Dose effects in electron beam irradiation of DNA-complex thin films

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Electron beam irradiation of double-stranded DNA (dsDNA)-surfactant thin films was investigated. Irradiation caused dissociation, leading to increasing thin film solubility in water and degradation of dsDNA. These two effects produced a maximum concentration of dsDNA in aqueous solution at 400 $\mu C/cm^2$ dose. These properties resulted in dual-mode resist characteristics of the DNA-surfactant films. At low dose, the DNA films functioned as positive resist while at high dose they worked as negative resist. The transition between the two regimes also occurred at 400 $\mu C/cm^2$. This implies that the cross-linking process (typical for negative resists) first requires the dissociation of the DNA-surfactant complex. © *2010 American Institute of Physics*. [doi:10.1063/1.3478227]

DNA biopolymers have attracted considerable interest[1–6] in fields ranging from biotechnology to nanoscience. Due to its molecular structure, DNA exhibits unique properties both in liquid and solid form.[7–12] The DNA-surfactant complex,[13,14] formed by mixing marine derived DNA solution with the surfactant cetyltrimethylammonium chloride (CTMA-Cl) solution and extracted as a precipitate, can be formed into thin films by spin coating or thermal evaporated,[15] enabling incorporation into high performance organic light emitting diodes (OLEDs). In the OLED structure, the DNA thin films are continuous, with no local patterning required. Indeed, no practical approaches for patterning DNA thin films for fabrication of microelectronic and photonic devices existed until the recent report[16] of DNA-CTMA direct patterning by electron beam (e-beam) irradiation. The irradiated DNA films were shown to function as either positive or negative resist depending on the solvent type used for development.

Radiation, including ions,[17] x-rays,[18,19] low energy[20] (3–20 eV) and ultrahigh energy[21] electrons (1 MeV) can induce mutation of DNA in solution by breaking the double helix. Possible mechanisms[16] in the irradiation and dissolution of DNA-CTMA solid thin films include the following: (1) e-beam dissociation of hydrophobic DNA-CTMA into DNA+CTMA (both hydrophilic); (2) DNA-CTMA molecules charged by electrons, becoming hydrophilic; (3) broken CTMA alkyl chains, damaging the hydrophobic tail; (4) DNA molecules broken into smaller, water soluble, fragments.

This paper explores the effect of electron irradiation dose on optical properties of DNA-CTMA in both liquid and solid form. The results provide additional insight into the irradiation/dissolution mechanisms and an improved process for the direct patterning of DNA films.

The process of extraction and purification of salmon sperm DNA, DNA-CTMA complex formation, and DNA (200 kDa)-CTMA thin film preparation have been previously reported.[14,16] While DNA is only soluble in water, the DNA-surfactant reaction precipitates from aqueous solution but is soluble in organic solvents. Most irradiations were performed using a 5 kV electron gun and currents from a few microamperes to 40 μA. The lithography experiments were carried out using a Raith 150 e-beam lithography system operating at 5 and 10 kV. The clearing dose was obtained using a matrix array of 20 μm circles.

Optical transmission and circular dichroism (CD) experiments were performed on thin film samples. Optical absorption and photoluminescence using double-stranded DNA (dsDNA) labels[22] (Picogreen™) were performed by dissolving the thin films in liquids. CD measures the chirality of the DNA-CTMA films, indicating the relative amount of dsDNA.[23] After e-beam irradiation, the films were immersed in deionized water or butanol for 15 min. It was previously reported[16] that K_2CO_3 aqueous solution produced the highest contrast in developing e-beam irradiated DNA. However, water was used in this study because its slower development rate gives much better control when preparing liquid samples.

Figure 1 shows the effect of e-beam irradiation on the DNA-CTMA optical properties in both solid form and in solution. Optical transmission of e-beam irradiated and unexposed DNA-CTMA thin films is shown in Fig. 1(a). Transmission spectra exhibit an ultraviolet (UV) absorption peak at ~260 nm, which is the wavelength normally associated with the DNA bases.[24] The unexposed DNA-CTMA film has the lowest 260 nm transmission (highest absorption). The transmission at 260 nm increases very quickly with dose, reaching a saturation level at ~100 $\mu C/cm^2$. Film transmission at 260 nm remains high over a wide range of doses (measured up to 1600 $\mu C/cm^2$).

It is reasonable to assume that in thin film form, the 260 nm absorption strength of dsDNA is related with the number of base pairs. Thus, the results indicate that after irradiation, a portion of the base pairs were separated making the film more transparent at 260 nm. The number of affected base pairs increases with dose and reaches saturation at ~100 $\mu C/cm^2$. The slight decrease in film transmission beyond this dose is likely caused by the onset of cross-linking. The crossed-linked DNA material has a broad absorption in the UV region, as seen for the dose of 1600 $\mu C/cm^2$.

Figure 1(b) shows the photoluminescent (PL) characteristics obtained from Picogreen™ in solutions of irradiated DNA-CTMA by pumping with a 488 nm Ar laser. The solutions were prepared by first irradiating the DNA-CTMA thin film, followed by dissolving the irradiated DNA complex in

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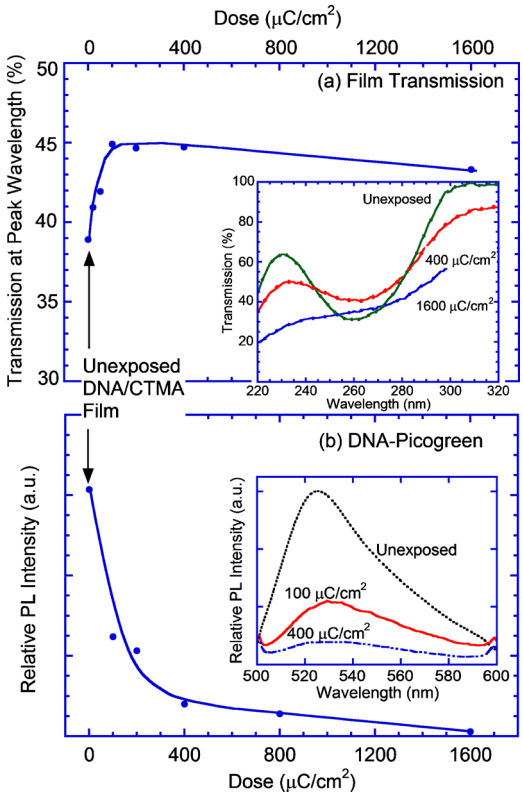

FIG. 1. (Color online) Characteristics of 5 kV e-beam irradiated DNA-CTMA as a function of electron dose: (a) thin film transmission intensity at 260 nm; inset: near-UV transmission spectra; (b) normalized peak photoluminescence intensity at 525–530 nm for DNA-CTMA thin films dissolved in either butanol (unexposed) or water (e-beam irradiated) solutions containing the dsDNA intercalating dye Picogreen™; inset: PL spectra for the unexposed and irradiated films.

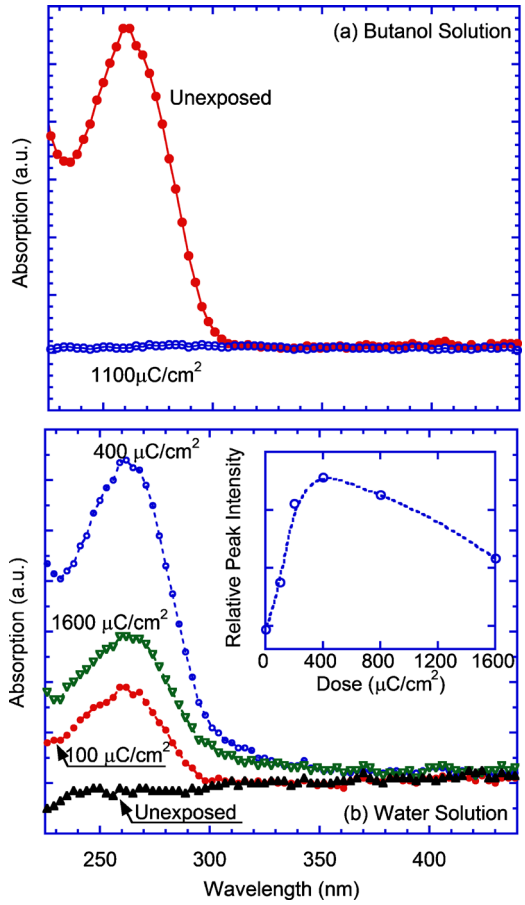

FIG. 2. (Color online) Absorption spectra comparison of 5 kV e-beam irradiated DNA-CTMA thin films dissolved in: (a) butanol; (b) water; inset: normalized absorption peak intensity as a function of electron dose.

water. The unexposed DNA-CTMA was dissolved in butanol. The PL intensities were normalized to the DNA absorption strength at 260 nm to yield a fair comparison, since not all of the irradiated DNA-complex can dissolve in water before reaching the clearing dose. Green emission peaking at ~530 nm was observed from the unexposed DNA-CTMA butanol solution. This indicates that the double helix nature of the material remains unaffected by the thin film formation and dissolution process. The PL intensity at 530 nm decreases rapidly with dose. At doses >400 $\mu C/cm^2$, the green emission becomes quite weak, slowly decreasing to zero emission at ~1600 $\mu C/cm^2$.

Figure 2 shows the absorption spectra for DNA-CTMA solutions. e-beam irradiated and unexposed DNA-CTMA thin films were immersed in butanol and water. It was observed that butanol dissolves the unexposed DNA-CTMA very quickly but does not dissolve the e-beam irradiated material. This is illustrated in Fig. 2(a), which shows that the solution obtained from the unexposed DNA-CTMA has strong absorption at 260 nm, whereas the solution with the irradiated DNA-CTMA has no absorption.

This phenomenon is reversed when using water as solvent: water dissolves the irradiated DNA-CTMA to an extent depending on dose, whereas it does not dissolve the unexposed DNA-CTMA. As expected, the water solution obtained from the unexposed DNA-CTMA film shows no absorption over the entire wavelength range [see Fig. 2(b)]. However, aqueous solutions obtained from irradiated DNA-CTMA films show a clear absorption peak at ~260 nm. The 260 nm absorption increases very quickly with dose and reaches a maximum at ~400 $\mu C/cm^2$, beyond which it decreases slowly with increased dose [see Fig. 2(b) inset].

Comparison of the thin film absorption of Fig. 1(a) and the absorption of aqueous solutions of Fig. 2(b) is complex. The absorption in thin films [Fig. 1(a)] is due to the combined effect of materials in the film, including dissociated DNA-CTMA complex, undissociated complex and possible cross-linked material. The nearly constant absorption at 260 nm for doses >100 $\mu C/cm^2$ implies a relatively constant number of base pairs. On the other hand, the absorption of irradiated DNA/CTMA dissolved in aqueous solution is a function of the conversion efficiency of DNA/CTMA to dsDNA and of the solubility of the irradiated product in water. Therefore, the initial sharp increase in absorption peak [shown in the inset of Fig. 2(b)] is due to production of dsDNA (which is water soluble), while the later decrease in absorption with dose is due to an increasing fraction of cross-linked insoluble material. The maximum absorption at 400 $\mu C/cm^2$ indicates the condition at which the dsDNA concentration in water is highest. As shown later, this coincides with the clearing dose value observed in lithography experiments.

Figure 3 shows CD spectra of DNA-complex thin films irradiated with different doses. The unexposed DNA-CTMA film exhibits characteristic CD spectral features of DNA in the UV region: a negative lobe at 250 nm and a positive lobe at 280 nm. This suggests the unexposed DNA-CTMA is a

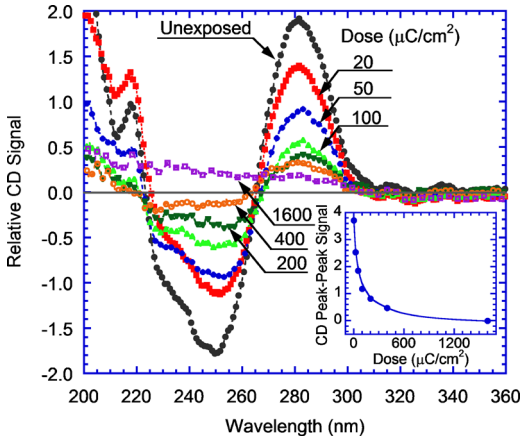

FIG. 3. (Color online) CD spectra for DNA-CTMA thin films irradiated with 5 kV electrons at different doses.

B-type double helix. Previous CD measurements[23] have shown that while the reaction of DNA with CTMA does produce some shift in the CD spectrum, the resulting structure is the same in thin film form and in solution. Current results show that the e-beam irradiated DNA-CTMA films maintain the same spectral characteristics. However, irradiating the DNA-CTMA film clearly affects the chirality of the material, with both positive and negative lobes decreasing with electron dose. The intensity of the CD signal (Fig. 3 inset) decreases rapidly even at low doses and becomes very weak at 400 $\mu C/cm^2$. When the dose increases to 1600 $\mu C/cm^2$, the CD signal completely disappears.

It is instructive to note the similarity between the results of the CD measurements on thin films (Fig. 3 inset) and the PL results obtained with the Picogreen™ solutions [Fig. 1(b)]. The similarity indicates that the amount of dsDNA dissolved in aqueous solution is proportional to the amount of undamaged DNA remaining in the thin film after irradiation.

Resist parameters contrast (γ) and clearing dose were obtained for irradiated DNA-CTMA thin films developed in water. D_{80} is the dose where 80% of the material is removed upon development, while the clearing dose occurs when all the initial material is removed. Remaining film thickness versus dose is shown in Fig. 4. Starting from a low irradiation

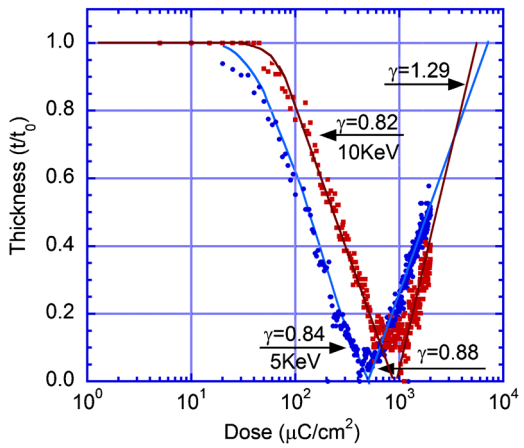

FIG. 4. (Color online) Contrast curves for DNA-CTMA resist development: remaining DNA-CTMA film thickness as a function of dose for 5 and 10 kV electrons.

dose, the DNA/CTMA films acted as a positive resist. The D_{80} and clearing doses increased from ~240 and ~400 $\mu C/cm^2$ for 5 kV to ~500 and ~900 $\mu C/cm^2$ at 10 kV. This is likely due to the longer range of the higher voltage electrons depositing energy outside the DNA/CTMA films. The contrast for these two conditions was very close at ~0.82–0.84. For doses larger than the clearing dose (400 and 900 $\mu C/cm^2$) for both electron energies, the resist process is reversed with films becoming increasingly insoluble in water or butanol. The γ for the 5 kV and 10 kV conditions are 0.88 and 1.29, respectively. The most likely explanation for this is that the higher doses lead to DNA cross-linking and the resulting increase in molecular weight leads to the decreasing solubility in either aqueous or organic solvents.

The dose effect in electron beam irradiation on DNA-CTMA thin films has been investigated. The e-beam irradiation process appears to induce dissociation of the DNA-CTMA complex, leading to increasing thin film solubility in water. Irradiation also causes the degradation of the DNA polymer. These two effects combine to produce a maximum concentration of dsDNA in aqueous solution at an intermediate dose of 400 $\mu C/cm^2$. These properties resulted in dual-mode (positive and negative) resist characteristics of the DNA-surfactant films. The transition between the two regimes occurred at the clearing dose of ~400 $\mu C/cm^2$. The shift to negative resist mode after the clearing dose for positive mode is reached implies that the cross-linking process (typical for negative resists) first requires the dissociation of the DNA-surfactant complex. This unique ability of DNA films to act as both positive and negative resist enhances the flexibility of the fabrication process for DNA-based devices.

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