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Bacterial Detection through Response of Photoswitchable-lipid-included Liposomes

Jin-Won Park [1]

[1]*Department of Chemical and Biomolecular Engineering, College of Energy and Biotechnology, Seoul National University of Science and Technology, Seoul, Republic of Korea, 01811.*

ABSTRACT: *This research aimed to investigate the bacterial detection through the fluidity change of liposome layer. Pyranine-encapsulated liposomes were prepared with palmitoyloleoylphosphatidylcholine or with palmitoyloleoyl phosphatidylglycerol and oleoylhydroxy phosphatidylglycerol. Each liposome contained Azobenzene-derivatized phosphatidylcholine (AzoPC) as 10 % molar ratio. AzoPC-contained liposome underwent the configuration change caused by the UV illumination. Only AzoPC-contained PC-liposome layer turned out more fluid by the interaction with the bacteria. Therefore, the quantitative bacteria-detection was achieved with the simple UV-exposure.*

Keywords: *Liposome layer; Fluidity; UV illumination; Bacteria detection.*

I. INTRODUCTION

Since some of bacteria are pathogenic, rapid and accurate detection are significant to prevent the bacterial infection [1]. The conventional methods such as plate counting, polymerase chain reaction, isothermal amplification, and enzyme-linked immunosorbent assay are frequently used, but they are either time-consuming or instrument-requiring [2,3]. In the last decade, numerous developments have been made to advance the detection. For example, quartz crystal microbalance, electrochemical conductivity, and optical intensity have been reported [4-6]. Still, these techniques need skilled ability and appropriate pretreatment.

Azobenzene-derivatized phosphatidylcholine (AzoPC) lipids are a kind of photoswitchable lipid used in the light-induced changes in membrane fluidity [7]. These lipids maintain a trans configuration in the absence of light and undergo trans-to-cis photoisomerization upon UV exposure (λ = 365 nm). The fluidity change has been known to cause the release of the encapsulated matters in the liposomes [8]. The interaction between bacteria and lipid membranes was facilitated by the fluidity change. Therefore, in this study, the bacterial detection was investigated through the fluidity change corresponding to the configuration caused by the simple UV-exposure to the solution.

II. MATERIAL AND METHODS

2.1. Liposome preparation

Palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylglycerol (POPG), oleoylhydroxy phosphatidylglycerol (OHPG), pyranine, and AzoPC were purchased from Merck (Rahway, NJ, USA). Two types of liposomes were prepared. One was pyranine-encapsulated POPC liposomes, and the other was pyranine-encapsulated POPG-OHPG liposomes. AzoPC were added as 10% molar ratio into both liposomes. For

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comparison, AzoPC free liposomes were prepared as well. The desired lipids were dissolved in chloroform, which was then evaporated under nitrogen stream. The lipids were hydrated into 1 mg/ml with DI water of 5 mM pyranine. And the hydration solution was extruded through 100 nm polycarbonate membrane to form liposomes. The size of liposomes was monitored using dynamic light scattering.

2.2. Bacteria addition and UV illumination

Escherichia coli (*E. coli*) were also from Merck. The *E. coli* concentration was considered into 20 to 100 cfu/g because 20 cfu/g was satisfactory criterion and 100 cfu/g was acceptable to ensure safe food [9]. After the *E. coli* addition followed by shaking the liposome solution, UV light (365 nm) illuminated to both liposome solution. For comparison, the identical aqueous-solution without *E. coli* was also added to the solutions and exposed to the light. Also, the illumination was separately performed to the solutions without any addition. Right after the UV illumination, two drops of pH 3 solution were added into the liposome solutions. Since pyranine was pH-sensitive dye, the fluorescence intensity was monitored.

III. RESULTS and Discussion

First, the illumination to both liposomes without AzoPC was performed for 20 minutes [10]. After the acid drops were added, the fluorescence intensity change was observed. In order to find out the effect of the illumination on the liposomes without AzoPC, the contrast experiments were also performed only without the illumination. The difference in the changes was little distinguishable with respect to time. Therefore, it was found that the illumination caused little change in the fluidity of non-AzoPC-included liposome layer (Figure 1). These results were compared with those of the AzoPC-included-liposomes.

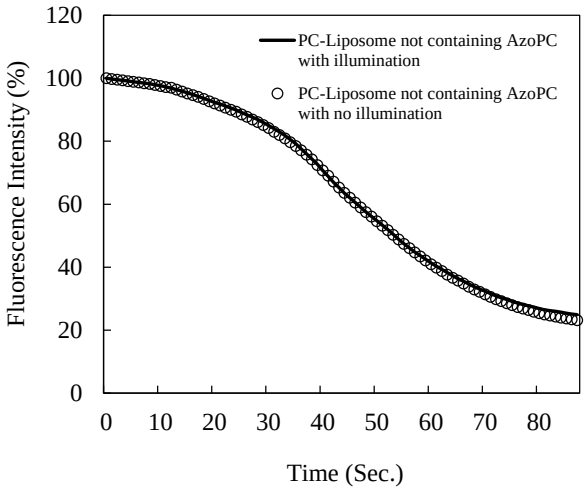

Figure 1. Fluorescence intensities of the AzoPC-free liposomes for the UV (365 nm) illumination presence.

The UV illumination was also performed to both liposomes containing AzoPC. It was observed that the illumination step generated a significant effect on the fluorescence intensity change. The change in the AzoPC conformation made an effect on the fluidity of the liposome layer, as known [11]. The release of the pyranine was facilitated by the conformation change, compared to the experiments in the absence of the illumination. For both liposomes, the change in the fluorescence intensity was almost identical with respect to time. The intensity change was shown in Figure 2 (on next page).

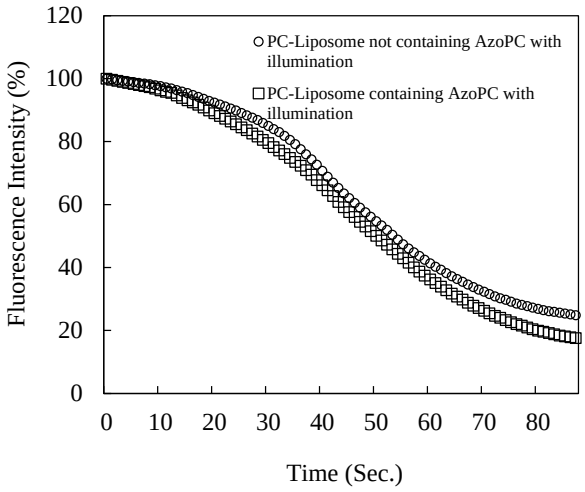

Figure 2. Fluorescence intensities of the AzoPC-free PC-liposomes and the AzoPC-contained PC-liposomes for the UV (365 nm) illumination presence.

The bacteria solution was added to the liposomes containing AzoPC into the desired concentration (20 to 100 cfu/g). After the bacteria addition, the illumination was performed to the liposomes followed by the observation of the fluorescence intensity. The PC-liposomes containing AzoPC showed the clearly more change in the intensity, while the PG-liposomes containing AzoPC showed little change from the bacteria absence. Therefore, it was found that the headgroups of liposomes were critical and interacted with bacteria. Furthermore, the interaction seemed to induce the more fluidity of the liposome layer because the more release of the pyranine across the liposome layer occurred. The change difference caused by bacteria between PC- and PG-liposomes containing AzoPC was shown in Figure 3.

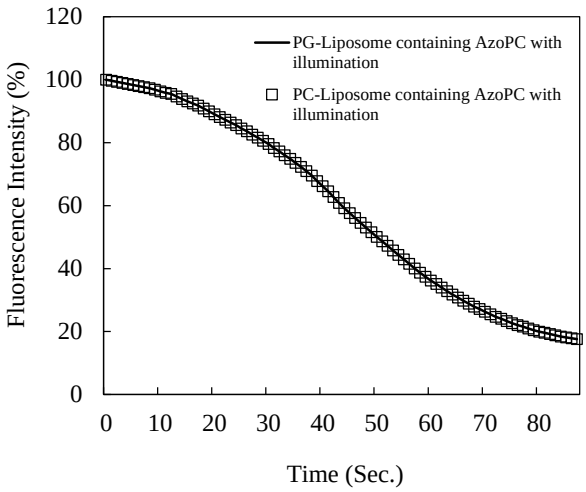

Figure 3. Bacteria-caused difference in fluorescence intensity change between PC- and PG-liposomes containing AzoPC.

The bacterial concentration was proportional to the release, as expected. This correlation is obviously related to the binding area available to the bacteria. The area was covered with the bacteria, and then the release increase became saturated (Figure 4).

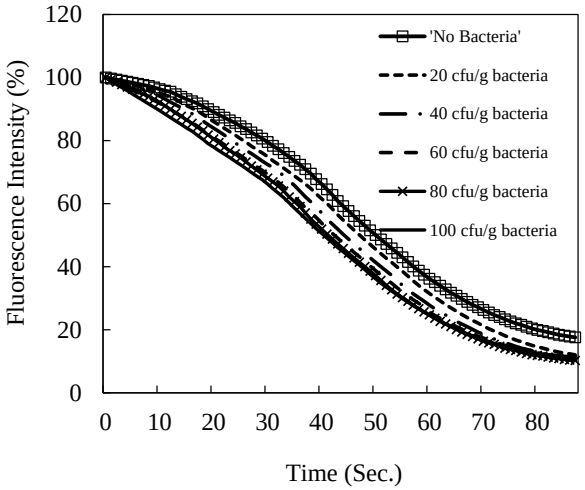

Figure 4. Fluorescence intensity change with respect to the bacteria concentration.

IV. CONCLUSION

The applications of role play activities in practicing English speaking skill in Vietnam are quite popular. However, each teacher has a different way to apply them in various contexts. Based on the features of my context that I explained above, I employed group work role play activities to engage my students in the speaking lessons and help enhance their accuracy in speaking English. This paper points out both strong and weak aspects of using group work role play in improving students' accuracy in speaking English. It answered my first research question:

V. REFERENCES

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