

Article

Decomposition of Glucose-Sensitive Layer-by-Layer Films Using Hemin, DNA, and Glucose Oxidase

Kentaro Yoshida ^{1,*}, Yu Kashimura ¹, Toshio Kamijo ², Tetsuya Ono ¹, Takenori Dairaku ¹, Takaya Sato ², Yoshitomo Kashiwagi ¹ and Katsuhiko Sato ^{2,3}

¹ School of Pharmaceutical Sciences, Ohu University 31-1 Misumido, Tomita-machi, Koriyama, Fukushima 963-8611, Japan; 714024@ohu-u.jp (Y.K.); t-ono@pha.ohu-u.ac.jp (T.O.); t-dairaku@pha.ohu-u.ac.jp (T.D.); y-kashiwagi@pha.ohu-u.ac.jp (Y.K.)

² Department of Creative Engineering, National Institute of Technology, Tsuruoka College, 104 Sawada, Inooka, Tsuruoka 997-8511, Japan; kamijo@tsuruoka-nct.ac.jp (T.K.); takayasa@tsuruoka-nct.ac.jp (T.S.)

³ Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3 Aoba, Aramaki, Aoba-ku, Sendai 980-8578, Japan; satok@m.tohoku.ac.jp

* Correspondence: k-yoshida@pha.ohu-u.ac.jp; Tel.: +81-24-932-8931; Fax: +81-24-933-7372. (K.Y.)

Abstract: Glucose-sensitive films were prepared by the layer-by-layer (LbL) deposition of poly(ethyleneimine) (H-PEI) solution and DNA solution (containing glucose oxidase (GOx)). H-PEI/DNA+GOx multilayer films were constructed using electrostatic interactions. The (H-PEI/DNA+GOx)₅ film was then partially decomposed by hydrogen peroxide (H₂O₂). The mechanism for the decomposition of the LbL film was considered to involve a more reactive oxygen species (ROS) that was formed by the reaction of hemin and H₂O₂, which then caused nonspecific DNA cleavage. GOx present in the LbL films reacts with glucose to generate hydrogen peroxide. Therefore, decomposition of the H-PEI/DNA+GOx)₅ film was observed when the thin film was immersed in a glucose solution. A (H-PEI/DNA+GOx)₅ film exposed to a glucose solution for periods of 24, 48 72, and 96 h indicated decomposition of the film increased with the time. The rate of LbL film decomposition increased with the glucose concentration. At pH and ionic strength close to physiological conditions, it was possible to slowly decompose the LbL film at a sub-millimolar glucose concentration.

Keywords: hydrogen peroxide response; layer-by-layer; multilayer thin film; glucose sensitive; stimuli-sensitive

1. Introduction

Layer-by-layer (LbL) films have been prepared by the alternate deposition of polyelectrolytes (polycation and polyanion) on a solid surface assisted by electrostatic interaction [1-3]. Various other interactions have also been recently employed to construct LbL films, such as hydrogen bonding [4,5] and sugar-lectin binding [6,7]. The materials employed for this purpose have included synthetic polymers [8,9], polysaccharides [10-12], protein [13-15], and DNA [16,17]. Such layered multilayer films have found application in separation and purification [18,19], sensors [20,21], and drug delivery systems (DDSs) [22-24].

We have recently reported that hydrogen peroxide (H₂O₂) induced the decomposition of LbL films composed of hemin-modified poly(ethyleneimine) (H-PEI) and DNA [25]. Hemin is an iron porphyrin molecule and the iron porphyrin produces more reactive oxygen species (ROS), such as hydroxy radicals (\cdot OH), by reaction with H₂O₂ [26,27]. The ROS causes non-specific DNA cleavage [28-30], and H-PEI/DNA LbL film was consequently decomposed by the addition of H₂O₂. H₂O₂ is generated by the reaction of substrates and oxidases. LbL films composed of oxidases have been applied in biosensors and stimuli-responsive devices [31-33]. In the present work, we report the

preparation of thin LbL films consisting of H-PEI and DNA with glucose oxidase (GOx), and the glucose-induced decomposition of these LbL film (Figure 1). Glucose-sensitive materials respond to the blood glucose level of a diabetic patient; therefore, LbL films composed of GOx can be applied to an insulin DDS [34,35]. The decomposition of LbL films reported in this study is slow due to the stepwise reactions (enzymatic reaction, generation of ROS by hemin, and nonspecific cleavage of DNA). If decomposition of the membrane is slow, then the drug encapsulated in a capsule membrane can be released gradually. The glucose-induced decomposition of the LbL film in this work was achieved by the addition of glucose and the decomposition dependence on the concentration of glucose was investigated.

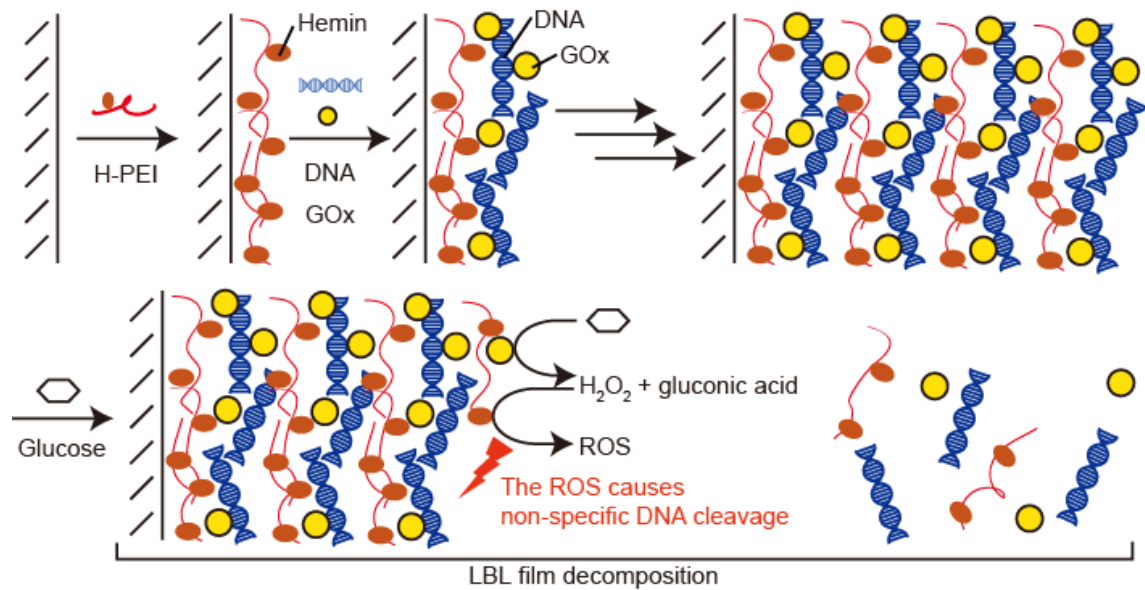


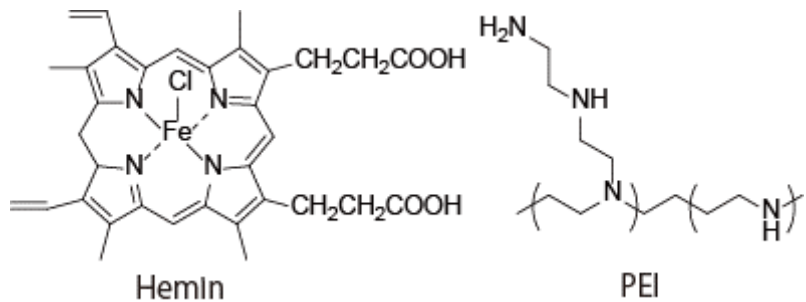
Figure 1. Preparation and decomposition of glucose-sensitive LbL films composed of hemin, DNA, and GOx.

2. Materials and Methods

2.1. Materials

Hemin and poly(ethyleneimine) (PEI) were obtained from Tokyo Chemical Industry Co. (Tokyo, Japan). DNA (calf thymus) was purchased from Nacalai Tesque Inc. (Tokyo, Japan). GOx was obtained from Sigma-Aldrich Chemical Co. (Wisconsin, USA). All other reagents used were of the highest grade and used without further purification.

H-PEI was synthesized as follows. PEI (100 mg) and hemin (37.9 mg) were dissolved in dimethyl sulfoxide, to which was added N-hydroxysuccinimide (8.02 mg) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (13.4 mg) at 4 °C. After 24 h, the reaction mixture was purified by dialyzing with water for 3 days and then freeze-dried. The content of hemin residues was 2.2% (molar ratio of hemin to primary amine), as determined by UV-vis absorption spectroscopy. Figure 2 shows the chemical structures of hemin, PEI, and H-PEI.



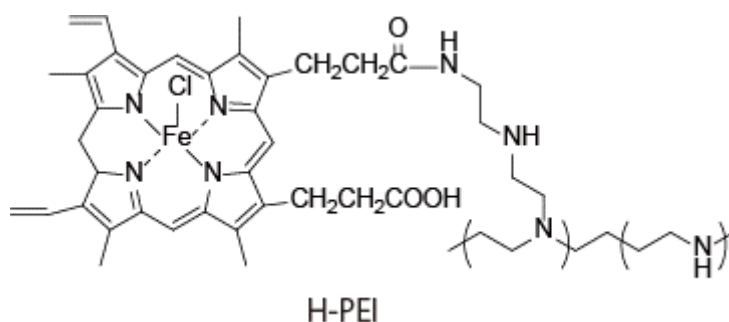


Figure 2. Chemical structures of hemin, PEI, and H-PEI.

2.2. Apparatus

A quartz crystal microbalance (QCM; eQCM 10M Garmry, Warminster, United Kingdom) was employed for gravimetric analysis of LbL films consisting of H-PEI and DNA. An 8 MHz AT-cut quartz resonator coated with a gold (Au) layer (surface area 0.2 cm²) was used as a probe, in which the adsorption of 1 ng of substance induced a -1.4 Hz change in the resonance frequency. The Au surface layer of the quartz resonator was cleaned using piranha solution (a mixture of H₂O₂ and H₂SO₄, 1:3 v/v) and thoroughly rinsed in pure water before use (CAUTION: piranha solution should be handled with extreme care). Atomic force microscopy (AFM) images were recorded using an AFM5200S (Hitachi High-Technologies Co., Tokyo, Japan) microscope. AFM images were acquired in contact mode at room temperature in air. UV-vis spectroscopy measurements were conducted using a V-560 (Jasco Co., Tokyo, Japan) spectrometer.

2.3. Preparation of LbL films

(H-PEI/DNA films were prepared on the cleaned quartz resonator for QCM analysis. The quartz resonator was immersed in 0.1 mg/mL H-PEI solution in 10 mM HEPES buffer containing 150 mM NaCl (pH 7.4) for 15 min to deposit the first H-PEI layer by the hydrophobic force of attraction. After being rinsed in buffer for 5 min to remove any weakly adsorbed H-PEI, the quartz resonator was immersed in 0.1 mg/mL DNA solution for 15 min to deposit DNA by electrostatic interaction. At that time, 0.1 mg/mL GOx was mixed in the DNA solution. The second H-PEI layer was deposited similarly on the surface of the quartz resonator. The deposition steps were repeated to build up LbL films. Circular glass slides (18 mm diameter) and quartz slides (50×9×1 mm) with LbL films were prepared in the same manner. UV-vis absorption spectra of the LbL films in the working buffer were recorded on a UV-vis spectrometer. For AFM observation, the circular glass slides used to prepare each of the (H-PEI/DNA+GOx)₅ films were rinsed with milli-Q water and dried for 24 h in a desiccator. AFM image were taken in AC mode using arrow NCR probe (TOYO Corporation, Tokyo, Japan) at room temperature in air.

2.4. Decomposition of LbL films

The H₂O₂-induced decomposition of the (H-PEI/DNA+GOx)₅ films was studied using UV-vis absorption spectroscopy. The LbL films prepared on quartz slides were exposed to 1, 10, and 100 mM H₂O₂ solution (pH 7.4) for 30, 60, 90, 120, 180, and 240 min and then rinsed with the working buffer for 5 min.

The glucose-induced decomposition of the (H-PEI/DNA+GOx)₅ films were monitored in the same manner. The LbL films prepared on quartz slides were exposed to 1, 10, and 100 mM glucose solution (pH 7.4) for 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24, 30, 35, and 48 h. The glucose-induced decomposition of the LbL films was studied by monitoring the resonance frequency change (ΔF) of the 5-bilayer film-coated quartz resonator in a flow-through cell of the QCM. All experiments were conducted at room temperature (ca. 20 °C).

3. Results and Discussion

Figure 3 shows the change in the resonance frequency (ΔF) of the QCM when H-PEI solution and the DNA/GOx mixed solution are immersed on the quartz resonator. The ΔF values decreased with deposition of both H-PEI and the DNA/GOx mixture, which indicated that the (H-PEI/DNA+GOx) film was successfully formed on the surface of the quartz resonator. From the QCM data, the amount of deposited LbL film was calculated to be $14.07 \pm 2.44 \mu\text{g cm}^{-2}$ for the (H-PEI/DNA+GOx)₅ film. It is considered that positively-charged H-PEI and negatively-charged DNA and GOx are deposited by electrostatic attraction, which builds up the LbL film on the quartz resonator surface. LbL films using DNA or GOx driven by electrostatic interactions have been reported by Zhang et al. [36] and Kakade et al. [37]. It is considered that DNA and GOx are both present in the negatively charged layers because DNA and GOx adsorb to positively charged polymers.

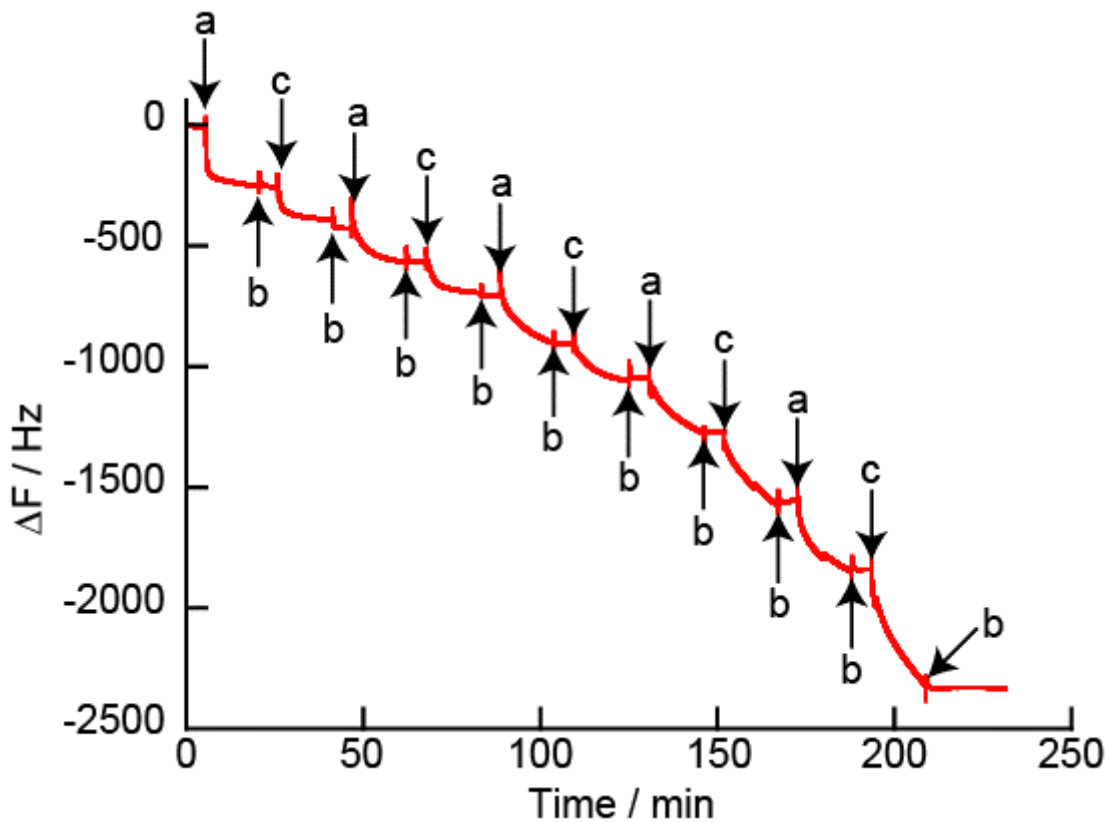


Figure 3. Change of the QCM frequency for the deposition of H-PEI/DNA+GOx)₅ film at pH 7.4. The resonator was exposed to (a) 0.1 mg/mL H-PEI, (b) 10 mM HEPES buffer solution, and (c) 0.1 mg/mL DNA (containing 0.1 mg/mL GOx).

Figure 4 shows an AFM image and a depth profile of a dried (H-PEI/DNA+GOx)₅ film. The circular glass slides (18 mm diameter) used to prepare each of the (H-PEI/DNA+GOx)₅ films were rinsed with milli-Q water and dried for 24 h in a desiccator. The thicknesses of the LbL films were determined by scratching the film-coated glass slide using a cutter and performing AFM depth profile scans over the scratch. The thicknesses of the (H-PEI/DNA+GOx)₅ films were estimated to be $45.68 \pm 15.95 \text{ nm}$.

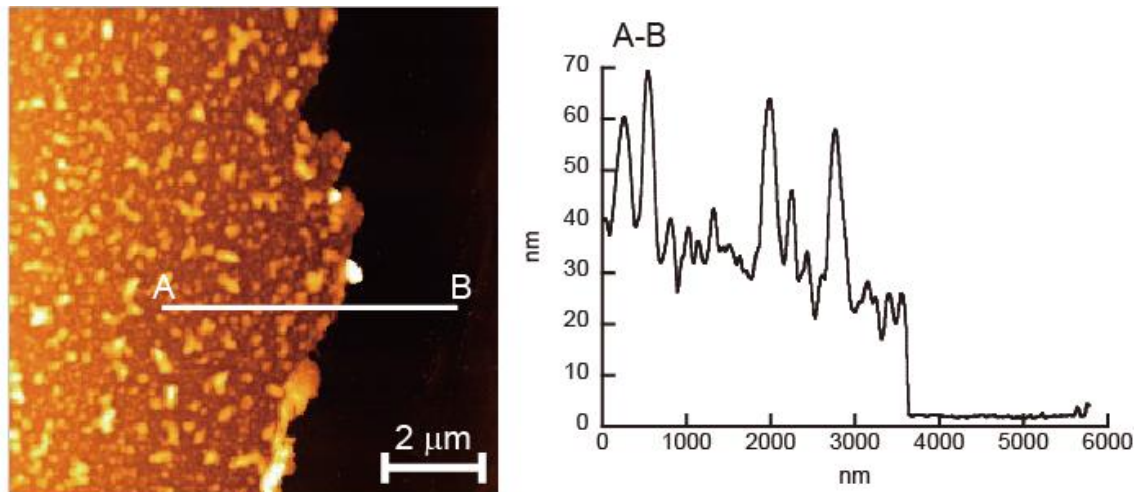


Figure 4. AFM image of the dried (H-PEI/DNA+GOx)₅ film.

Figures 5a and 5b show UV-vis absorption spectra for the (H-PEI/DNA+GOx)₅ film and exposure to 100 mM H₂O₂ solution in 10 mM HEPES buffer containing 150 mM NaCl (pH 7.4), respectively. The hemin solution has an absorption at 390 nm. H-PEI is adsorbed on the surface of the DNA layers, and the membrane has an absorption maximum derived from hemin. The disappearance of the absorption at 390 nm was confirmed after the (H-PEI/DNA+GOx)₅ film was exposed to 100 mM H₂O₂ solution for 29 h. The iron porphyrin contained in hemin produces ROS. The H₂O₂ causes the hemin to be degraded, so that the absorption maximum derived from hemin in the film was significantly reduced. The absorbance of the (H-PEI/DNA+GOx)₅ film at 260 nm also decreased when immersed in 100 mM H₂O₂ solution. The DNA solution has an absorption at 260 nm. If ROS are generated near the LbL films, then nonspecific cleavage of DNA is triggered. Yoshida et al. reported the decomposition of H-PEI/DNA nanofilm by H₂O₂ [25]. Similarly, decomposition of the (H-PEI/DNA+GOx)₅ film by H₂O₂ resulted in a decrease in the absorption maximum derived from DNA.

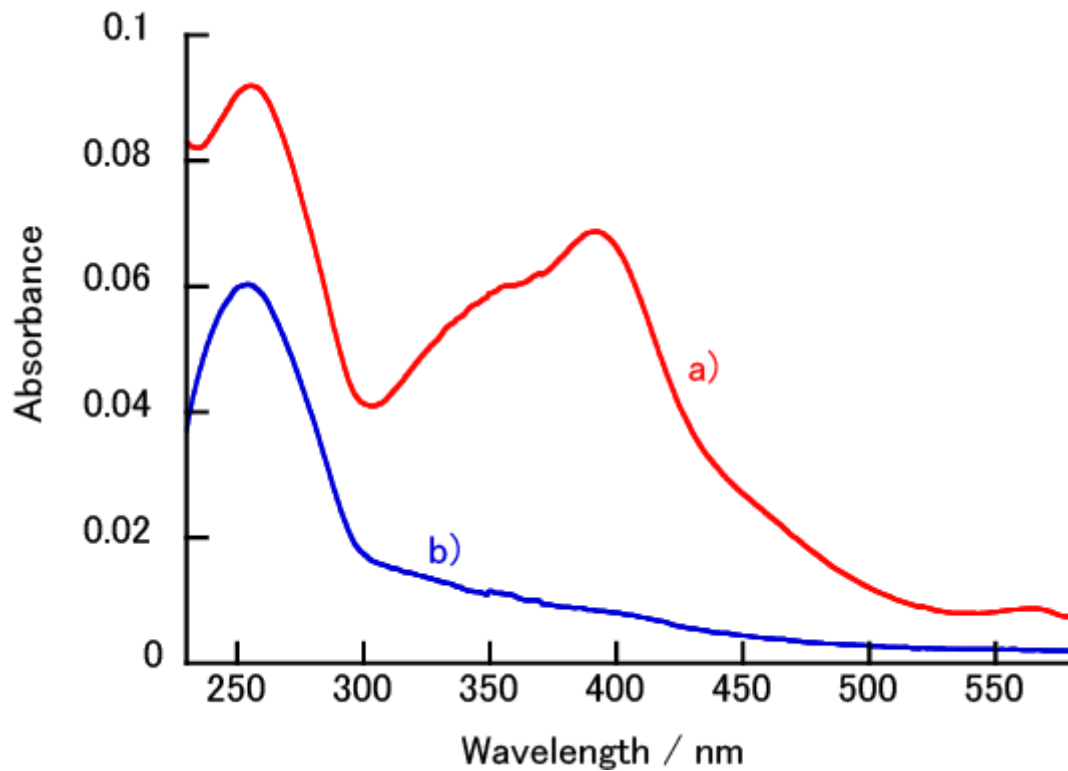


Figure 5. UV-vis absorption spectra for the (H-PEI/DNA+GOx)₅ film (a) before and (b) after exposure to 100 mM H₂O₂ solution (pH 7.4) for 29 h.

The H₂O₂-induced decomposition of (H-PEI/DNA+GOx)₅ films was investigated using UV-vis absorption spectroscopy (Figure 6). The extent of H₂O₂-induced decomposition of the LbL films was determined using Eq. (1).

$$\text{Decomposition (\%)} = \left(1 - \frac{\text{absorbance at 260 nm of (H-PEI/DNA+GOx)}_5 \text{ film when immersed in hydrogen peroxide solution for } t \text{ h.}}{\text{absorbance at 260 nm of (H-PEI/DNA+GOx)}_5 \text{ film}}\right) \times 100 \quad (1)$$

When (H-PEI/DNA+GOx)₅ films were exposed to 1 and 10 mM H₂O₂ solutions, it was not possible to confirm the decomposition of the LbL films. When the (H-PEI/DNA+GOx)₅ film was exposed to 100 mM H₂O₂ solution, the LbL film was ca. 35% decomposed after 4 h. DNA and GOx are present in the polyanion layers in the LbL film. While H₂O₂ causes DNA to be partially degraded by nonspecific cleavage, a portion of the LbL films composed by the electrostatic affinity between GOx and H-PEI remains. Therefore, the (H-PEI/DNA+GOx)₅ film was partially decomposed by H₂O₂.

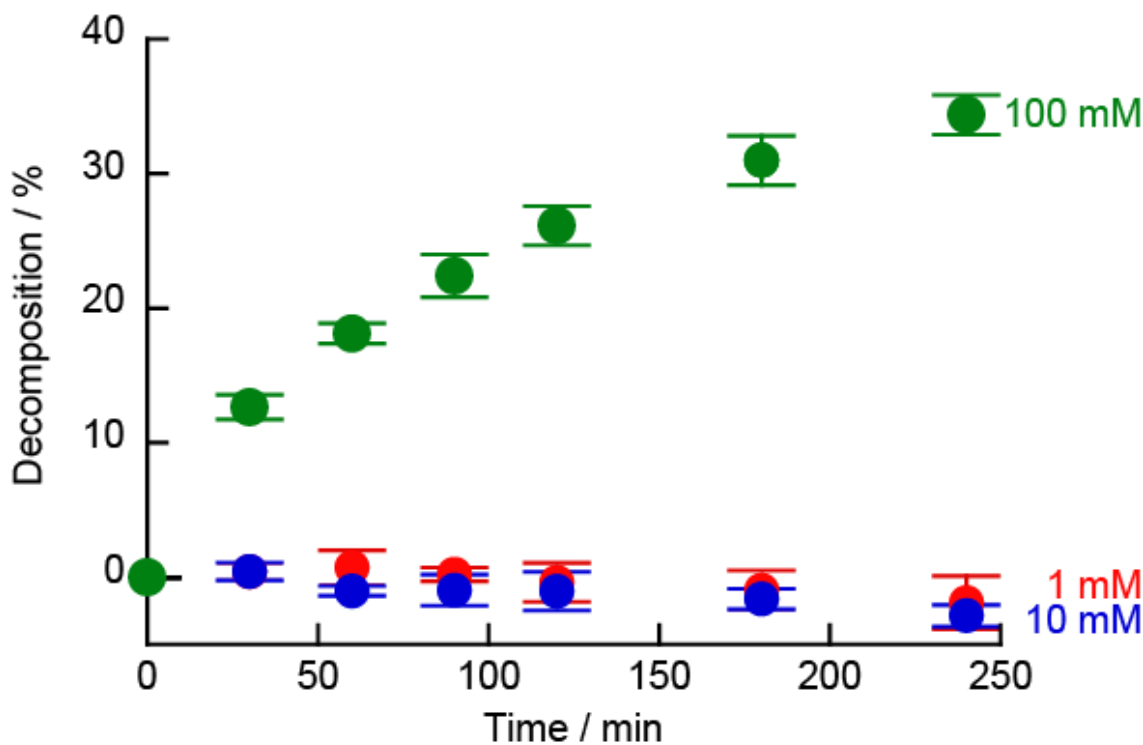


Figure 6. H₂O₂-induced decomposition of (H-PEI/DNA+GOx)₅ films investigated using UV-vis absorption spectroscopy. The LbL films were exposed to 1 mM (red), 10 mM (blue), and 100 mM (green) H₂O₂ solutions (pH 7.4) for up to 240 min.

The glucose-induced decomposition of (H-PEI/DNA+GOx)₅ films was investigated using the QCM (Figure 7). The extent of the decomposition was calculated from the change in the resonance frequency. When the (H-PEI/DNA+GOx)₅ film was exposed to 100 mM glucose solution for 24, 48 72, and 96 h, the extent of decomposition was 9.97%, 16.3%, 23.1%, and 30.5%, respectively. A longer immersion time in 100 mM glucose solution resulted in more decomposition of the LbL films. Hemin is an iron porphyrin molecule and the active cofactor for various enzymes such as catalase and peroxidase [38]. GOx present in the LbL films reacts with glucose to generate gluconic acid and H₂O₂. H₂O₂ present in the LbL film reacts with hemin to generate ROS. Nonspecific cleavage of DNA by ROS promotes partial degradation of the LbL film. The decomposition rate of the film immersed in the glucose solution was slower than that immersed in the H₂O₂ solution. GOx and glucose are enzymatic reactions; therefore, H₂O₂ is generated slowly and the degradation of the LbL film is delayed.

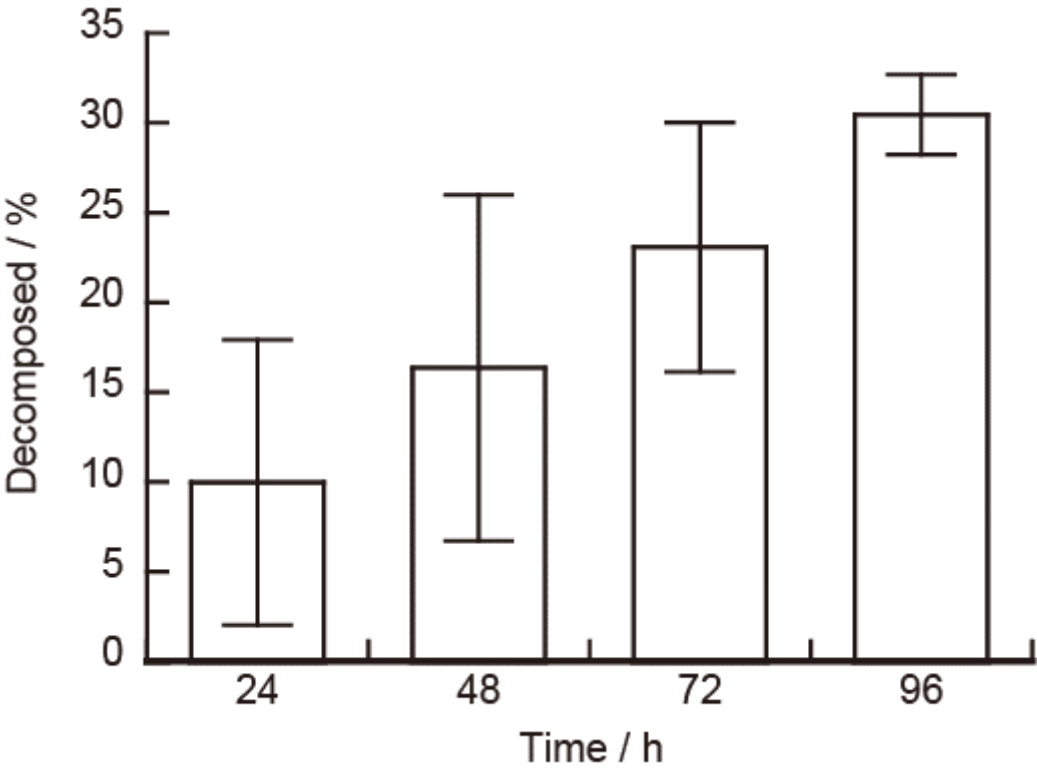


Figure 7. Glucose-induced decomposition of (H-PEI/DNA+GOx)₅ films was investigated using a QCM. The LbL film was exposed to 100 mM glucose solution (pH 7.4) for up to 96 h.

The glucose-induced decomposition of (H-PEI/DNA+GOx)₅ films was investigated using UV-vis spectroscopy (Figure 8). The glucose-induced LbL films were determined using Eq. (2).

$$\text{Decomposition (\%)} = \left(1 - \frac{\text{absorbance at 260 nm of (H-PEI/DNA+GOx)}_5 \text{ film when immersed in glucose solution for } t \text{ h.}}{\text{absorbance at 260 nm of (H-PEI/DNA+GOx)}_5 \text{ film}}\right) \times 100 \quad (2)$$

When (H-PEI/DNA+GOx)₅ film was exposed to 1, 10 and 100 mM glucose solutions for 48 h, the extent of decomposition was 23.9%, 29.8%, and 36.0%, respectively. Therefore, the rate of LbL film decomposition increased with the glucose concentration. At pH and ionic strength close to physiological conditions, it was possible to slowly decompose the LbL film at sub-millimolar glucose concentration. However, even when the LbL film was immersed in the glucose solution for a long time, more than half of the LbL film remained. This is the same result as that when immersed in the H₂O₂ solution. This suggests that the degradation of the LbL film is due to the DNA present in the thin film. The LbL film could be degraded with a sub- millimolar concentration of glucose solution but not with a sub- millimolar H₂O₂ solution. Most of the H₂O₂ produced by GOx could be present in the vicinity of the membrane, so that the film could be decomposed even in a sub- millimolar concentration of glucose solution.

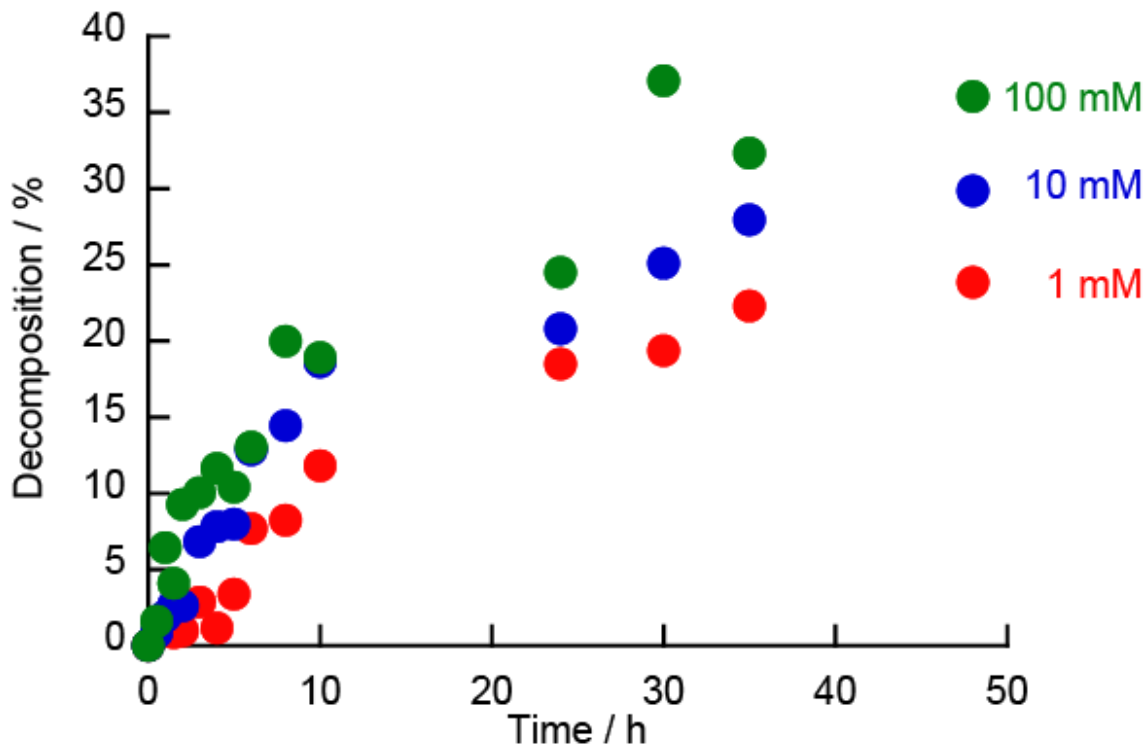


Figure 8. Glucose-induced decomposition of (H-PEI/DNA+GOx)₅ films investigated using UV-vis absorption spectroscopy. The LbL films were exposed to 1 mM (red), 10 mM (blue), and 100 mM (green) glucose solutions (pH 7.4) for up to 48 h.

4. Conclusions

(H-PEI/DNA+GOx)₅ LbL films were prepared by alternate immersion of a substrate in H-PEI solution and DNA solution (containing GOx). When the (H-PEI/DNA+GOx)₅ films were immersed in a H₂O₂ solution, partial decomposition of the LbL films was observed. The iron porphyrin in hemin produces more ROS from reaction with H₂O₂ [26,27]. The ROS causes non-specific DNA cleavage; therefore, decomposition of the LbL films composed of DNA was observed. Furthermore, partial degradation was observed when the membrane was immersed in a glucose solution. GOx present in LbL films produces H₂O₂ from glucose. These LbL films decomposed under physiological conditions with various glucose concentrations, which suggests that a glucose-stimuli-responsive nanofilm could be realized. We have developed other to glucose- and pH-responsive thin films [39,40]; however, the (H-PEI/DNA+GOx)₅ film has a very slow decomposition. If a drug could be encapsulated in this thin film, then there is a possibility that a system capable of drug release over a long time period, depending on the substrate, could be realized.

Author Contributions: K.Y. and K.S. designed the work. K.Y. and Y.K. collected the materials. K.Y. and Y.K. conducted experimental works on detailed LbL film data. T.K. and T.S. performed the AFM observations. K.Y., T.D., T.O. and K.S. were involved in providing experimental advice and operations. The manuscript was prepared by K.Y. and K.S.

Funding: This work was supported Kakenhi Grants-in-Aid (Nos. JP18K06791 and JP18K19936) from the Japan Society for the Promotion of Science (JSPS) and the Ohu University Research Fund.

Acknowledgments: This work was partially supported by Kakenhi Grants-in-Aid (Nos. JP18K06791 and JP18K19936) from the Japan Society for the Promotion of Science (JSPS). The Ohu University Research Fund is also acknowledged for financial support. The authors thank staff of Tsuruoka College for assisting with the AFM observations.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Decher, G.; Hong, J.D. Buildup of ultrathin multilayer films by a self-assembly process, 1 consecutive adsorption of anionic and cationic bipolar amphiphiles on charged surfaces. *Makromol Chem Macromol Symp* 1991, 46, 321–327.
2. Decher, G. Fuzzy Nanoassemblies: Toward layered polymeric multicomposites. *Science* 1997, 277, 1232–1237.
3. He, T.; Jańczewski, D.; Guo, S.; Man, S.M.; Jiang, S.; Tan, W.S. Stable pH responsive layer-by-layer assemblies of partially hydrolysed poly(2-ethyl-2-oxazoline) and poly(acrylic acid) for effective prevention of protein, cell and bacteria surface attachment. *Colloids Surf. B Biointerfaces* 2018, 161, 269–278.
4. Bellanger, H.; Casdorff, K.; Muff, L.F.; Ammann, R.; Burgert, I.; Michen, B. Layer-by-layer deposition on a heterogeneous surface: Effect of sorption kinetics on the growth of polyelectrolyte multilayers. *J. Colloid Interface Sci.* 2017, 500, 133–141.
5. Zhao, W.; Xu, J.J.; Chen, H.Y. Electrochemical biosensors based on layer-by-layer assemblies. *Electroanalysis* 2006, 18, 1737–1748.
6. Hoshi, T.; Anzai, J.; Osa, T. Controlled deposition of glucose oxidase on platinum electrode based on an avidin/biotin system for the regulation of output current of glucose sensors. *Anal. Chem.* 1995, 67, 770–774.
7. Dai, Z.; Wilson, J.T.; Chaikof, E.L. Construction of pegylated multilayer architectures via (strept)avidin/biotin interactions. *Mat. Sci. Eng. C* 2007, 27, 402–408.
8. Zelikin, A.N.; Becker, A.L.; Johnston, A.P.R.; Wark, K.L.; Turatti, F.; Caruso, F. A general approach for DNA encapsulation in degradable polymer microcapsules. *ACS Nano* 2007, 1, 63–69.
9. Shchukin, D.G.; Patel, A.A.; Sukhorukov, G.B.; Lvov, Y.M. Nanoassembly of biodegradable microcapsules for DNA encasing. *J. Am. Chem. Soc.* 2004, 126, 3374–3375.
10. Itoh, Y.; Matsusaki, M.; Kida, T.; Akashi, M. Enzyme-responsive release of encapsulated proteins from biodegradable hollow capsules. *Biomacromolecules* 2006, 7, 2715–2718.
11. Chinnayelka, S.; McShane, M.J. Glucose-sensitive nanoassemblies comprising affinity-binding complexes trapped in fuzzy microshells. *J. Fluoresc.* 2004, 14, 585–595.
12. Hoffman, K.; Tieke, B. Layer-by-layer assembled membranes containing hexacyclen-hexaacetic acid and polyethyleneimine N-acetic acid and their ion selective permeation behavior. *J. Membr. Sci.* 2009, 341, 261–267.
13. Huang, Z.; Li, M.; Li, N.; Tang, X.; Ouyang, Z. Antibacterial properties enhancement of layer-by-layer self-assembled nanofiltration membranes. *J. Nanosci. Nanotechnol.* 2018, 18, 4524–4533.
14. Sato, K.; Yoshida, K.; Takahashi, S.; Anzai, J. pH- and sugar-sensitive layer-by-layer films and microcapsules for drug delivery. *Adv. Drug Deliver. Rev.* 2011, 63, 809–821.
15. Choi, D.; Hong, J. Layer-by-layer assembly of multilayer films for controlled drug release. *Arch. Pharm. Res.* 2014, 37, 79–87.
16. Nolan, C.M.; Serpe, M.J.; Lyon, L.A. Thermally modulated insulin release from microgel thin films. *Biomacromolecules* 2004, 5, 1940–1946.
17. Donath, E.; Sukhorukov, G.B.; Caruso, F.; Davis, S.A.; Möhwald, H. Novel hollow polymer shells by colloid-templated assembly of polyelectrolytes. *Angew. Chem. Int. Ed.* 1998, 37, 2201–2205.
18. Mercato, L.L.D.; Ferraro, M.M.; Baldassarre, F.; Mancarella, S.; Greco, V.; Rinaldi, R.; Loporatti, S. Biological applications of LbL multilayer capsules: From drug delivery to sensing. *Adv. Colloid Interface Sci.* 2014, 207, 139–154.
19. Tong, W.; Song, X.; Gao, C. Layer-by-layer assembly of microcapsules and their biomedical applications. *Chem. Soc. Rev.* 2012, 41, 6103–6124.
20. Biesheuvel, P.M.; Mauser, T.; Sukhorukov, G.B.; Möhwald, H. Micromechanical theory for pH-dependent polyelectrolyte multilayer capsule swelling. *Macromolecules* 2006, 39, 8480–8486.
21. Kozlovskaya, V.; Sukhishvili, S.A. Amphoteric hydrogel capsules: multiple encapsulation and release routes. *Macromolecules* 2006, 39, 6191–6199.
22. Levy, T.; De'jugnat, C.; Sukhorukov, G.B. Polymer microcapsules with carbohydrate-sensitive properties. *Adv. Funct. Mater.* 2008, 18, 1586–1594.
23. Geest, B.G.D.; Jonas, A.M.; Demeester, J.; Smedt, S.C.D. Glucose-responsive polyelectrolyte capsules. *Langmuir* 2006, 22, 5070–5074.

24. Chinnayelka, S.; McShane, M.J. Microcapsule biosensors using competitive binding resonance energy transfer assays based on apoenzymes. *Anal. Chem.* 2005, 77, 5501–5511.
25. Prevot, M.; Déjugnat, C.; Mchwald, H.; Sukhorukov, G.B. Behavior of temperature-sensitive PNIPAM confined in polyelectrolyte capsules. *Chem. Phys. Chem.* 2006, 7, 2497–2502.
26. Selin, V.; Ankner, J.F.; Sukhishvili, S.A. Diffusional response of layer-by-layer assembled polyelectrolyte chains to salt annealing. *Macromolecules* 2015, 48, 3983–3990.
27. Schmidt, D.J.; Moskowitz, J.S.; Hammond, P.T. Electrically triggered release of a small molecule drug from a polyelectrolyte multilayer coating. *Chem. Mater.* 2010, 22, 6416–6425.
28. Travascio, P.; Li, Y.S.D. DNA-enhanced peroxidase activity of a DNA-aptamer-hemin complex. *Chem. Biol.* 1998, 5, 505–517.
29. Yao, Y.; Mao, Y.; Huang, Q.; Wang, L.; Huang, Z.; Lu, W.; Chen, W. J. *Hazard. Mater.* 2014, 264, 323–331.
30. Jiang, B.; Yao, Y.; Xie, R.; Dai, D.; Lu, W.; Chen, W.; Zang, L. *Appl. Catal. B: Environ.* 2016, 183, 291–297.
31. Breen, A.P.; Murphy, J.A. Reactions of oxyl radicals with DNA. *Free Radical Bio. Med.* 1995, 18, 1033–1077.
32. Hertzberg, R.P.; Dervan, P.B. Cleavage of DNA with methidiumpropyl-EDTA-iron(II): Reaction conditions and product analyses. *Biochemistry* 1984, 23, 3934–3945.
33. Sigman, D.S. Nuclease activity of 1,10-phenanthroline-copper ion. *Acc. Chem. Res.* 1986, 19, 180–186.
34. Kawanishi, S.; Oikawa, S.; Murata, M.; Tsukitome, H.; Saito, I. Site-specific oxidation at GG and GGG sequences in double-stranded DNA by benzoyl peroxide as a tumor promoter. *Biochemistry* 1999, 38, 16733–16739.
35. Drechsel, D.A.; Patel, M. Role of reactive oxygen species in the neurotoxicity of environmental agents implicated in Parkinson's disease. *Free Radical Bio. Med.* 2008, 44, 1873–1886.
36. Liou, G.Y.; Storz, P. Reactive oxygen species in cancer. *Free Radical Res.* 2010, 44, 479–496.
37. De Gracia, L.C.; Joshi-Barr, S.; Nguyen, T.; Mahmoud, E.; Schopf, E.; Fomina, N.; Almutairi, A. Biocompatible polymeric nanoparticles degrade and release cargo in response to biologically relevant levels of hydrogen peroxide. *J. Am. Chem. Soc.* 2012, 134, 15758–15764.
38. Rehor, A.; Hubbell, J.A.; Tirelli, N. Oxidation-sensitive polymeric nanoparticles. *Langmuir* 2005, 21, 411–417.
39. Broaders, K.E.; Grandhe, S.; Fréchet, J.M. A biocompatible oxidation-triggered carrier polymer with potential in therapeutics. *J. Am. Chem. Soc.* 2011, 133, 756–758.
40. Yoshida, K.; Awaji, K.; Shimizu, S.; Iwasaki, M.; Oide, Y.; Ito, M.; Dairaku, T.; Ono, T.; Kashiwagi, Y.; Sato, K. Preparation of microparticles capable of glucose-induced insulin release under physiological conditions. *Polymers* 2018, 10, 1164.
41. Inoue, H.; Anzai, J. Stimuli-sensitive thin films prepared by a layer-by-layer deposition of 2-iminobiotin-labeled poly(ethyleneimine) and avidin. *Langmuir* 2005, 21, 8354–8359.
42. Pasternack, R.F.; Goldsmith, J.I.; Szép, S.; Gibbs, E.J. A spectroscopic and thermodynamic study of porphyrin/DNA supramolecular assemblies. *Biophys. J.* 1998, 75, 1024–1031.
43. Suenaga, H.; Nango, M.; Sugiyama, T.; Iwasaki, T.; Takeuti, Y.; Shinkai, S. DNA cleavage by a polyethylenimine-appended porphyrin. *Chem. Lett.* 2000, 29, 306–307.
44. Sato, K.; Takahashi, M.; Ito, M.; Abe, E.; Anzai, J. H₂O₂-induced decomposition of layer-by-layer films consisting of phenylboronic acid-bearing poly(allylamine) and poly(vinyl alcohol). *Langmuir* 2014, 30, 9247–9250.
45. Sato, K.; Awaji, K.; Ito, M.; Anzai, J. Preparation of H₂O₂-induced poly(amidoamine) dendrimer release nanofilms. *Colloid Polym. Sci.* 2017, 295, 877–882.
46. Sato, K.; Takahashi, M.; Ito, M.; Abe, E.; Anzai, J. Glucose-induced decomposition of layer-by-layer films composed of phenylboronic acid-bearing poly(allylamine) and poly(vinyl alcohol) under physiological conditions. *J. Mat. Chem. B* 2015, 3, 7796–7802.
47. Sato, K.; Shimizu, S.; Awaji, K.; Hitomi, O.; Anzai, J. Lactate-induced decomposition of layer-by-layer films composed of phenylboronic acid-modified poly(allylamine) and poly(vinyl alcohol) under extracellular tumor conditions. *J. Colloid Inter. Sci.* 2018, 510, 302–307.