing the origin of the proliferative assemblies. However, experiments have not yet been conducted to clarify the nature or function of these internal components.

Thus, the present study demonstrated that exposure to specific gasses induced the emergence of living cells from non-living DNA crown cell seeds.

This finding suggests that gas may represent an overlooked pathway in the shift from non-living to living systems, with gasses possibly acting as a trigger for structural re-organization within DNA crown cell seeds and potentially conferring seeds with a self-replicating ability. Such seeds grew in agar medium and subsequently generated gas-crown cells.

Therefore, monolaurin, which was not contained in crown cell seeds, does not appear to be involved in the autonomous behavior of seeds. Instead, it may be associated with gas production or the regulation of structural properties.

Several mechanisms could account for these observations. One possibility is that gas exposure alters the hydration or charge redistribution dynamics in the seeds, thereby promoting the assembly of membrane-like boundaries or internal compartments.

Alternatively, gas molecules may provide energy inputs that facilitate molecular rearrangement, similar to the role of environmental fluctuations in early Earth scenarios.

These interpretations align with a previous hypothesis, which proposed that that life may have emerged through cycles of drying, gas exposure, and re-hydration, although such mechanisms have not been demonstrated experimentally.

The present results therefore expand the conceptual framework of chemical evolution by introducing gas-associated emergence as a viable route toward living systems.

However, among the four gases identified, it is currently unclear which is responsible for the generation of gas crown cells.

Several of these gasses are commonly associated with biological activity, which raises important questions about the chemical processes occurring within the culture systems.

In particular, DMS is widely used in a variety of fields. It is also produced by marine microorganisms and is associated with biological processes in sulfur-rich environments. Moreover, it is considered one of the most significant and promising gasses in astrobiology, and is regarded as a potential indicator of extraterrestrial life. Indeed, its presence on some planets has been interpreted as a possible indicator of life.

Comparison with prime studies further underscores the novelty of the finding. Traditional proto-cell research has focused primarily on lipid vesicles, coacervates, or aqueous-phase DNA assemblies. While DNA-based artificial cells have been reported in typical require controlled chemical conditions and have not been reported to induce gas-phase activation. In contrast, the emergence of the cells observed in this study occur spontaneously upon gas exposure, without the need for enzymatic catalysts or phospho-lipid components.

This distinguished DNA crown cells as a unique platform for studying the transition between non-living and living states. Moreover, gas-induced emergence of cells suggests a new dimension in origin-of-life theory, with atmospheric components potentially playing a more direct role in early cell realization than previously considered.

Despite the significance of these observations, several limitations remain.
First, it is unclear how Gas crown cells proliferate. In primary culture, they were observed to proliferate in a colony-like form (Figure 9), whereas in secondary culture, they grew in a microorganism-like manner (Figure 14).

Although assembly formation was not observed in Gas crown cells, it is possible that they share similar adhesive or structural characteristics with synthetic DNA crown cells. If this is the case, then the phenomenon could be interpreted as follows.

In primary cultures, Gas crown cells are adsorbed by each other and are maintained in the assembly, resulting in a colony-like morphology. In the secondary culture, the structure becomes dispersed, leading to a more typical microorganism-like growing pattern.

These observations suggested that Gas crown cells do not proliferate as isolated single cell, rather, once a multi-cellular assembly is established, the assembly functions as a unit and undergoes proliferation.

Other important questions are whether Gas crown cells differ from DNA crown cells that proliferate within egg white, from partner-associated antibiotic crown cells, and which gas is most effective for promoting the proliferation of Gas crown cells. It remains unclear whether a single gas or multiple gasses are involved, and whether differ types of gasses may alter the characteristics of Gas crown cells. This study focused on dimethyl sulfide, which has been identified as one of the associated gasses.

The most critical issue is how gas is involved in the proliferation of Gas crown cells, and whether their proliferation is gas-dependent or gas-independent remains unresolved.

Numerous types of DNA crown cells have already been produced, and gas crown cells can likewise generated without limitation. Practical applications have already demonstrated, including the production of antibiotics and yogurt fermentation, demonstrating a clear contribution to the medical sciences and the food industry. The ability of gas crown cells to generate an ever wider range of cellular forms suggests that this method may support advances in many additional fields.

Thus, gas crown cells potentially represent a scientifically and

industrially valuable system that may contribute substantially to human welfare.

Further studies should investigate various kinds of gas crown cells using present methods and clarify whether gas is linked directly to the emergence of both DNA and gas crown cells, as well as to the formation of unique cellular structures and cell functions such as antibiotic production. In addition, it will be important to clarify how these gasses are produced and which gasses are generated by gas crown cells under different conditions.

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