Final Research report to the AOARD grant in 2008

Title of Research Project: Stability Improvements of DNA Photonic Devices

Principal Investigator: ***Naoya Ogata***

Professor Emeritus,
Ogata Research Laboratory, Ltd.
3-3-7-704 Aoba, Chitose, Hokkaido 066-0015, Japan

Date of this report: December 20th,2008

Summary of Research

Recent research results on DNA-lipid complexes have shown various attractive features on E/O or O/E devices, optical memories, switches and sensors by intercalating optical dye into DNA double helix、while DNA devices absorbed water under high humidity which led to decreases of optical functions. However, it is possible to improve the stability of DNA devices by encapsulating the DNA-lipid complexes into sol-gel materials or synthetic polymers so that water permeation is prevented by glass or synthetic polymers to stabilize and to keep the optical functions for a long time. This research aims at stability improvements of the DNA photon devices by sealing the DNA devices either by sol-gel glass or polymer blending. Following research results were obtained:

1. Encapsulation of DNA devices in Sol-Gel materials

Encapsulation of dye-intercalated DNA-CTMA complex by sol-gel process was carried out by dissolving the dye-intercalated DNA-lipid complex into ADEKA so-gel materials(structures are not opened) derived from tetraethoxy silane $(EtO)_4Si$ (TEMOS) having acrylate or epoxy groups with stirring at room temperature in such concentration as 1g to 100ml TEMOS. The TEMO solution was spin-coated onto Teflon-coated glass plate to obtain films by irradiating UV light to cause crosslinking reactions of the sol-gel materials provided by the ADEKA company in Japan. Clear and transparent films containing DNA-lipid complexes which were intercalated by optical dyes were obtained. Circular dichroism (CD) spectra of the glass beads containing DNA-CTMA proved that the DNA chains kept the double helical structure even after the encapsulation into glass networks and moisture absorption was prevented to stabilize fluorescence intensity of the DNA devices

2. Hyblidization of DNA devices byPolymer Blending

Hybrid films of dye-intercalated DNA-CTMA and various synthetic polymers were prepared to prevent moisture absorption to keep fluorescence quantum yields under various relative humidity. It was found that much improvements of water absorption and quantum yield were attained by blending dye-intercalated DNA-CTMA with PMMA, so that the DNA devices can be applied for practical uses. Especially, fluorinated PMMA completely prevented the permeation of water to stabilize the DNA photonic functions.

Keywords: DNA/DNA-lipid complex/chiral lipid/sol-gel process/stabilization of DNA devices

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
17 NOV 2009	**FInal**	**05-06-2008 to 05-07-2009**

4. TITLE AND SUBTITLE
Stability Improvements of DNA Photonic Devices

5a. CONTRACT NUMBER
FA48690814039

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Naoya Ogata

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Ogata Research Laboratory,1-3-1 Kashiwadai-minami,Chitose, Hokkaido 066-0009,chitose,JP,066-0009

8. PERFORMING ORGANIZATION REPORT NUMBER
N/A

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AOARD, UNIT 45002, APO, AP, 96337-5002

10. SPONSOR/MONITOR'S ACRONYM(S)
AOARD

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AOARD-084039

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Recent research results on DNA-lipid complexes have shown various attractive features on E/O or O/E devices, optical memories, switches and sensors by intercalating optical dye into DNA double helix、while DNA devices absorbed water under high humidity which led to decreases of optical functions. However, it is possible to improve the stability of DNA devices by encapsulating the DNA-lipid complexes into sol-gel materials or synthetic polymers so that water permeation is prevented by glass or synthetic polymers to stabilize and to keep the optical functions for a long time. This research aims at stability improvements of the DNA photon devices by sealing the DNA devices either by sol-gel glass or polymer blending.

15. SUBJECT TERMS
bioderived polymer, Polymer Chemistry, Photonics

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**17**	

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, thymin, guanine,and thitoshin 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, that is called as 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 have been 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 [1]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 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. Physical properties of DNA-lipid complexes are greatly dependent on kinds of lipids which influence moisture absorption. Chiral lipids may induce a liquid crystalline structure to prevent moisture absorption. Based on this expectation. various chiral lipids were prepared from α-amino acids to form DNA-lipid complexes[7,8]

This report also describes a novel hybridization method of the dye intercalated DNA molecules by means of so-called so-gel process in order to increase stabilities and durability of DNA photonic devices under environmental changes. The sol-gel process has been applied to prepare supper conducting ceramics which request homogeneous mixing of various metal oxides. Typical methods of the sol-gel process are to use various metal alkoxides which are homogeneously dissolved in organic solvents such as alcohol, followed by casting the solution to obtain films which are treated to acidic water to hydrolyze the metal alkoxides, thus leading to network structures of metal oxides. 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. UV curable sol-gel materials were kindly supplied by the Adeka company. The miscibility of the Adeka so (chemical structure is not open, while it may contain acrylate and epoxy groups by IR analyses.) with DNA-lipid complexes was investigated. Also, hybridization of DNA-lipid complexes which were intercalated with optical dyes was carried out by blending synthetic polymers such as poly(methylmethacrylate) by a solution blending method for the stability improvements of the DNA photonic functions..

2. EXPERIMENTALS

2.1 Preparation of DNA-Lipid Complex Films

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 below:

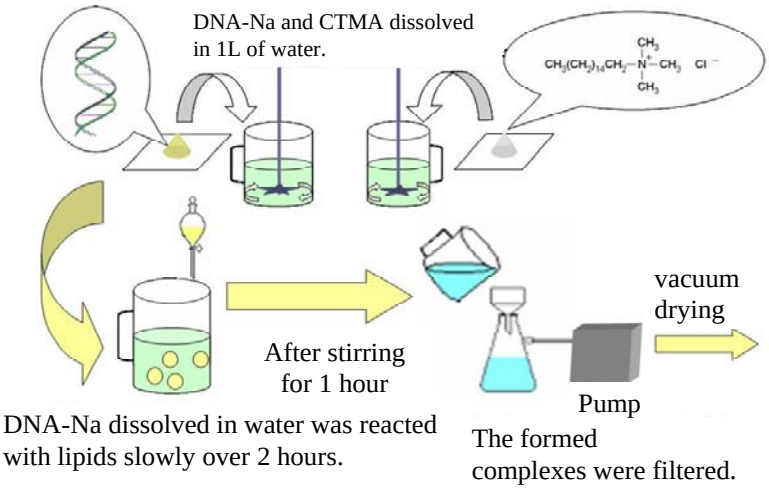


Figure 1 Preparative method of DNA-lipid complex films

2.2 DNA－Lipid Hybrid Films Derived from Chiral Lipids

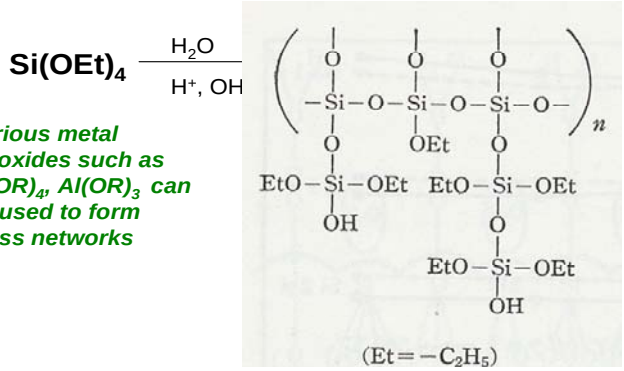
Physical properties of the DNA-lipid complexes are strongly dependent on kinds of lipid in terms of number of alkyl chain length of lipids[7]. It was reported[8] that when a cationic lipid having chiral structure such as cholesteryl 3-N-((dimethylamino)ethy)carbamate (DC-Chol) was used to form a binary complex of DNA and cationic lipid、a self-assembled structure of DNA-DC-Chol complex was obtained. It is expected that more simple chiral lipids may induce to construct a strong self-assembled structure of DNA hybrid film. Based on this expectation, chiral amino acid-derived lipids were synthesized as reported in 2006.

The chiral lipids derived from L-glutamic acid (L-GluC10) and L-phenylalanine (L-PhAlC10) were insoluble in water, so the preparation of the DNA-lipid complex was performed by following method: 1g of DNA dissolved in 100ml of water, followed by adding dropwisely 100ml of ethanol solution of 1g of L-GluC10 or L-PhAlC10, and the DNA-L-GluC10 or DNA-L-PhAlC10 complexes were precipitated, followed by filtration and washing with water and drying. Structure of these DNA-chiral lipid complexes were confirmed by IR and NMR analyses.

2.4 Encapuslation of DNA-lipid complexes by sol-gel process or polymers

DNA optical devices have to be protected to avoid moisture absorption of DNA molecules which are very much hydrophilic to keep photonic or electronic functions. Typical methods of the sol-gel process are to use various metal alkoxides which are homogeneously dissolved in organic solvents such as alcohol, followed by casting the solution to obtain films which are treated to acidic water to hydrolyze the metal alkoxides, thus leading to network structures of metal oxides. The concept of the sol-gel process is shown below:

Sol-Gel Process



Various metal alkoxides such as $Ti(OR)_4$, $Al(OR)_3$ can be used to form glass networks

When alkoxy group contains acryoyl group –COCH=CH_2 , a photo-crosslinking becomes possible by irrdiating UV light.

Dye-intercalated DNA-lipid complexes were encapsulated to form interpenetrated networks with the DNA complexes and inorganic glass by using a photo-crosslinkable sol provided by the ADEKA company and homogeneous clear films were obtained by spin-coating on a glass, followed by irradiating UV light. Solution blending of the dye-intercalated DNA-CTMA complex with synthetic polymers such as poly(methylmetacrylate) , fluorinated methylmethacrylate (3FMA) or polycarbonate was carried out by dissolving both dye-intercalated DNA and synthetic polymers in solvents such as chloroform or hexafluoroisopropanol, followed by casting onto a glass plate and drying to obtain hybrid films.

Water absorption of the encapsulated DNA photonic devices and fluorescence intensity changes under various humidity were measured either by weight increases and by spectroscopic analyses.

3. RESULTS AND DISCUSSION

3.1 Applications of sol-gel process for DNA photonic devices

Problems of DNA photonic devices are related to moisture absorption of DNA molecules which are very much hydrophilic, and adsorbed water influences the dye-intercalated structures of DNA molecules to decrease quantum yields of fluorescence intensity. Therefore, it is necessary to protect the dye-intercalated state of DNA molecules by sealing off water penetration.

Encapsulation of dye-intercalated DNA-CTMA complex by sol-gel process was carried out by dissolving the dye-intercalated DNA-lipid complex into ADEKA so-gel materials(structures are not opened) derived from tetraethoxy silane $(EtO)_4Si$ (TEMOS) having acrylate or epoxy groups with stirring at room temperature in such concentration as 1g to 100ml TEMOS. The TEMO solution was spin-coated onto Teflon-coated glass plate to obtain films by irradiating UV light to cause crosslinking reactions of the Adeka sol-gel materials Clear and transparent films containing DNA-lipid complexes which were intercalated by photonic dyes were obtained. It is seen in Fig. 2 that the dye-intercaleted DNA which was encapuslated into the Adeka sol material kept the dye (ethyzium bromide, EtBr) into the film of the Adeka glass material after immersing the film in water.

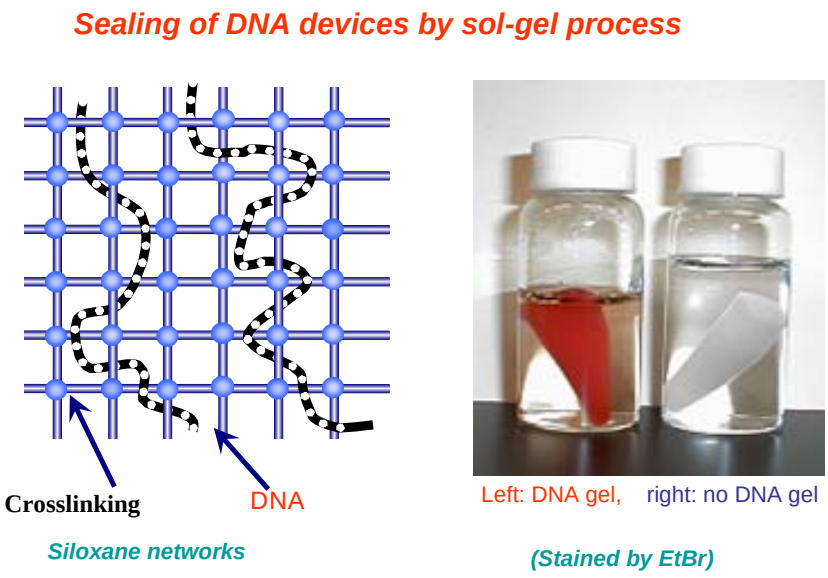


Fig. 2 Encapuslation of DNA device in the Adeka sol-gelmaterial

Various lipids having different alkyl chains as counter cations for DNA were used to improve the miscibility of the DNA-lipid complexes with the Adeka sol materials. However, all of these DNA-lipid complexes showed a micro-phase

as shown in Fig. 3, which is the case of DNA-CTMA complex(0.1wt%) in the Adeka sol material.

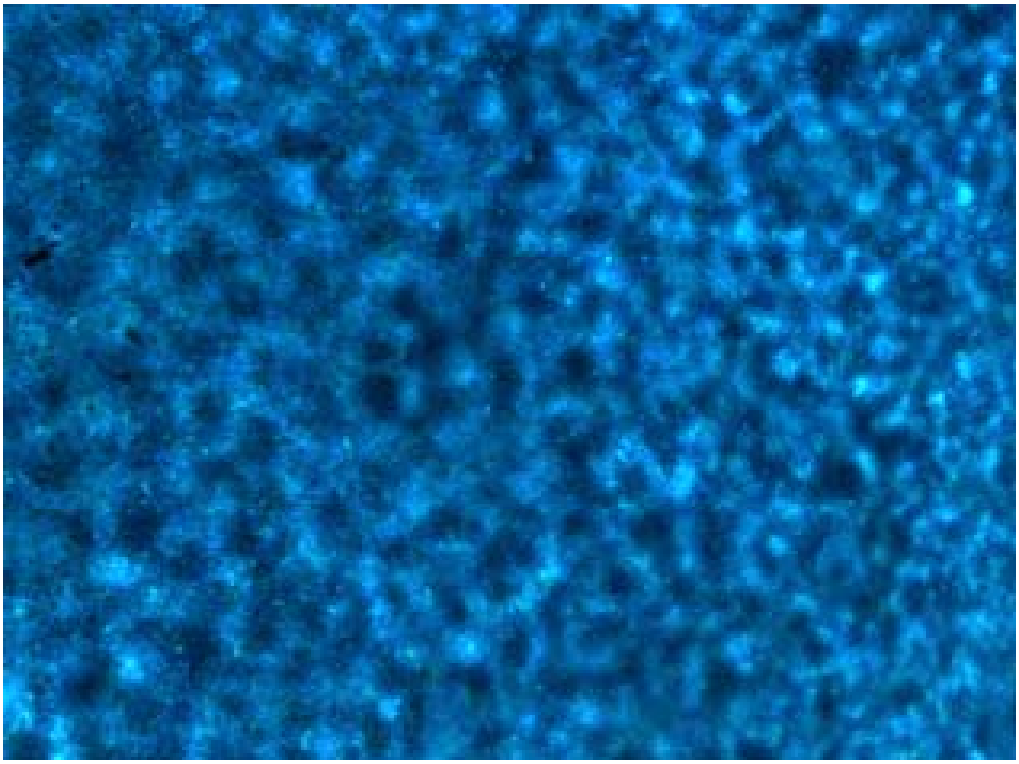
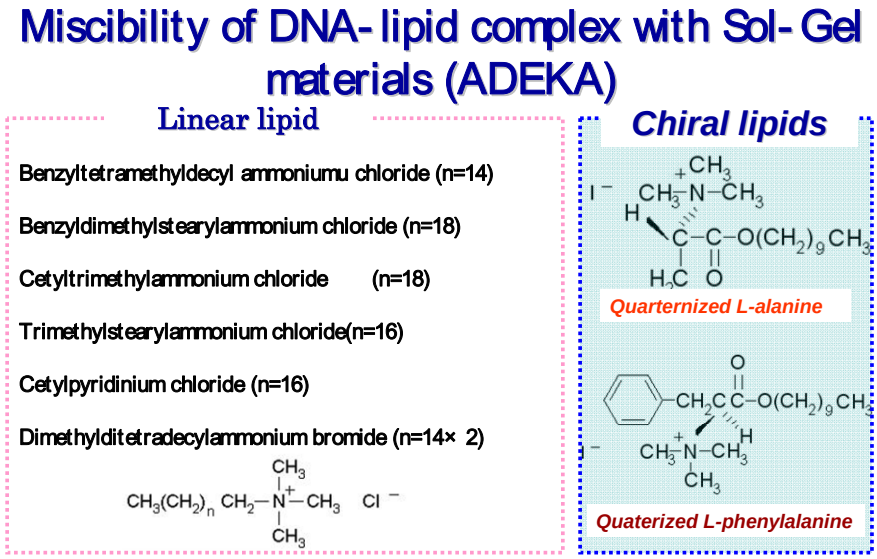


Fig. 3 Microscopic observation of the DNA-CTMA(0.1wt%) and the Adeka sol material

Circular dichroism (CD) spectra of the glass beads containing DNA-CTMA proved that the DNA chains kept the double helical structure even after the encapsulation into glass networks. In order to improve the miscibility of DNA-lipid complexes into the Adeka sol material, following chiral lipids were used as counter cations of DNA, since these DNA-chiral lipid complexes indicated specific mechanical strength as was reported in 2006. Various structures of lipids for the DNA complexation are summarized in Table I.

Table I Various lipids for the complexation with DNA



Surprisingly, the miscibility of these DNA-chiral lipid compleses was greatly improved to mixed ratios of more than 20wt% and homogeneous films having no micro-phase separation were obtained as shown in Fig. 4.

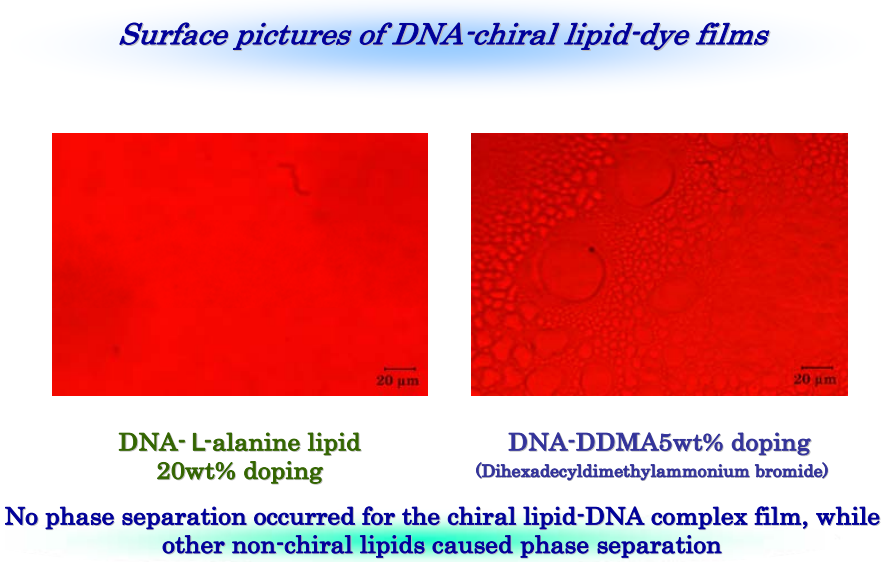


Fig. 4 Surface microscopic pictures of DNA-lipid-dye films

Figure 5 shows water absorption of the glass-networks containing 0.1 wt% of DNA-CTMA complex, while Figure 6 indicates quantum yields of fluorescence light of the glass networks containing 0.1wt% dye-intercalated DNA-CTMA complex. The used dye was 4-[4-(dibutyl amino)styryl]-1-methyl)pyridinium iodide (DAMSDPI). The water absorption of the DNA-CTMA complex film increased with increasing relative humidity, reaching 40% under 100% humidity, while almost no increase of water absorption under 100% humidity was observed for the encapsulated DNA-dye complex. Figure 6 indicates dependency of quantum yields of fluorescence light on relative humidity that no change of the quantum yields was observed for the encapsulated DNA device. These results clearly indicate that water permeation into the glass networks was prevented and photo-stability increased as was expected.

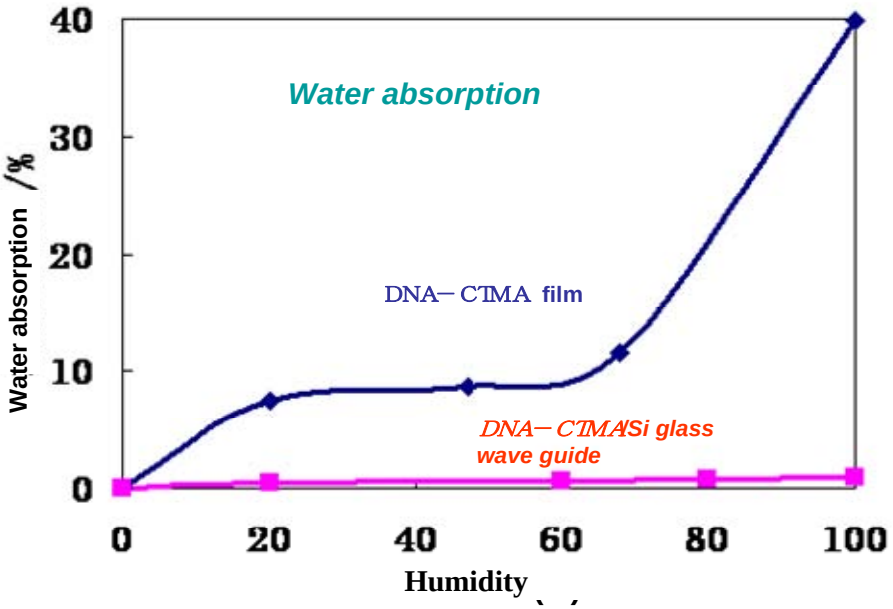


Figure 5 Water absorption of DNA-CTMA encapsulated into glass

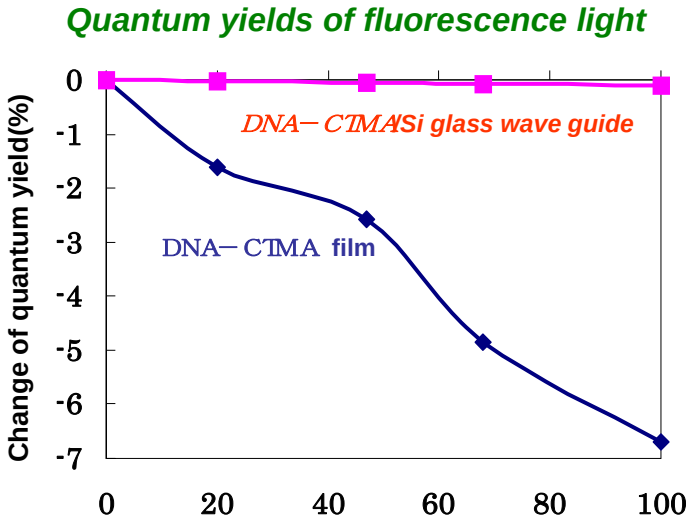


Figure 6 Quantum yields of fluorescence light at different humidity

Doped dye trans-4-[4-(dibutylamino)styryl]-1-methylpyridinium iodide

Spectrum analyses of the fluorescence light of glass films containing DNA-CTMA-dye by irradiating UV light were carried out as shown in Fig.7 which indicates that narrowing of half width of fluorescence spectrum was observed at 612 nm with a threthold value at 1 mJ/cm^{-2} for the glass films containing DNA-dye , indicating lasing effect , while no narrowing and no threthold value of the spectrum were observed for glass films containing dye only without DNA. Therefore, it is clear that the DNA is responsible for the fluorescence light enhancement.

However, miscibility of normal lipids having long alkyl chains like CTMA into the ADEKA sol-gel material is limited to about only 0.5wt% because of hydrophobic characters of long alkyl chains. Therefore, more polar lipids derived from amino acids such as L-alanine (L-Al) and L-phenyl alanine (L-PhAl) having C10 alkyl chain as ester group were used in order to enhance miscibility of DNA-lipid complexes with the ADEKA so-gel materials which contain photo-crosslikable moieties by UV irradiation. L-alanine and L-phenylalanine-derived lipids with decane unit (abbreviated asL-AlC10 and L-PhAlC10) which were synthesized as mention in section 2.2, were used to encapsulate the DNA-lipid complexes into glass networks by using the ADEKA sol-gel materials.

The miscibility of these polar lipids derived from α-amino acids was greatly improved up to more than 20wt%.,and clear transparent films were obtained after spin-coating, followed by irradiating with UV light. The good miscibility of chiral lipids derived from α-amino acids with ADEKA sol materials is not clear. Presumably, Chiral lipids may fit with helical direction of DNA molecules.

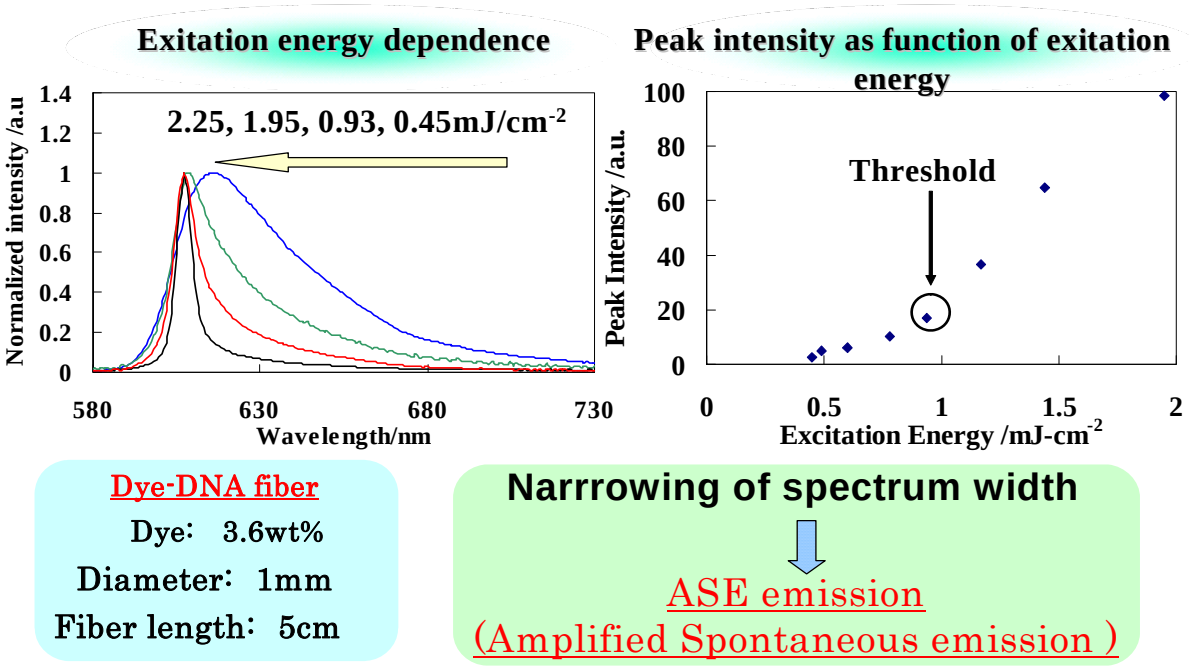


Figure 7 Fluorescence light emission from the encapsulated DNA-CTMA-dye

These DNA-amino acid derived lipid complexes could de dissolved up to 20wt% without a phase separation and homogeneous transparent films were obtained. These polar DNA complexes may have a good affinity with Si-O- units in the ADEKA sol materials. Further research on the miscibility mechanism is required.

Figure 8 indicates that the fluorescence intensity of the glass films containing each 5wt% of L-PhAlC10 and L-AlC10 (dye concentration was adjusted as the same amount of 5wt%) was greatly enhanced in comparison with that of dye only and that from L-PhAlC10 were almost twice as high as that of L-AlC10, in spite of the same dye concentration. Presumably, the phenyl group of PhAlC10-DNA may have electron-electron interactions with the dye to enhance spontaneous amplified emission of fluorescence light from the glass films. Thus, the sol-gel process to encapsulate DNA photonic devices opened a novel route to stabilize the DNA devices under various environmental conditions.

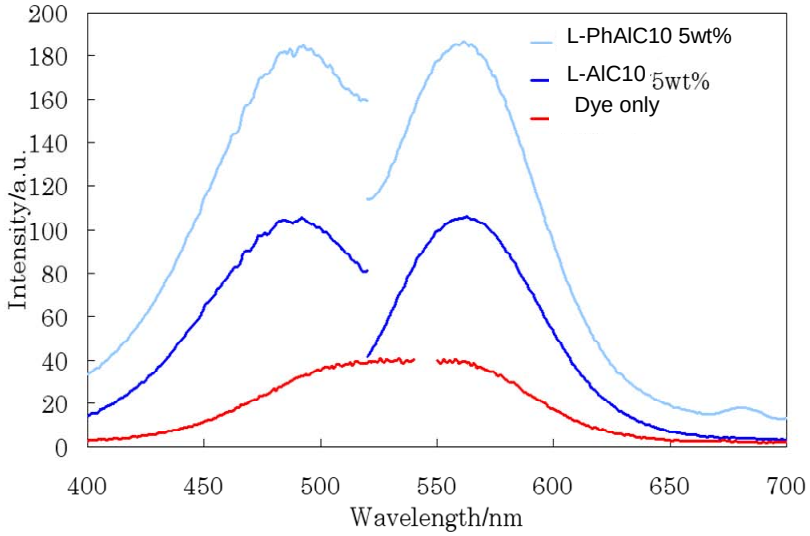


Fig.8 Fluorescence spectra of glass films containing 5wt% of L-PhAlC10 and L-AlC10, which were intercalated by dye(4-[4-(Dibutyl amino)styryl]-1-methyl)pyridinium iodide (DAMSDPI)

3.2 Hybridization of dye-intercalated DNA with synthetic polymers

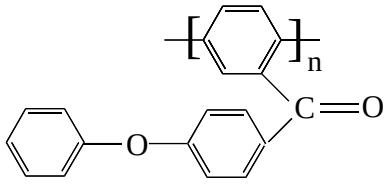
The dye (DAMSDPI)-intercalated DNA-CTMA complex was dissolved in solvents such as chloroform, hexafluoroisopropoanol in 10 wt% concentrations, followed by adding various synthetic polymers in mixing ratios of 1/10 of DNA/polymers, as shown in Table I. The mixed solutions were dried to evaporate solvents to obtain films. No phase separation occurred by blending these polymers shown in Table I and homogeneous films were obtained.

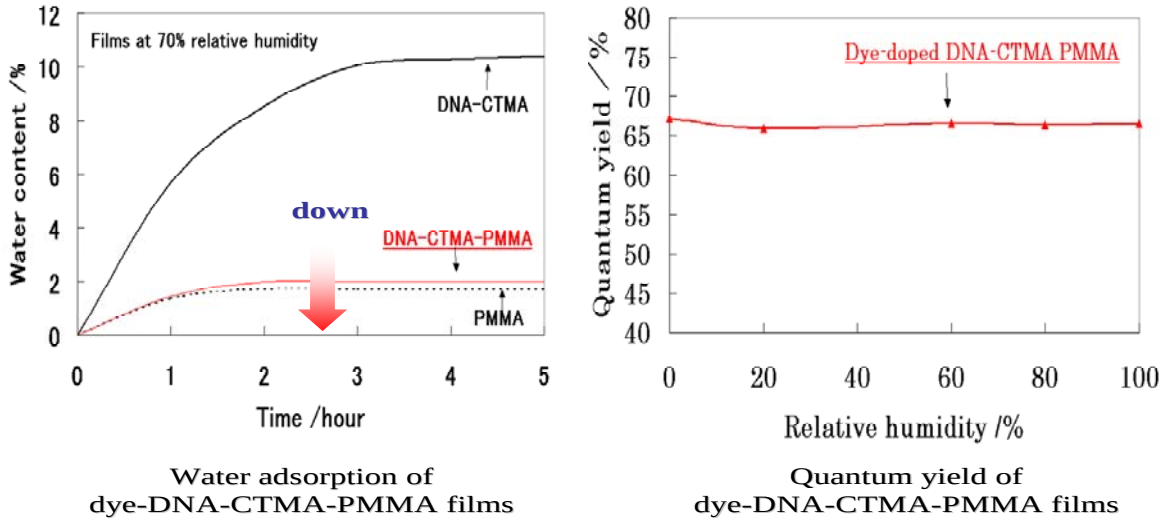
Hybrid films of dye-intercalated DNA-CTMA and PMMA were measured in terms of water absorption at 70% relative humidity and fluorescence quantum yields at various relative humidity and results are summarized in Fig. 9. It is seen in Fig, 9 that much improvements of water absorption and quantum yield were attained by blending dye-intercalated DNA-CTMA with PMMA, so that the DNA devices can be applied for practical uses.

Table I Blending of dye-intercalated DNA-CTMA with synthetic polymers

Solvent	Ethanol	Chloroform	Dichloroethane	HexaF-2-isopropoanol	Films appearance
Synthetic polymers					
DNA-CTMA	○	X	X	○	Clear film
Polycarbonet SP	X	○	○	X	Half transparent film
Nylon CM8000	X	○	○	○	White film
PMMA	X	○	○	○	Transparent film
Parmax2000	X	X	○	X	White film

○ : soluble, X:Insoluble, Parmax2000: poly(p-phenylene) attached with phenoxy benzophenone





Water adsorption of dye-DNA-CTMA-PMMA films

Quantum yield of dye-DNA-CTMA-PMMA films

Fig. 9 Stability improvements of dye-intercalated DNA films under variour relative humidity

In-situ polymerization was applied for blending DNA devices into polymers. Benzyl methacrylate was used as a monomer and a DNA-benzyl alkyl (C12) lipid complex was dissolved in benzyl methacrylate in an amount of 10wt%. The *in-situ* polymerization was carried out by irradiating the solution with UV light in the presence of an UV radical intiator. The solution became a solid rod after the UV irradiation for 1hr at ambient temperature, which was dissolved in chloroform for solvent casting to obtain films. Moisture absorption of the film is shown in Fig. 10, while fluorescence intensity of the film was measured as function of relative humidity changes as shown in Fig. 10. It is seen in Fig. 10 that much improvements for moisture absorption and fluorescence intensity changes were achieved by the *in-situ* polymerization method of benzylmethacrylate (PBMA) in the presence of DNA-CTMA-dye complex in comparison with PMMA, as PMBA is more hydrophobic than PMMA.

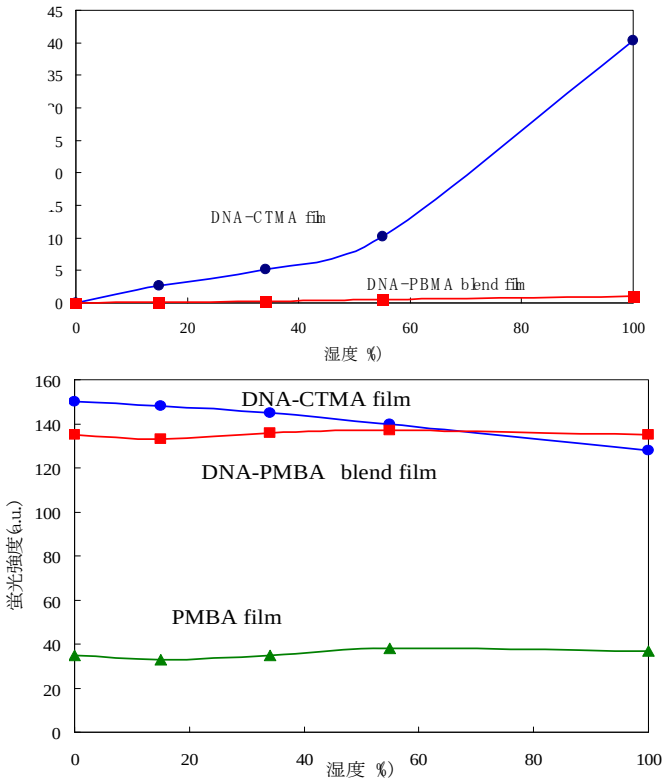


Fig. 10 Moisture absorption and fluorescence intensity changes of DNA-PBMA blend film

Since PMMA was quite miscible with DNA-CTMA to form an homogeneous film, more hydrophobic fluorinated PMMA such as a copolymer of CF_2=CFCOOEt(3FMA) and CH_2=C(CH_3)$COOCH_3$(1/1) was used for blending with DNA-CTMA-dye complex (20wt%). This copolymer is now practically being used for optical fiber for light transmission. A solvent for casting used was hexafluroisopropanol. After casting the solution, a clear film was obtained and no phase separation occurred. Fig. 11 shows moisture absorption of the film under various relative humidity, which indicates no water permeation occurred for this film. Fig. 12 indicates that fluorescence intensity change under various relative humidity did not occur at all for this films. Thus, stability improvements of the DNA devices were achieved by using blending miscible hydrophobic polymers with the DNA devices.

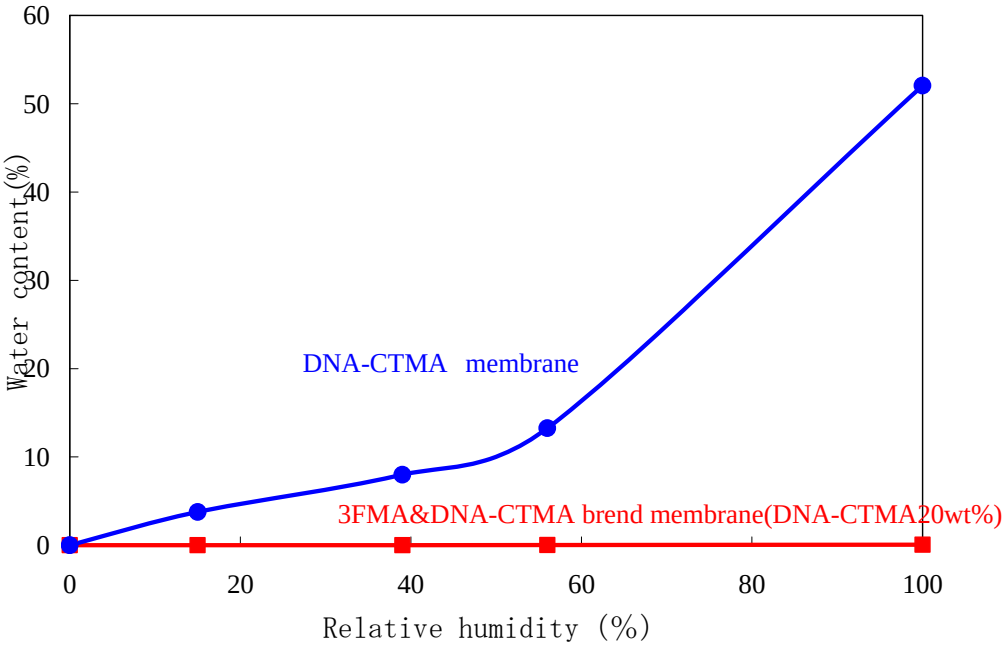


Fig. 11 Moisture absorption of 3FMA-DNA film(DNA-CTMA 20wt%)

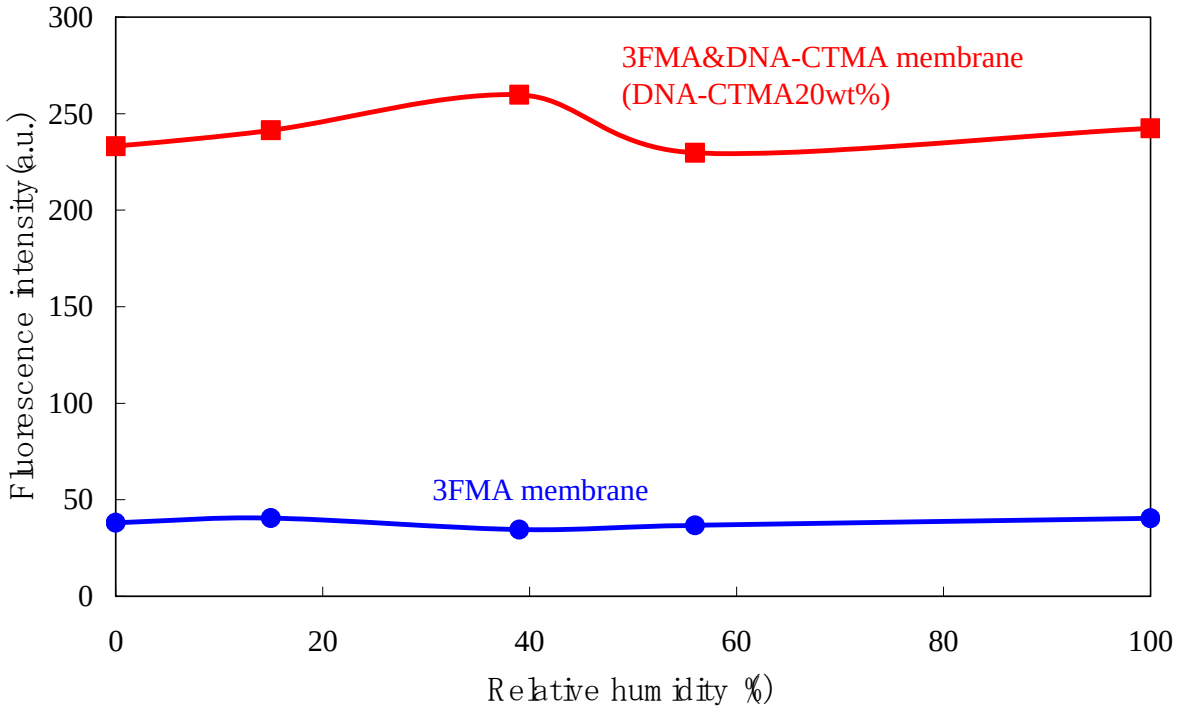


Fig.12 Fluorescence intensity change under various relative humidity

4. CONCLUSION

Stability and durability of the DNA devices which were obtained by an intercalation of dye into DNA-CTMA complex ,were greatly improved by the encapuslation into a glass network which was prepared by a sol-gel process and also by polymer blending methods in fluorinated PMMA.

ACKNOWLEDGMENT

Thanks are due to the Asian Office of US Air Force Research and Development for financial supports and especially to Dr. James G.Grote of US Air Force Research Laboratory for technical discussion.

REFERENCES

[1] L. Wang, J. Yoshida, N. Ogata, S. Sasaki and T. Kamiyama: "Self-assembled supramolecular films derived from marine deoxyribonucleic acid (DNA) – cationic lipid complexes: large-scale preparation and optical and thermal properties", *Chem. Mater.*, **13,** 1273-1281, 2001.

[2] J. Yoshida, L. Wang, S. Kobayashi, G. Zhang, H. Ikeda and N. Ogata: "Optical properties of photochromic-compound derived from dye-doped marine-biopolymer DNA-lipid complex films for switching applications", *Proc. SPIE*, **5351,** 260-268, 2004.

[3] W. M. Heckman, J. Grote, P. P. Yaney and F. K. Hopkins: "DNA-based nonlinear photonic materials", *Proc. SPIE*, **5516,** 47-51, 2004.

[4] P. P. Yaney, E. M. Heckman, D. E. Diggs, F. K. Hopkins and J. Grote: "Development of chemical sensors using polymer optical waveguides fabricated with DNA", *Proc. SPIE*, **5724,** 224-233, 2005.

[5] A. Watanuki, J. Yoshida, S. Kobayashi, H. Ikeda and N. Ogata: "Optical and photochromic properties of spiropyran-doped marine-biopolymer DNA-lipid complex films for switching applications", *Proc. SPIE*, **5724,** 234-241, 2005.

[6] J. Yoshida, A. Watanuki, S. Kobayashi, H. Ikeda and N. Ogata: "Potential switching application based on the photochromism of spiropyran-doped marine-biopolymer DNA-lipid complex films", *Tech. Digest, 10th Optoelectronics and Communication Conference (OECC2005)*, 342-343, 2005, Seoul, Korea.

[7] K. Yamaoka and N. Ogata, :"Effect of lipids on physical properties of DNA-lipid complexes", *Kobunshi Ronbunshu*,**61**,384-390, 2004.

[8] C. M. Wu, W. Kiou, H. L. Chen, T. L. Lin, and U. S. Jeng, "Self-assembled structure of the binary complex of DNA and cationic lipid", Macromol., **37**, 4974-4980,2004.

Expense report of the AOARD research grant in 2008

Period of Performance: from March 1 to December 31,2008

Organization Ogata Research Laboratory, Ltd		
Principal Investigator/Project Director Prof. Dr. Naoya Ogata/Prof.Dr.Naoya Ogata	**AOARD Program Manager** Dr. Misoon Mah	
		Funds Cost Shared Proposer
Labor Cost	A. Senior Personnel	$5,000
	B. Other Personnel	$15,000
	C. Fringe Benefits	$2,000
	Total Labor Cost	$22,000
Direct Cost	D. Equipment	$0
	E. Travel	$550
	F. Materials and Supplies	$1,450
	G. Publications and Reports	$0
	H. Consultant Services	$0
	I. Computer Services	$0
	J. Sub Contracts	$0
	Total Direct Cost	$2,000
Overhead Cost	K. Facilities & Administration Expense	
	Total Overhead	$6,000
Sub Total	*Total Labor, Direct & Overhead Cost*	$30,000
Total Project Value	*Total Funds Shared + Requested Funds*	**$30,000**

Reported by Naoya Ogata, signed

Date of this report: December 31st,2008