Immmobilization of DNA by UV irradiation and its utilization as functional materials

Masanori Yamada¹, ², Kozue Kato¹, Kazuna Shindo¹, Motoyoshi Nomizu¹, Nobuo Sakairi¹, Hiroyuki Yamamoto² and Norio Nishi¹
¹Division of Bioscience, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan and ²Faculty of Textile Science and Technology, Shinshu University, Ueda 386-8567, Japan

ABSTRACT
The water-insoluble DNA film was successfully prepared by UV irradiation. The DNA film was stable in water. It could effectively accumulated the DNA-binding intercalating materials, such as ethidium bromide, dibenzo-p-dioxin and benzo[a]pyrene, in their aqueous solutions. On the other hand, DNA was immobilized onto nonwoven cellulose fabrics, also by the UV irradiation. The DNA immobilized cloth was found to bind silver ions. The DNA-cloth containing silver ion showed antibacterial activity. The water-insoluble DNA prepared by UV irradiation has a potential ability to serve as biomaterials for medical, engineering and environmental objects.

INTRODUCTION
DNA, one of the most important materials in life science, can be regarded as naturally occurring and highly specific functional biopolymer. However, utilization of DNA as a functional material was quite limited. There are several reports to utilize DNA.¹,² Although fibers and films can be prepared from DNA, they are water-soluble and have a low mechanical strength. Recently, we found a new method for the immobilization of DNA using alginic acid or collagen.³⁻⁶ Films or gels composed of DNA and alginic acid can be prepared by the addition of calcium ion, and the resulting DNA-alginic acid films were found to adsorb DNA-binding intercalating materials, such as ethidium bromide and benzo[a]pyrene.³

On the other hand, Beukers et al. reported the formation of thymine-thymine dimer structure induced by UV irradiation.⁷⁻⁹ This convenient method of UV irradiation has been used for the immobilization of DNA onto cellulose powder.¹ In the present study, we report the insolubilization and immobilization of DNA by UV irradiation, and utilization of the products as functional materials.

RESULTS AND DISCUSSION
Double stranded DNA from salmon milt (MW; ca. 5 × 10⁶, Yuki Fine Chemical Co. Ltd., Japan) was dissolved in water. The resulting DNA solution was casted onto the glass plates and dried at room temperature, then UV irradiations was carried out. The water-insoluble DNA film on glass plates was striped by soaking in water. This DNA film is stable in aqueous solution and really water-insoluble even when it was soaked in aqueous solution for a long time.

The insoluble DNA by UV irradiation could be solubilized by treating with hot water at 100°C for 15 minutes. By the agarose gel electrophoresis for this solubilized sample, the molecular weight of the insoluble DNA was found to be very high. The cross-linkings between many DNA molecules are suggested for the formation of the insoluble DNA film by UV irradiation.

The insoluble DNA film was placed in the aqueous solution containing intercalating materials such as ethidium bromide, benzo[a]pyrene and dibenzo-p-dioxin.
dibenzo-p-dioxin. Benzo[a]pyrene and dibenzo-p-dioxin have been designated as endocrine disruptors.

The concentrations of intercalating materials in the solution were measured by UV-VIS spectroscopy. The results indicate that intercalating materials were removed by the insoluble DNA from the solution. Figure 1 shows the absorption spectra of ethidium bromide solution in the absence or presence of insoluble DNA. When the insoluble DNA was added to the solution, the peak at 480 nm disappeared. Benzo[a]pyrene and dibenzo-p-dioxin were also found to be removed in the similar experiments. These results suggest that the highly cross-linked insoluble DNA can capture the intercalating materials as well as a double stranded DNA. The insoluble DNA may develop for environmental science to remove the DNA intercalating material such as dibenzo-p-dioxin and benzo[a]pyrene designated as endocrine disruptors.

![Absorption spectra of ethidium bromide solution](image)

Figure 1 Absorption spectra of ethidium bromide solution (10 μM) in the absence (a) and presence (b) of insoluble DNA.

Next, we attempted the immobilization of DNA onto the nonwoven cellulose fabrics by UV irradiation. DNA aqueous solution was casted onto the nonwoven fabrics and dried at room temperature. After the UV irradiation the nonwoven fabrics were washed with water to remove the non-immobilized DNA and dried at room temperature. The amount of immobilized DNA onto the nonwoven fabrics increased depending on the time length of UV irradiation and the amount of immobilized DNA reached a constant value after 15 minutes. The maximum amount of immobilized DNA onto the nonwoven fabrics (1g) was approximately 20 mg.

The DNA-cloth containing silver ion was prepared by immersing in aqueous silver nitrate solution, then washed with water and dried at room temperature. According to atomic absorption spectrometry, the amount of silver ion bound to the cloth increased depending on the amount of silver nitrate. The maximum amount of bound silver ion onto DNA-cloth (1g) was approximately 5mg.

The antibacterial activity of the DNA-cloth containing silver ion was evaluated using Escherichia coli (E. Coli) and Staphylococcus aureus (S. aureus). The DNA-cloth containing silver ion was found to significantly inhibit growth of the both bacteria. The DNA-cloth without silver ion did not affect growth of the bacteria. The DNA-cloth containing silver ion was suggested to be widely applicable as an antibacterial materials.

ACKNOWLEDGMENTS

This work was supported by the Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (No. 11694114, No. 11450359 and No. 10555327) and also by Hokkaido Foundation for the Promotion of Scientific and Industrial Technology (Hokscitec).

REFERENCES