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Covalent attachment of deoxyribonucleic acid (DNA) to diazo-resin (DAR) in self-assembled multilayer films

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Summary

Construction of covalently attached multilayer films was achieved by irradiating layer-by-layer self-assembled multilayer films of DNA and DAR with ultraviolet (UV) light.

Introduction

Due to its biological characteristics, DNA is currently exploited for the tumor gene detection, gene therapy and various biosensors [1]. As a kind of biomolecular materials with special polyanionic double-helix structure, DNA can be electrostatically incorporated into simple chemical microenvironments, especially into a self-assembled film. This has received considerable attention and a lot of efforts have been paid for its functionalization. An aligned cast film of electrostatically capped DNA-lipid complex presented novel properties of anisotropic electric conductivity and ability to capture some drugs [2]. But it is difficult to construct alternate multilayers with multi-components by means of casting films.

Consecutively alternating self-assembling provided a convenient technique and was widely applied in preparation of multilayers with multi-components [3]. This method has also been employed for the fabrication of multilayers of DNA and polyelectrolytes [4]. Liu [5] studied the interaction between DNA in multilayers and low-weight dye molecules in solution. However, DNA is easy to be desorbed in some salt solutions, which will restrict its practical application in assembled systems. So, it is necessary to strengthen the stability of the multilayers. A novel approach to improve the stability is to fabricate covalently attached multilayer films through the photoreaction between the adjacent layers in the multilayers. Zhang [6] et al showed confidently covalent connection between DAR and PAA, PSS and some dye anions in self-assembling multilayers, respectively, and their stability was greatly strengthened in inorganic salt solution. In order to obtain a stable composite multilayer of DNA and polyelectrolytes, herein we reported the preparation of covalently attached alternate multilayers of DNA and DAR through UV light irradiation. The stability of the multilayer was confirmed by etching it in aqueous salt solution.

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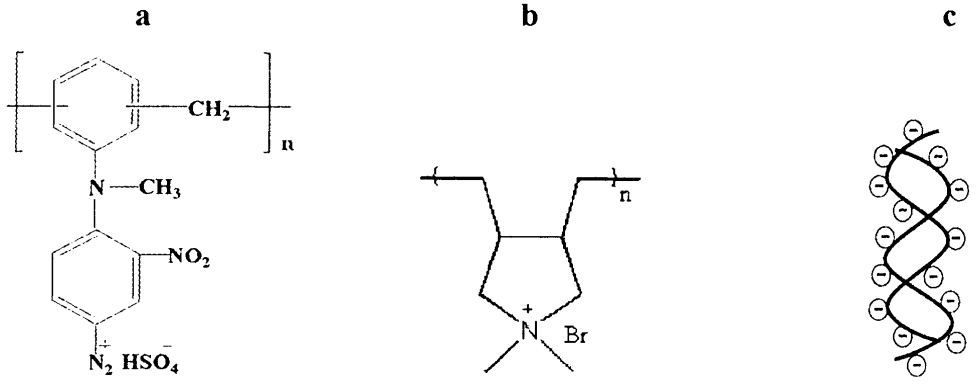
Experimental

Materials

The following chemicals were used as supplied. Poly(diallyldimethylammonium chloride) (PDDA) (Sheme 1.)was purchased from Aldrich(Mw. ca. 10000-20000). Polycationic DAR was kindly provided by Cao WX [7]. The average molecular weight of the DAR was ca. 2500. Salmon sperm DNA Sodium Salt (DNA-Na^+) fragment was purchased from AMRESCO and used without any further purification. Deionzed water was used and the pH value of the DNA solution and the rinsed water were adjusted to 3.0.

Scheme 1. Structures of materials used in the deposition process. (a) DAR; (b) PDDA; (c) DNA



Instruments

UV-Visible spectra were obtained by using a Shimadzu 3100 PC spectrophotometer. Fourier transform infrared (FT-IR) spectra were collected at a 4 cm^{-1} resolution on a Bruker IFS66V FT-IR spectrometer equipped with a MCT detector, and typically 2048 interferograms were coadded to yield spectra in a high signal-to-noise ratio. For the Grazing-reflection-absorption FT-IR (GRA-FTIR) measurement, a reflection attachment (Spectra-Tech. FT-80 RAS) was employed at the incident angle of 78°, together with a JEOL IROPT02 polarizer. A 16 w medium mercury lamp (with a filter at 365 nm) was used to irradiate the films at a distance of 6.5 cm.

Multilayer construction and covalently attached connection

Quartz wafer used to assemble DNA/DAR was rinsed as our previous report [6]. They were cleaned and coated with PDDA by immersing the plate into a 1 % aqueous PDDA solution for 20 min, rinsed with deionic water and dried by pure nitrogen. The covalently attached DNA films on the substrate were fabricated as following method. Dipped the PDDA-coated substrate alternately into aqueous solutions of DNA (1 mg/ml and pH = 3.0) and DAR (1 mg/ml) for 20 min, with intermediate deionic water (pH = 3.0) rinsing and N_2 drying. Repeating these two steps in a cyclic fashion could form multilayer films. Next, the above-fabricated films were exposed under UV light (wavelength = 365 nm) for a given time to ensure that the photoreaction proceeded completely. In this way, the partially covalently attached multilayer films were obtained. The deposition process of the films was conducted in the dark to avoid the decomposition of the DAR.

Results and Discussion

Figure 1 showed the UV-Vis spectra of 2, 4, 6, 8 and 10 layer pairs of DNA/DAR

assembled on a quartz wafer (based on DAR), respectively. The absorption band at 380 nm could be assigned to the contribution of π - π * transition of the diazonium group of DAR. As shown in the inset of Figure 1, the linear increasing

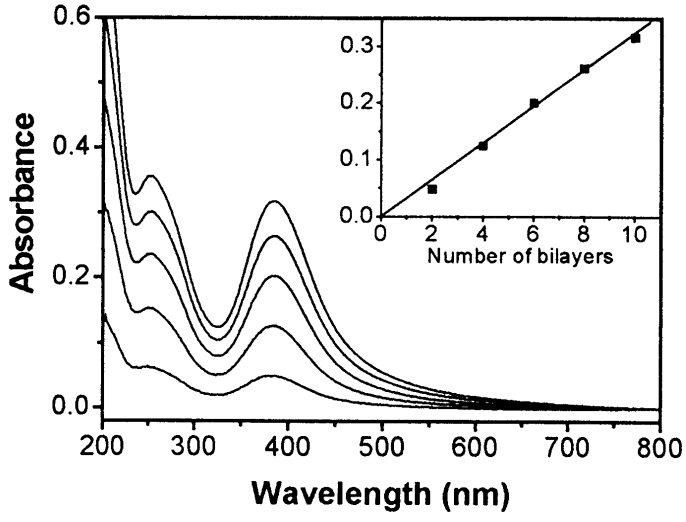


Figure 1. UV-Vis absorption spectra of multilayer films of DNA/DAR with the number of 2,4,6, 8 and 10 layer pairs (based on DAR) from the bottom to up, respectively. The inset shows linear increasing of the absorbance at 380 nm with the number of layer pairs.

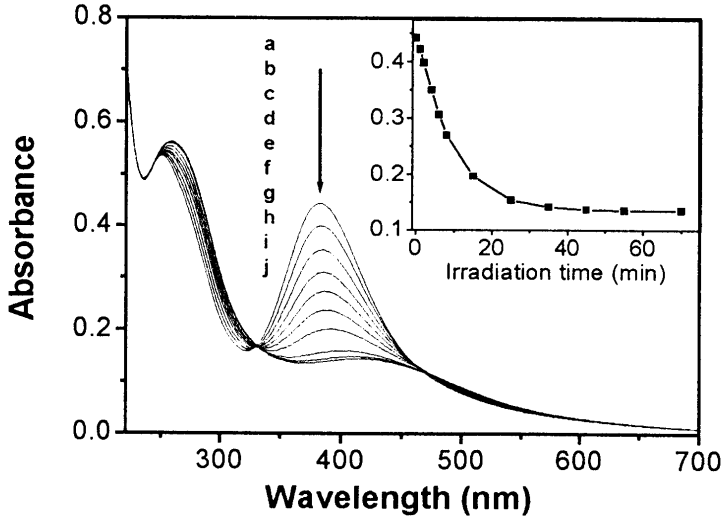


Figure 2. UV-Vis absorption spectra of 11 layer pairs of DNA/DAR upon UV light irradiation (wavelength = 365 nm) at a distance of 6.5 cm for (a) 0 min, (b) 2 min, (c) 4 min, (d) 6 min, (e) 8 min, (f) 10 min, (g) 15 min, (h) 25 min, (i) 35 min and (j) 45 min. The inset shows the absorbance change at 380 nm with irradiation time.

of the absorbance at 380 nm with the number of layer pairs indicated an even progressive deposition.

Then, the above-fabricated films were exposed under UV light for a certain time to ensure that the photoreaction proceeded completely. In order to avoid the condensation and cross-linking of DNA, the UV light with wavelength at 365 nm was selected. After irradiation with a 16 w medium power mercury lamp at a

distance of 6.5 cm to the assembled film containing 11 layer pairs of DNA/DAR, Figure 2 showed a dramatic change in UV-Vis spectra against irradiation time, from which we could clearly note that the absorbance at 380 nm decreased due to the decomposition of the diazonium group and the change tended to be limited within 35 min (shown as the inset of figure 2). Similar to the photoreaction of diazonium with sulfonate and carboxylate [6], we considered here that DAR covalently attached to DNA through the photoreaction of diazonium in DAR with phosphate in DNA. The isosbestic points appeared at 331 and 244 nm supported the speculation.

FT-IR was employed to further confirm the change taking place in the film before and after irradiation. Figure 3 showed GRA-FTIR spectra of a multilayer film containing 6 layer pairs of DNA/DAR, which deposited on gold-evaporated glass slide that pre-coated with polycationic PDDA. For the specimen before irradiation, two quite strong vibration bands appeared at 2246 and 2216 cm^{-1} could be assigned to the asymmetric and symmetric stretching modes of diazonium, respectively, as presented in Figure 3 (a) [8]. And bands at 1591 cm^{-1} and 1134 cm^{-1}, which were attributed to the stretching mode of phenyl group containing

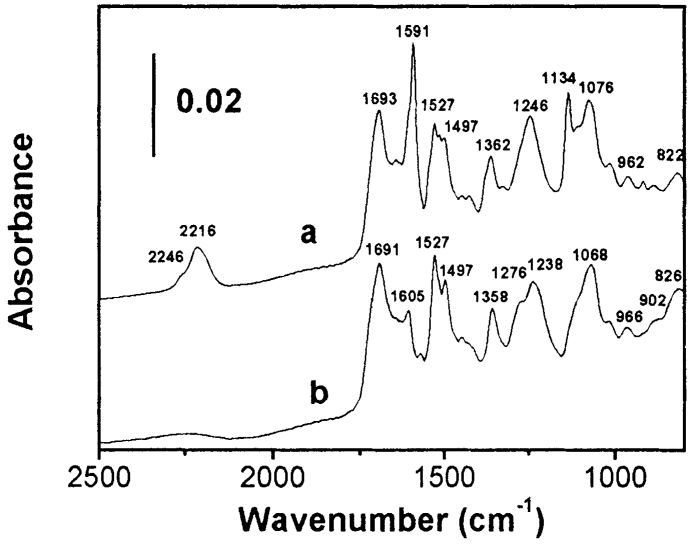
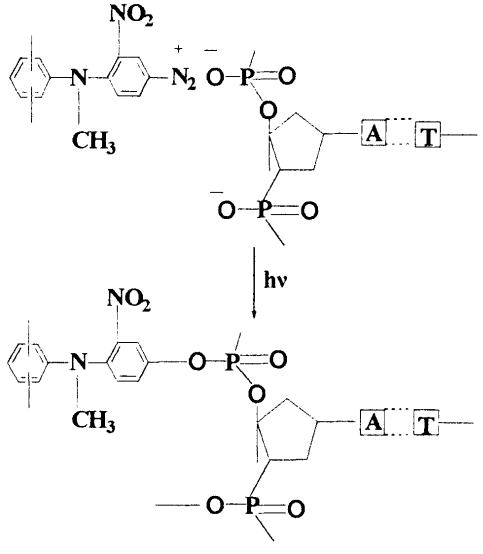


Figure 3. GRA-FTIR spectra of 6 layer pairs before (a) and after (b) UV irradiation on gold-evaporated glass slide.

substituted diazonium group in DAR [6] and N–O stretching mode between diazonium and phosphate groups due to strong ionpair effect ($—N{\equiv}N^{+}{\rightarrow}PO_4^{-}$) [9], respectively. However, after irradiation, the former shifted to 1605 cm^{-1} and the latter disappeared completely as shown in Figure 3 (b), indicating the decomposition of the diazonium groups in the film, which was consistent with the change in UV-Vis spectra. Furthermore, for the vibrations of phosphorus in DNA, a quite strong band, appeared at 1246 cm^{-1} before irradiation, could be assigned to the P=O stretching, which were separated into two bands at 1276 and 1238 cm^{-1} after the irradiation. This result implied the influence of irradiation to DNA. These two bands were corresponding to the stretching mode of P=O bonded to the aromatic ring and the O—Phenyl stretching mode of pentavalent phosphate aromatic ester, respectively [10]. Thus, we could conclude that DNA was photo-polymerized to DAR in solid multilayer film. Actually, there was a possibility that the nitrophenyldiazonium moiety reacted with other sites of DNA.

But in our case, it should mainly react with phosphate moiety. The reason was that on one hand, the multilayer film was constructed by employing electrostatic interaction as driving force, which associated cationic nitrophenyldiazonium moiety and anionic phosphate moiety between adjacent layers more tightly. On the other hand, the reaction took place in solid layer-by-layer self-assembled multilayer film. In addition, the reaction was carried out immediately after the film preparation in order to avoid other by-reactions as possible as could be done. We compared the stability of the multilayer films before and after irradiation by immersing the corresponding films into a 20 % aqueous NaCl solution. The structure of the multilayers in this investigation was DNA/DAR/DNA/DAR/DNA by alternate deposition on PDDA modified quartz plate. Through UV-Vis absorption spectra [11], we found that, before irradiation treatment, the absorbance at 260 nm decreased during etching the multilayer and reached a steady value in 20 min, and the reducing percent based on the total DNA was estimated to be about 30 %. This suggested that almost all the surface DNA layer desorbed in the aqueous salt solution within 20 min. However, for the irradiated film, after immersing into the same solution for the same time, the decrease of absorbance at 260 nm was less than 1 %, showing the stability was greatly improved.

Scheme 2. Schematic representation for the linkage between DNA and DAR upon UV irradiation.



Conclusion

In summary, upon UV irradiation, photoreaction took place between the phosphate moiety of DNA and diazonium moiety of DAR and resulted in the covalent connection between them in the alternate deposited multilayer film as shown in Scheme 2. The covalently attached film greatly increased its stability in some electrolyte solutions and this might provide a simple and efficient way to stabilize DNA multilayers.

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