Research Article ISSN 2771-666X

Journal of Advanced Materials Science and Engineering

Emergence of Autonomous Artificial DNA by Gas-Exposure

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Received: 17 Sep 2026; **Accepted:** 27 Oct 2026; **Published:** 07 Nov 2026

Citation: Shoshi Inooka. Emergence of Autonomous Artificial DNA by Gas-Exposure. J Adv Mater Sci Eng. 2026; 6(2): 1-5.

ABSTRACT

Artificial DNA (Type GFP model, 121 bp) was defined as a minimal sequence capable of driving autonomous molecular organization. When this artificial DNA was exposed to gas, autonomous artificial DNA (Auto-artificial DNA) emerged. When Auto-artificial DNA was cultured, it proliferated and subsequently generated cell-like structures termed AA crown cells. Thus, AA crown cells are not the origin of proliferation, but rather a developmental stage arising autonomously from DNA. These findings demonstrate that a minimal synthetic DNA fragment can trigger autonomous structural development in a simple molecular system and establish gas exposure as a novel trigger for a self-organizing nucleic acid system.

Keywords

DNA crown cells, Sphingosine-DNA, G-crown cells, Gas powder, Gas-exposed DNA, Auto-artificial DNA, AA-crown cells.

Introduction

Self-organization in minimal systems provides a promising route to understanding how autonomous structures can emerge without cellular machinery. Recent studies have shown that simple combinations of amphiphilic molecules and nucleic acids can generate dynamic assemblies; however, the ability of synthetic, ultra-short DNA fragments to initiate higher-order structural development remains largely unexplored. To investigate this possibility, the author designated the Type GFP model (121 bp) artificial gene as a minimal sequence capable of autonomous activity. Gas exposure is known to alter the reactivity of organic molecules, but its influence on nucleic acids, including artificial genes and plasmids, has not been systematically explored.

The author previously developed methods for preparing self-replicating artificial cells, termed DNA crown cells, by combining sphingosine, DNA, and adenosine [1-5] and several kinds of artificial cells based on DNA crown cells, such as antibiotic-producing crown cells and crown cells nema, have subsequently been prepared [6-17]. One of these types is the G-Sph crown cell, which is prepared using gas-exposed sphingosine instead of sphingosine [18-22]. Culture of the DNA fraction (SO-DNA) extracted from G-Sph crown cells results in the emergence of self-developing crown cells (SD-crown cells). This behavior suggests that SO-DNA acquires functional units capable of self-organization. The findings also suggested that the gases within G-Sph crown cells may be transmitted to DNA within the G-Sph crown cells, resulting in the generation of SO-DNA [23]. Further, it was demonstrated that gas-exposed DNA gives rise to Auto-DNA and that Auto-DNA forms Auto-Developing crown cells (AD-crown cells) [24]. Here, Auto-DNA (*E. coli*) refers to gas-exposed genomic DNA derived from *E. coli* that autonomously forms AD-crown cells.

In the present study, the author examined whether autonomous artificial DNA (Auto-artificial DNA) could be formed by gas exposure and subsequently form Auto-artificial crown-like cells (AA-crown cells). AA-crown cells are autonomous crown–like cellular structures generated from Auto-artificial DNA. The findings expand the conceptual boundaries of artificial gene function, and Auto-artificial DNA provides a unique platform for exploring minimal autonomous systems and the potential roles of DNA in non-genetic biological functions.

Materials and Methods

Materials

Artificial DNA fragments (Type GFP, 121 bp) were commercially synthesized by eurofins Genomics Co. Ltd. (Tokyo, Japan) and were dissolved in distilled water (46 µg/1 mL). Gas-exposed artificial DNA (Auto-artificial DNA) was prepared by gas-powder

treatment [20]. Agar plates and agar medium (SMA) were obtained from AS ONE (Japan).

Methods

Preparation of Auto-artificial DNA

Auto-artificial DNA was prepared according to the method used to prepare G-Sph, using cultures of gas powder (antibiotic-producing crown cells) [20].

In brief, egg white powder-enclosed bovine crown cells containing blueberry extract were cultured. When monolaurin was added to the cultures, gases, including dimethyl disulfide (DMDS), were produced [18]. A dish was then placed above the cultures inside a bag and 0.2 mL of artificial gene solution was added to the dish. In some cases, a detection tube was used to measure DMDS concentrations (Figure 1). DMDS production during gas-powder culture was monitored using a Kitagawa detection tube (Komyo Rikagaku Kogyo, K.K., Kawasaki, Japan) for DMDS (0.21–100 ppm). After 2 days of culture at 37°C, DNA in the dish was collected by adding 0.2 mL of distilled water and a spatula. The DNA was designated as Auto-artificial DNA and stored in a freezer at approximately 20°C until use.

Culture of Auto-artificial DNA

A total of 50 µL of Auto-artificial DNA (approximately 1.5 µg in total) was spread onto an agar plate using a culture spreader and incubated at 37°C for 1 day.

Secondary culture

Objects that had grown after 1 day of culture were collected using 6 mL of distilled water, and 200 µL of the resulting suspension was spread onto a new agar plate using a culture spreader and incubated at 37°C for 1 or 2 days.

General observations

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

Results and Discussion

Figure 1 shows the setup of the dish during culture for the collection of Auto-artificial DNA. A dish containing Auto-artificial DNA was placed above a gas-powder culture containing monolaurin. The reagent layer turned approximately 50% white. After 2 days of culture, the reagent layer turned completely white, indicating that the DMDS concentration had reached the upper detection limit (100 ppm).

Figure 2 shows a microscopic image of Auto-artificial DNA. Direct gas exposure induced the spontaneous emergence of microscopic rounded objects with thin membrane-like structures within 48 h. These particle-like objects appeared to be autonomous and formed AA-cells during culture.

Figure 3 shows colony-like structures that formed during primary culture of Auto-artificial DNA. Auto-artificial DNA produced microscopic colony-like spots on agar medium within 18 h. The colonies exhibited circular or irregular shapes and expanded gradually, demonstrating proliferative behavior.

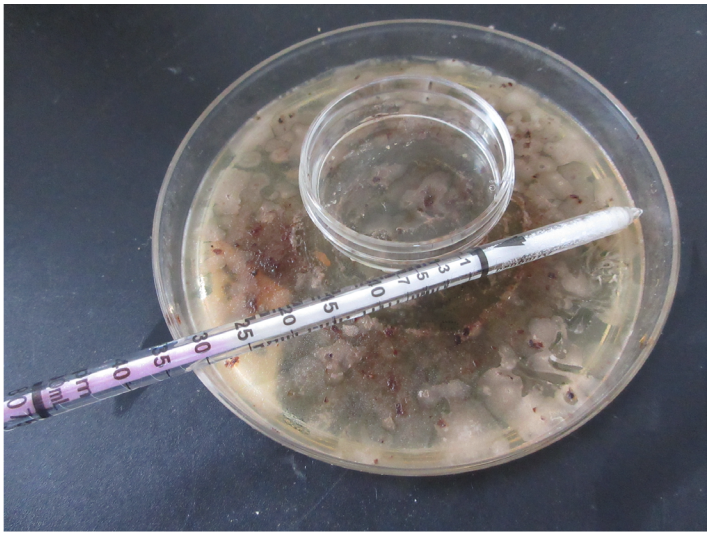


Figure 1: Setup of the dish during culture for the collection of Auto-artificial DNA. The reagent layer turned approximately 50% white.

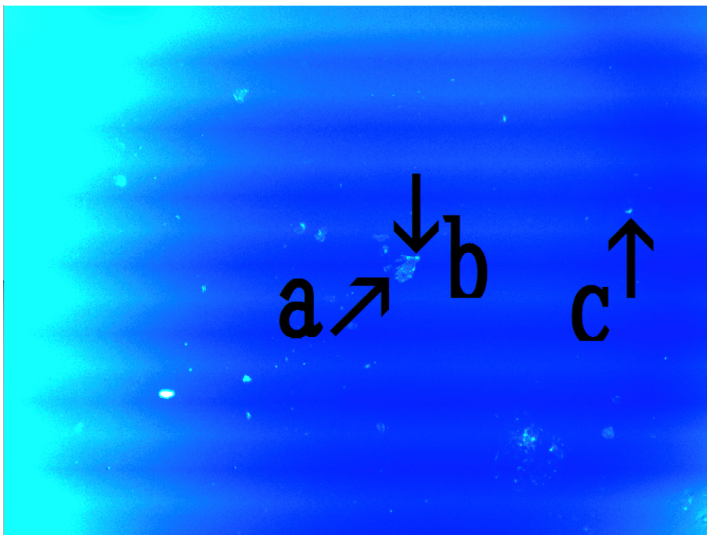


Figure 2: Microscopic image of Auto-artificial DNA. Rounded objects with thin membrane-like structures (Figure 2a) were observed. These particle-like objects (Figure 2b) were autonomous and formed AA-cells during culture.Approximate size of Fig 2c is 8-10µm.

Figure 4 shows the microscopic appearance of primary colonies derived from Auto-artificial DNA culture. Microscopic observation of primary colonies revealed large, rounded objects. AA-cell proliferation appeared to initiate from these objects.

Secondary colony formation demonstrating sustained autonomous proliferation is shown in Figure 5. Material transferred from primary colonies generated secondary colonies. Secondary colonies showed more uniform size and morphology, suggesting stabilization of the autonomous proliferation system across culture cycles.

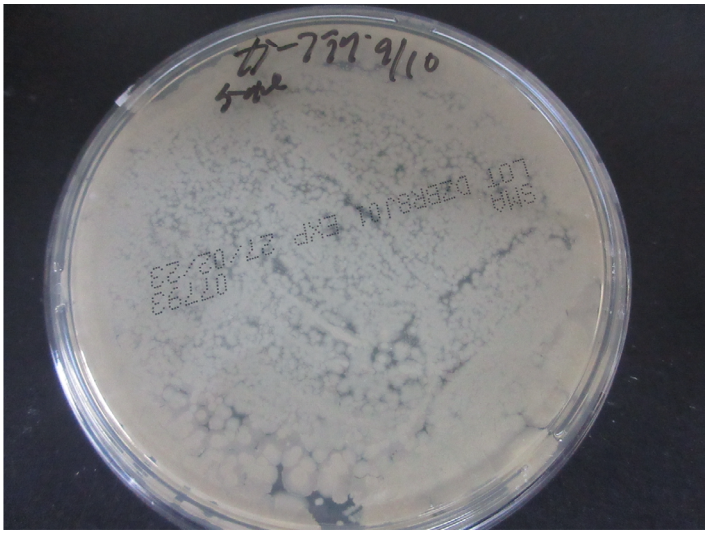


Figure 3: Colony-like structures formed during primary culture of Auto-artificial DNA. Auto-artificial DNA produced microscopic colony-like spots on agar medium. The colonies exhibited circular or irregular shapes.

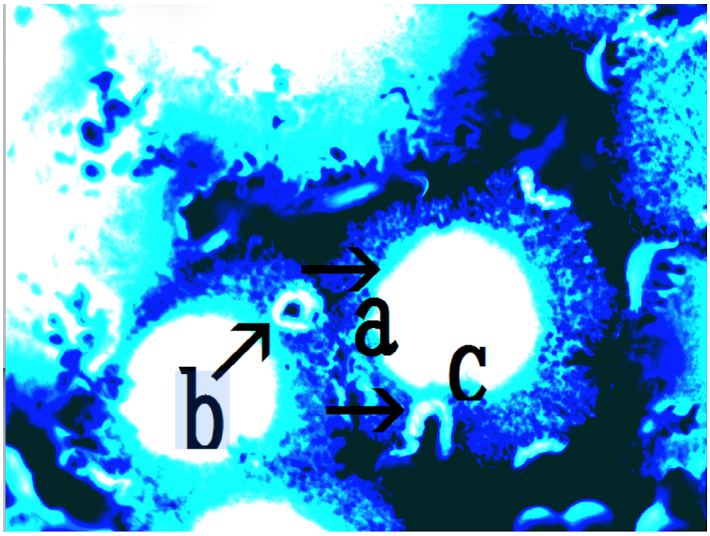


Figure 4: Microscopic appearance of primary colonies derived from Auto-artificial DNA culture. Microscopic observation of primary colonies revealed large, rounded objects (Figure 4a). Cell formation (Figure 4b) and proliferation (Figure 4c) from these objects were revealed by microscopic observation. Approximate size of Figure 4b is 170μm.

Figure 6 shows the microscopic appearance of secondary colonies derived from primary culture. These secondary colonies contained abundant wiggly objects and small rounded objects that differed from those observed in primary culture. The persistence of these structures confirms that Auto-artificial DNA maintained autonomous proliferative capacity through multiple generations of culture.

The present study demonstrated that when the minimal artificial gene Type GFP (121 bp) was exposed to gas, Auto-artificial DNA emerged and initiated autonomous structural development (forming AA-crown cells). The emergence of AA-crown cells indicates that self-organization can be triggered by gas exposure of ultra-short synthetic DNA fragments without enzymatic reactions, metabolic pathways, or pre-existing cellular components. The findings expand the functional scope of artificial genes beyond their informational or template functions, suggesting that minimal sequences can act as drivers of structural organization.

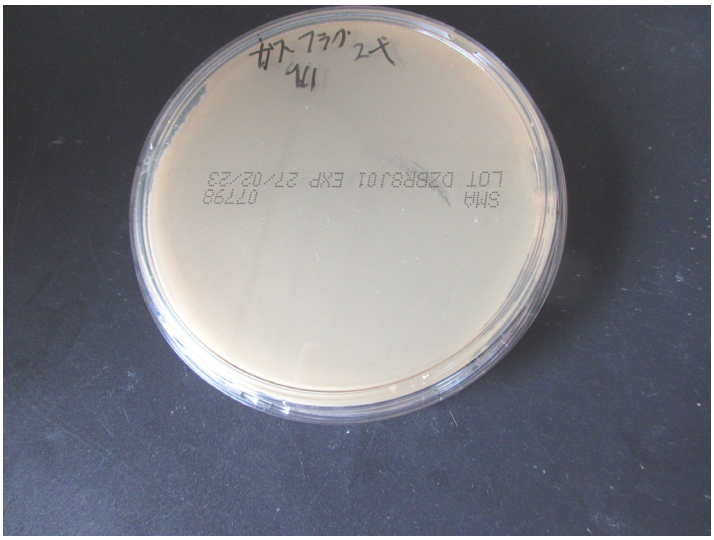


Figure 5: Secondary colony formation demonstrating sustained autonomous proliferation. Secondary colonies showed more uniform size and morphology.

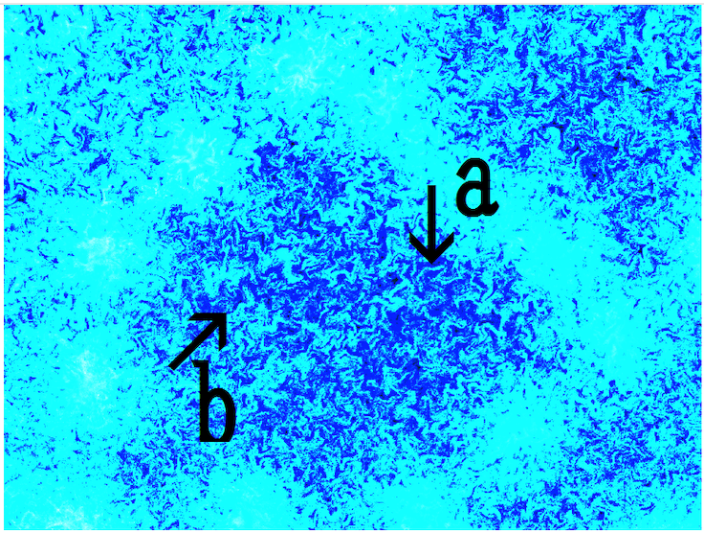


Figure 6: Microscopic appearance of secondary colonies derived from primary culture. Secondary colonies contained abundant wriggly objects (Figure 6a) and small rounded objects (Figure 6b; approximate size, 10 μm).

Moreover, a previous experiment demonstrated that directly gas-exposed DNA (Auto ***E. coli*** **genomic** DNA; Auto DNA) acquired the autonomous ability to generate colony-like structures (AD-crown cells) [24]. In the present study, the Type GFP model artificial gene (121 bp) was used to prepare gas-exposed artificial DNA (Auto-artificial DNA). Thus, the present and previous experiments indicate that both gas-exposed genomic DNA and

gas-exposed artificial DNA exhibit autonomous properties. These findings suggest that gas exposure can induce autonomous properties in DNA of different sizes and types.

The sequence of emergence of autonomous DNA and crown cells was similar for Auto-DNA and Auto-artificial DNA. First, Auto-DNA or Auto-artificial DNA emerged, followed by the formation of AD-crown cells or AA-crown cells, respectively. However, potential differences included variations in morphology and envelope formation between AD-crown cells and AA-crown cells, suggesting that the autonomous properties of Auto-DNA and Auto-artificial DNA are not identical and that multiple types of autonomous DNA may exist.

Neither Auto-DNA nor Auto-artificial DNA contained sphingosine. The author previously proposed a hypothesis regarding the role of sphingosine in autonomous behavior [23]. However, the role of sphingosine may now be redefined. Rather than being essential for the emergence of autonomous DNA, sphingosine may enhance morphological organization and stability, suggesting that it modulates the mode of self-organization.

Overall, the concept of Auto-DNA and Auto-artificial DNA expands the functional role of DNA beyond its conventional genetic capacity. In this system, DNA acts as a driver of autonomous assembly, contributing to emergent biological behavior without requiring intact cellular machinery. This positions Auto-DNA and Auto-artificial DNA within the emerging field of systematic autonomous DNA systems, which explores DNA-based self-organization and minimal autonomous units.

In conclusion, the present findings demonstrated that ultra-short artificial DNA, in addition to genomic DNA, acquires autonomous properties following gas exposure. To put this finding into perspective, particles derived from Auto-artificial DNA grew on agar plates in a manner resembling microorganism growth and formed AA-cell-like colonies over successive generations.

Overall, artificial DNA acquired autonomous properties following gas exposure alone. The findings suggest that systems generating life-like behavior may be investigated based on two compounds (Gas and DNA). Thus, Auto-artificial DNA may contribute to understanding how minimal components can generate life-like behavior. The discovery of Auto-artificial DNA may also contribute to new applications of artificial genes.

Future studies should determine whether circular DNA (plasmids), which differs structurally from both genomic DNA and the artificial DNA examined here, can acquire autonomous properties following gas exposure and whether AA-cells proliferate through the metabolism of specific substances or through chemical reactions.

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