Progress of DNA Biotronics and Other Applications

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Abstract

Crosslinking reactions of DNA film by UV irradiation were investigated in terms of structural changes which indicated the formation of –O-P-O- bond. The UV-cured DNA films were applied to medical uses for cell culture and wound-healing of skin, which were very effective for medical applications.

Keywords: DNA/DNA-lipid complex/Cell culture/Wound-healing effect

1. Introduction

Deoxyribonucleic acid (DNA) is carrying genetic information of all living things and is well known to form a double helical structure in which layers of 4 nucleic acids, namely adenine, thymine, guanine,and cytosin are stacked. DNA has a huge molecular weight of over billions and it can form a clear film, while DNA is water-soluble with sodium counter ions, which are not appropriate for applying DNA to material sciences such as electronic devices. However, DNA molecules become insoluble in water, yet become soluble in polar organic solvents such as ethanol, when sodium cations are replaced with quarternized ammonium salts, lipids which contain long alkyl chains to form DNA-lipid complexes, and clear and tough films are easily obtained by solvent casting of ethanol solutions[1].

Pure DNA was isolated from Salmon roe in an amount of over 1,000 ton/year in a semi-commercial plant, so that applications of DNA as materials are now possible in such areas as photonics, separation process or biomedical materials. Recent research results on DNA-lipid complexes have shown various attractive applications such as E/O or O/E devices, optical memories, switches and sensors[1-4]. It was reported[2] to study on possibility of basic optical characteristics, such as refractive indices, absorbance and fluorescence intensity, and photochromic properties, of spiropyran-doped DNA-cetyltrimethylammonium (CTMA) complex films, which were derived from DNA from Salmon, which showed potential applications to optical switches[5, 6]. Although DNA-lipid complexes showed promising potentials for optical functional devices such as switching or signal processing devices, their response speeds were relatively slow to apply them to practical uses. It was shown[5, 6] that much faster response speed (switching times) could be attained by increasing the excitation light intensity. Thus, applications of DNA photonic devices have been widely studied in the world.

However, problems of DNA optical devices are related to moisture absorption of DNA molecules which

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are very much hydrophilic, and adsorbed water influences the dye-intercalated structures of DNA molecules. Therefore, it is necessary to protect the dye-intercalated state of DNA molecules by sealing off water penetration. It was reported by us that a novel hybridization method of the dye intercalated DNA molecules by means of so-called so-gel process was effective to increase stabilities and durability of DNA photonic devices under environmental changes. The concept of the sol-gel process is applied to DNA devices as follows: encapsulation of dye-intercalated DNA-lipid complex by sol-gel process was carried out by dissolving the dye-intercalated DNA-lipid complex into tetraethoxy silane $(Et)_4Si$ (TEMOS) with stirring at room temperature to encapuslate DNA photonic devices. Also, hybridizations of DNA-lipid complexes which were intercalated with optical dyes were successfully carried out by blending synthetic polymers such as poly(methylmethacrylate) by a solution blending method.

This paper describes further applications of DNA for material sciences such as photonics and electronics and bio-medicals.

2. Experimentals

2.1 Preparation of DNA-Lipid Complex Films[7,8)]

Figure 1 shows the preparative method of DNA-lipid complex films. Single-chain trimethylammonium type lipid (CTMA hereafter) was used to form DNA-lipid complexes. First, refined DNA was dissolved in distilled water. Lipid solution dissolved in distilled water was mixed with the DNA aqueous solution. Then, the DNA-lipid complex was washed in distilled water, followed by drying process in a vacuum oven for 24 hours at 40^0C. After drying process, the DNA-lipid complex was dissolved in mixed solution of EtOH:$CHCl_3$=1:4, together with optical dye compounds. Finally, the solution was poured onto a Teflon-coated dish, followed by evaporating the solvent to obtain films, as schematically as Fig. 1.

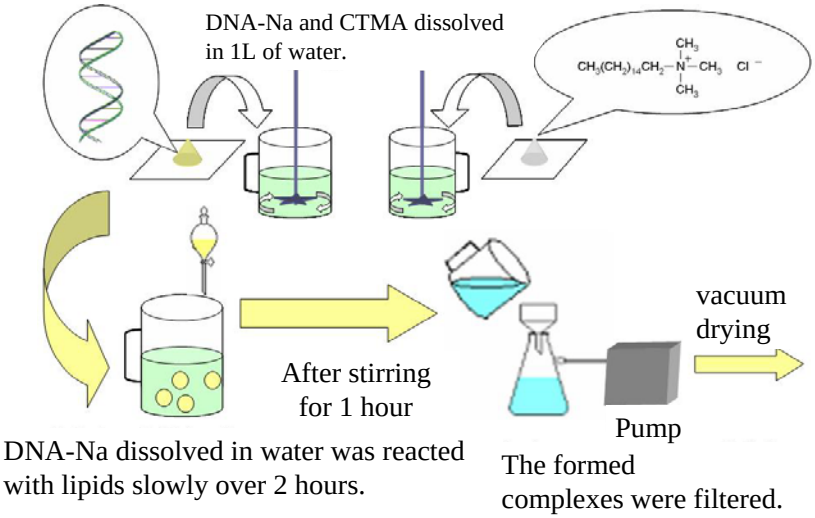


Fig. 1 Preparative method of DNA-lipid complex films

2.2 Chelation of DNA with noble metals or rare earth metal compounds.

Chelation of DNA with noble metal compounds was carried out by dissolving pure DNA in water in an amount of 1wt%, followed by adding noble metal compounds such as $HAuCl_4$, $NaAuCl_4$, $PdCl_2$ which were dissolved in water in amounts of equal mol of metal ions to 1 mol DNA(calculated as unit of 4 nucleic acid bases (adenine thymine, cytosine and guanine)). Also, rare earth metal compounds such as $EuCl_3$, $TbCl_3$ and $NdCl_3$ were used for the chelation with DNA in a similar way. No precipitation occurred for DNA-Au ion or rare earth metal ion complexes, while a pinky and bulk precipitate was formed for DNA-Pd complex. However, DNA remained in these aqueous solutions as UV-Vis spectra indicated absorptions owing to DNA molecules, which is indicated in Fig. 2. However, absorption peaks for DNA-Eu or DNA-Pd were shifted toward lower wave length in comparison with DNA-Na, indicating chelating effect of these metal ions with DNA.

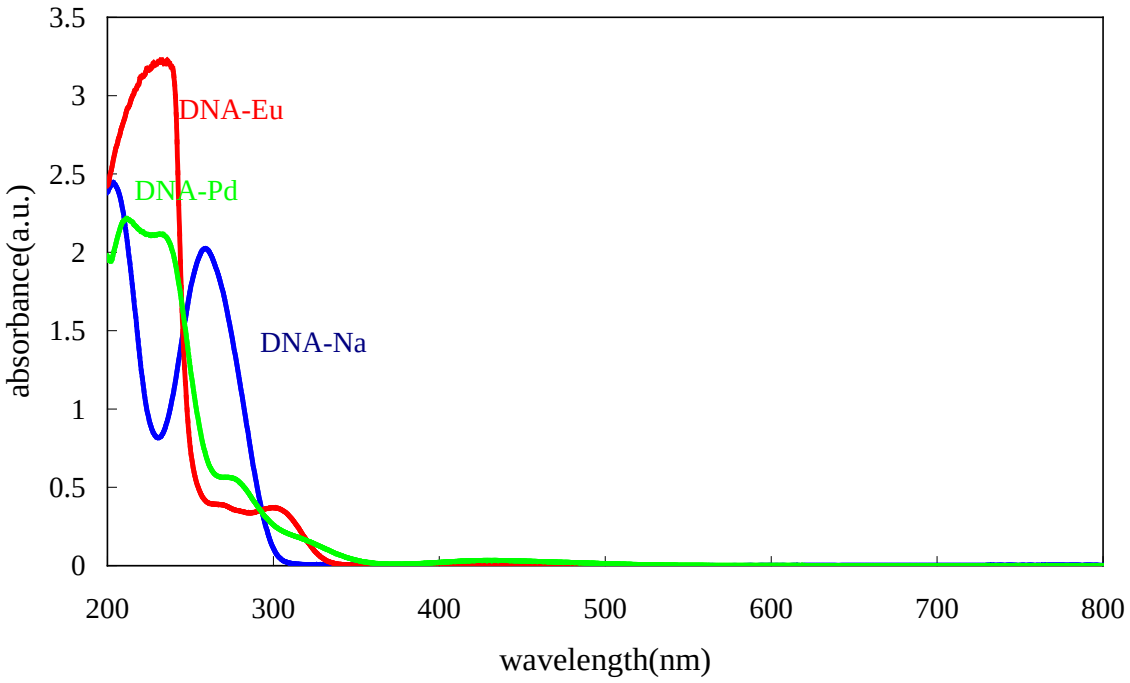


Fig. 2 UV-Vis spectra of aqueous solutions of DNA-metal complexes

DNA-CTMA was dissolved in ethanol in an amount of 1wt%, followed by adding noble metal or rare earth metal ions as described before. Addition of $PdCl_2$ to the ethanol solution of DNA-CTMA caused a pinky and bulky precipitate which was eliminated to measure UV-Vis spectra, as shown in Fig.3.

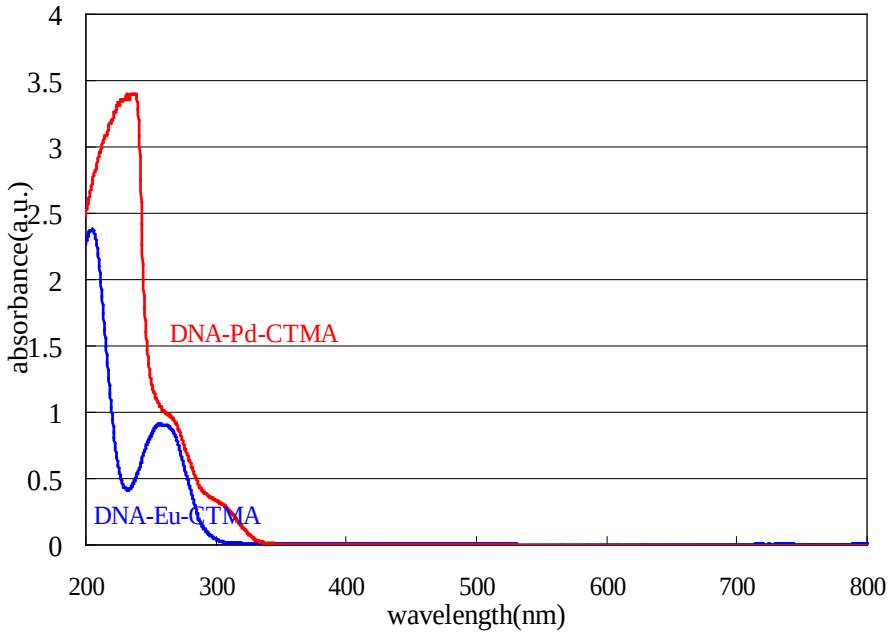


Fig. 3 UV-Vis spectra of DNA-CTMA-metal complexes in ethanol solutions

It is seen in Fig. 3 that DNA-CTMA-Pd complex showed a much lower absorption peak in comparison with DNA-CTMA-Eu complex, indicating much strong chelate effect of Pd to DNA –CTMA molecules as in the case of DNA in an aqueous solution.

3. Results and Discussion

3.1 Biomedical Application of DNA Films

Salmon-derived DNA is soluble in water, so it is possible to prepare clear and transparent films of the DNA by evaporating aqueous solution of the DNA under a reduced pressure at 50^{o}C. Since the DNA is biocompatible and non-toxic, it is highly expected that the DNA films can be applied for biomedical applications such as cell culture. However, since the DNA is soluble in water, it is necessary to insolubilize the DNA films in water because an aqueous cell culture medium is used for cell growth. Several methods of water-soluble DNA for insolubilization in water such as replacements of sodium cation of DNA by quarternized ammonium cation or metal polycations such as calcium cation. However, these insolubilization methods of the DNA may accompany with toxic problems for cell growth. The best insolubilization method would be UV irradiation of the DNA films for crosslinking reactions, so UV irradiation conditions of the DNA films were investigated in terms of structural analyses.

3.2 UV crosslinking of DNA films

High molecular weight DNA of over 6.6 million can be prepared as a transparent film by casting an aqueous solution (5%wt) of the DNA and by slowly evaporating water at 40^{o}C on a Tefron-coated glass plate. It has been known that an ultra-violet (UV) light irradiation of DNA caused cross-linking reactions among DNA molecules to insolubilize DNA molecules in water. It has been assumed[9] that the crosslinking reactions of the DNA molecules might be related with a dimerizaion reaction of thymin within

the DNA molecules. However, since the DNA molecules are well-known to form a stable double helical structure, the dimerization reaction of two different thymin molecules may not be able to collide each other as the thymin molecules are fixed in the double helix of the DNA molecules through hydrogen-bonding with adenine. Precise reaction conditions for the cross-linking reactions have not been ever reported, so the UV-curing conditions were investigated in terms of applications of water-insoluble DNA films for biomedical applications such as cell culture membranes.

A Molitex UV lamp was used for the crosslinking reaction of DNA molecules. This UV lamp is so powerful that no UV curing agents are necessary to start the crosslinking reactions. UV crosslinking reactions of the DNA containing UV curing agents (1wt%) and the pure DNA films were compared in terms of insolubilization in water and results are summarized in Table I, which indicates that the crosslinking reaction of DNA occurred by irradiating only 1 min without curing agents when the UV powerful lamp was used.

Table I UV curing conditions of DNA films

Initiators(1wt%)	Solubility in water	Time of irradiation
Darocure	All insoluble	30sec., 1,3,6,10,20min.
Ilgacure	All insoluble	30sec.,1,3,6,10,20min.
None	All insoluble	1,2,3,5,10,15,30min.

UV power : 160mW/cm^2 , Irradiation distance between lamp and DNA films : 10cm

Differential infrared spectra of non-irradiated and irradiated DNA films were measured as shown in Fig. 4, which indicated strong enhanced peaks at 1226 and 1062cm^{-1}. These peaks correspond to –O-P-O- bond. Therefore, the UV-crosslinking reaction of DNA is assumed to be owing to an exitation of P=O bond to form P-O radicals, resulting in the formation of O-P-O bonds, and in crosslinking among DNA molecules.

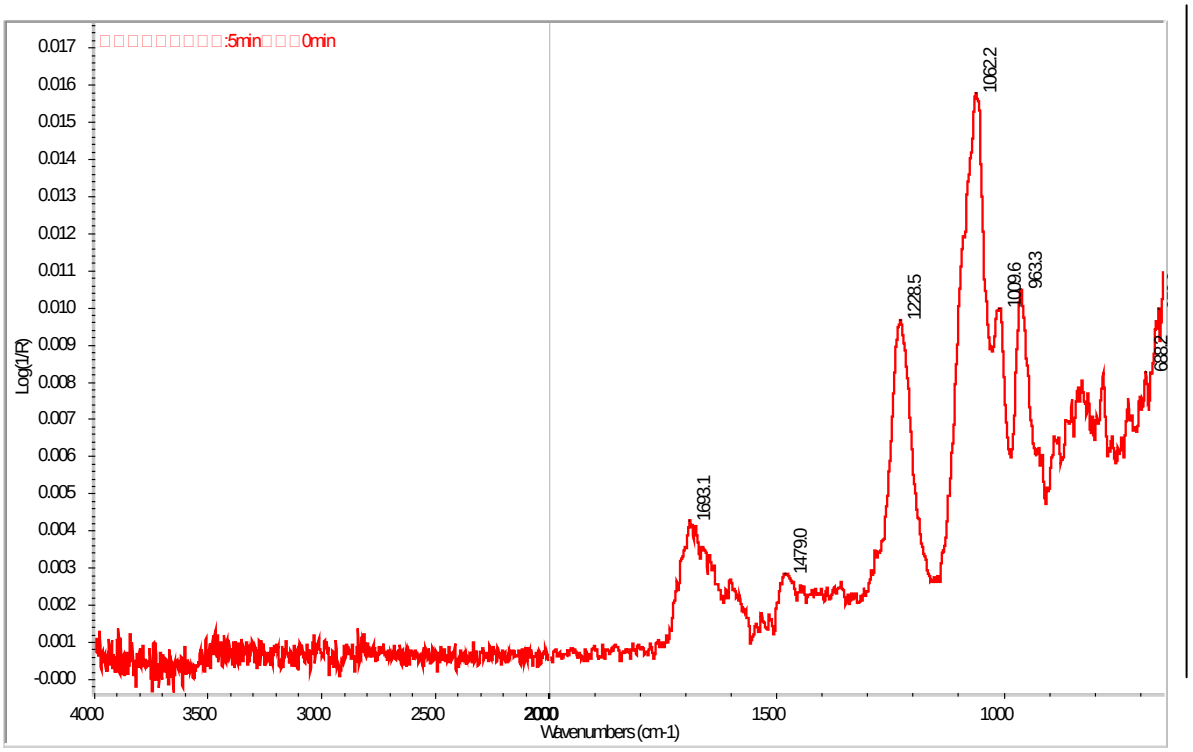


Fig.4 Differential infrared spectrum between non-irradiated and irradiated

Surface pictures by a scanning electron microscope of these UV-cured DNA films are shown in Fig.5 which shows the surface of DNA film of 5μ and 20μ in thickness, respectively..

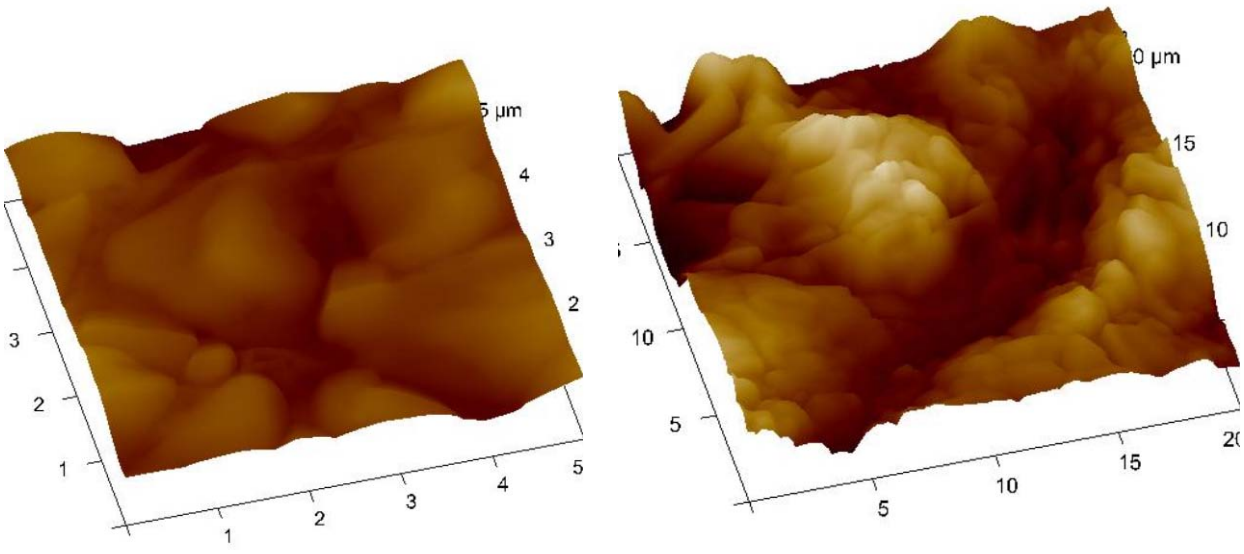


Fig. 5 Surface pictures (SEM) of DNA films (left:5μ, right: 20μ)

It is clearly seen in Fig. 5 that the surface of thick DNA film of 20 μ showed much rough surface structure in comparison with the surface of DNA film of 5μ. It is presumed that a thick DNA film would cause a much shrinkage with increasing thickness of the DNA films.

3.3 Cell Culture on DNA films

Cell culture on the UV-irradiated DNA films was carried out by using rat cartilage cells(ATDC5) at 37^{o}C in an incubator under CO_2 atmosphere. As shown in Fig. 6, cells grew on the surface of DNA film in comparison with a control which is a normal glass plate. The cell growth rates were measured by counting number of grew cell on the DNA film. Results are summarized in Fig. 7. It is seen in Fig. 7 that the growth rates were dependent on the thickness of the DNA films, indicating thinner DNA films were better in terms of the growth rate of the cells.

The thickness dependence of the cell growth would be related with the surface structure of the DNA films as described in previous section. More precise research is needed in terms of cell adhesion and surface structures of DNA films

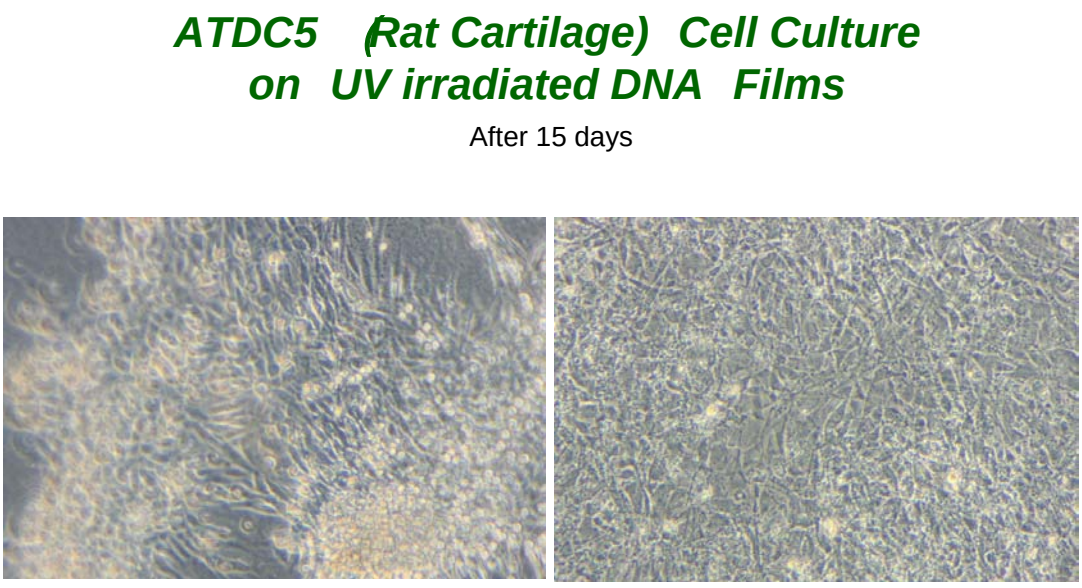


Fig.6. Cell culture of ATDC5 cells on UV-cured DNA film

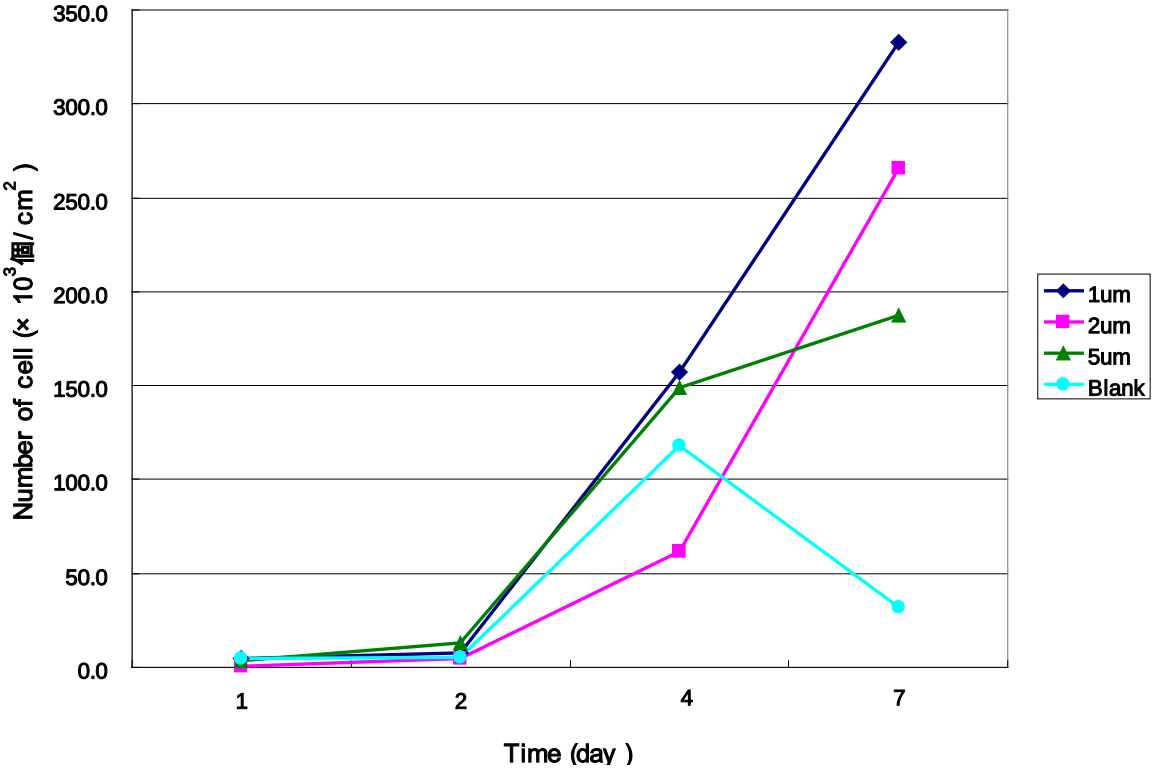


Fig. 7 Cell (ATDC 5) growth number as functions of incubation days

3.4 Wound-healing Effect of DNA and Crosslinked DNA films

It was confirmed before that gas permeation of DNA and crosslinked DNA films was very high in terms of oxygen, carbon dioxide and water vapor gases and also no toxicity of DNA was confirmed as was expected since the DNA was isolated from Salmon roe. Animal testing for wound-healing effects of DNA films was carried out by using wounded skin of rats and results are summarized in Fig. 8. The wounded rat skin without attached DNA film did not recover after 14 days, while the wounded rat skin was almost recovered as shown in Fig. 8.

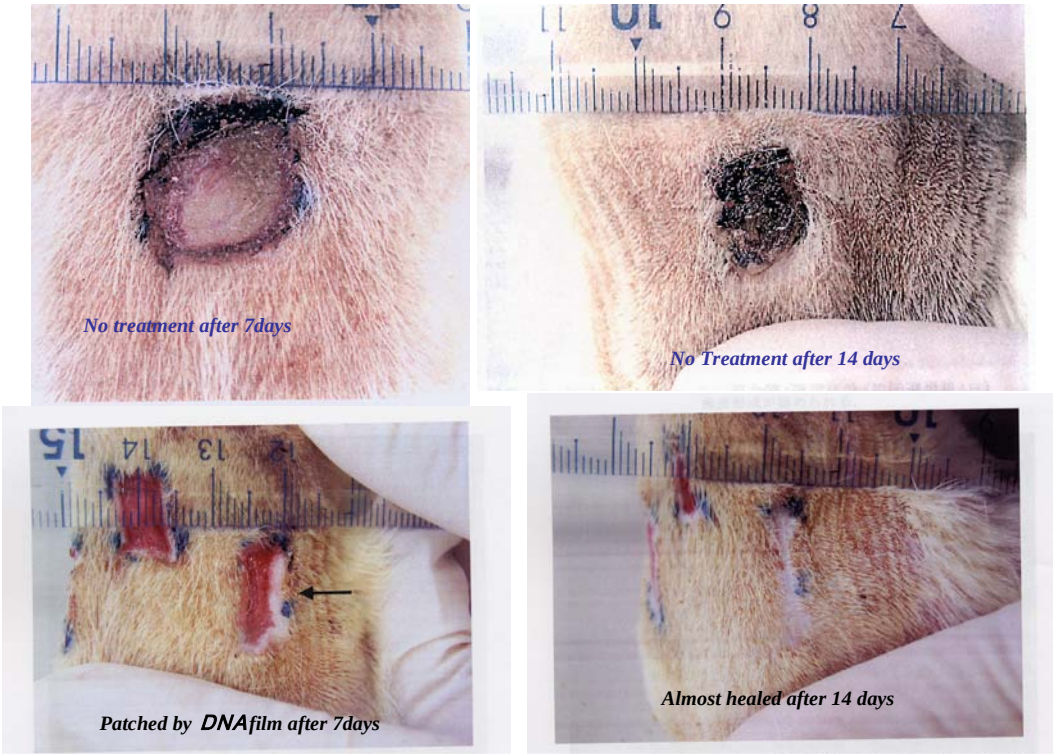
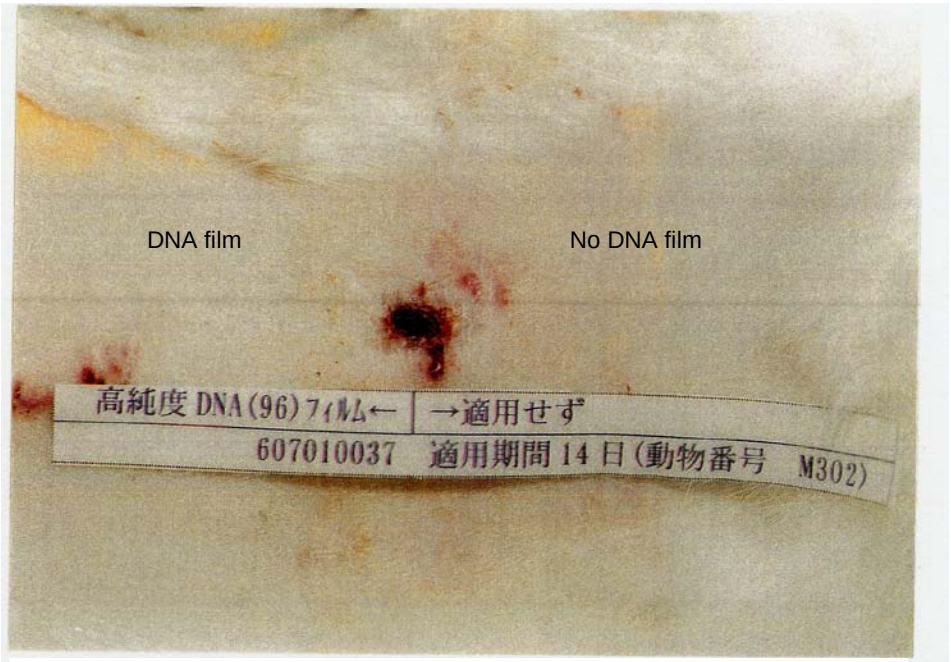


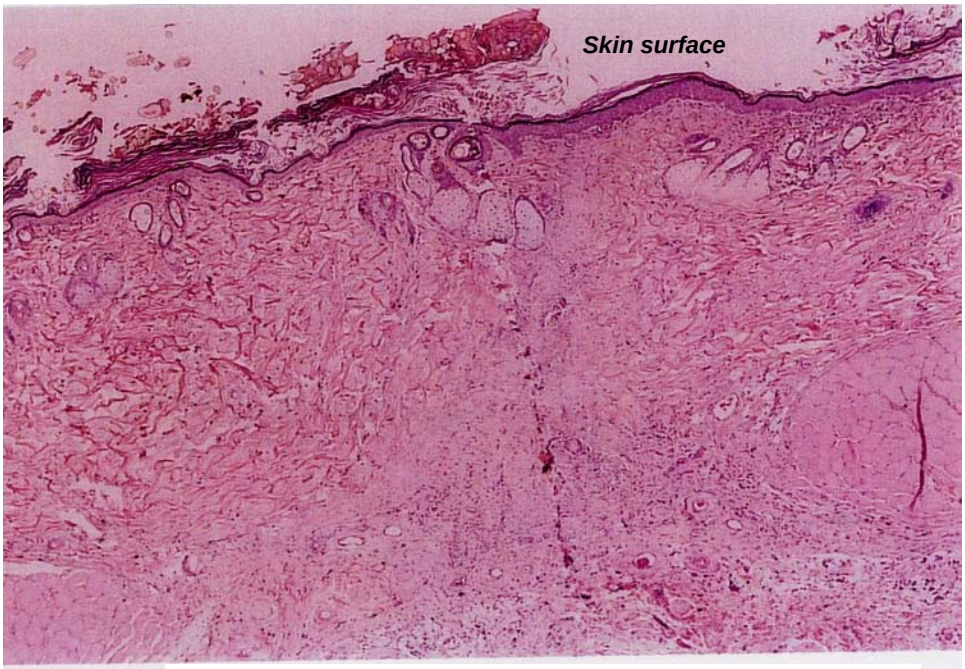
Fig. 8 Wounded skin recovery of a rat skin

A half part of wounded skin was attached with an UV-cured DNA film and another half part of the wounded skin of a rate was left as it was and recovery states of the wounded skin of a rate were compared as shown in Fig.9, which showed the DNA film-attached part of the wounded skin recovered much faster than no DNA film-attached part. Cross-section of the wounded skin tissue of a rate is also shown in Fig. 9 which indicate that the DNA-film attached part of the wounded skin was almost recovered.

These animal testing results strongly suggest that the DNA films are very effective for wounded skin recovery. The rapid recovery of the wounded skin might be related with good permeations of oxygen, carbon dioxide and water vapor of the DNA films.



Wound of a rat skin after 14 days, patched by DNA film



Cross section of wound rat skin after 14days, patched by DNA film

Fig 9 Comparison of wounded skin attached by DNA and no-DNA films

Gas permeation data of DNA films are summarized in Fig. 10[10] which indicates that oxygen and carbon dioxide gas can permeate DNA films as well as water vapor, especially the film derived from DNA-CTMA has a very high oxygen permeation. The high oxygen gas permeation may result in a rapid healing effect of skin wound. Further medical testing may be necessary for long term uses. especially for human body.

Gas permeation data of DNA films

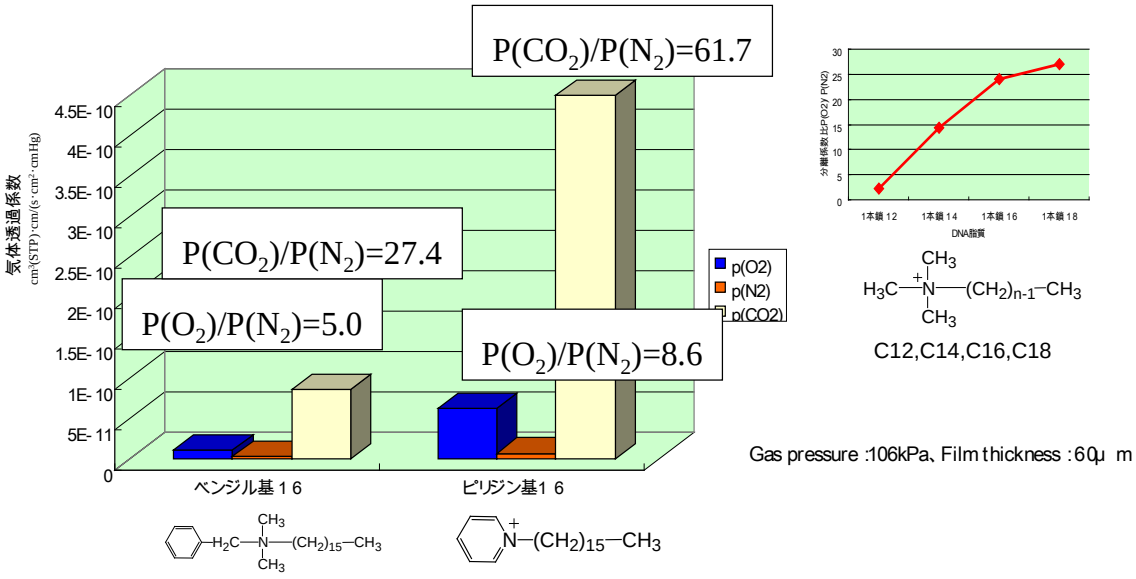


Fig. 10 Gas permeation data of various DNA films

4. Conclusion

UV crosslinking reactions of DNA films by UV irradiation occurred among DNA molecules with the formation of –O-P-O- bond and no dimerization of thymin took place among DNA molecules.

UV cured DNA films are applicable for various biomedical uses such as cell culture and wound-healing because of excellent biocompatibility. The applications of DNA will be expanded to not only tissue engineering for regeneration of organs, but also drug delivery systems (DDS).

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