교육학석사 학위논문

Optimizing DNA printing on surface for luminescent silver nanodot transfer

발광하는 은 나노닷 전이를 위한 표면의 DNA 프린팅 최적화에 대한 연구

2022 년 8 월

서울대학교 대학원

과학교육과 화학전공

홍 성 재

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이 논문을 교육학석사 학위논문으로 제출함

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2022 년 8 월

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Abstract

Optimizing DNA printing on surface for luminescent silver nanodot transfer

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Various silver nanodot properties have been studied and characterized in solution. However, their properties on the surface have not been studied enough yet. In this research, after printing single-stranded DNA (ssDNA) on the glass coverslip surface and transferring polyacrylic acid stabilized silver nanodots (PAA-AgNDs) to the printed DNA, we have examined the emission spectra of ssDNA encapsulated silver nanodots on the surface. On the glass

surface, even using the same ssDNA and PAA-AgNDs as in solution, emitters with different wavelengths are predominantly generated depending on the surface condition, such as system humidity, surface modifying materials, and surface pH. When the surface has a strong affinity for DNA, DNA can be kinetically trapped on the surface and cannot be reorganized with silver nanodots as flexibly as in the solution. Even using the same DNA and silver nanodots, different types of emitters can be generated by controlling the surface conditions.

Keywords: Silver nanodots, Surface properties, DNA printing, Kinetic trap, Surface DNA-silver reorganization

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Contents

Ⅰ. Introduction

1.1. Silver nanodots and their applications

Silver nanodots are reduced silver atom species that protected by different types of protection groups, such as solid matrix, peptide, polymer, and single-stranded DNA (ssDNA)[1]. Silver nanodots have been studied because of their small size, fluorophore function, excellent photophysical properties, and applications utilized in various fields of scientific research[1, 2 3 4 5 6]. They have been used as cellular staining materials with an excellent signal to noise ratio[7] or DNA detection probes[8] in a biological field. They have been also used in vivo imaging due to their high molar extinction coefficient[9]. Not only the function related to bio-cellular imaging, but silver nanodots also can measure the size of nanocage by using different emitters to measure the Förster resonance energy transfer (FRET) between the pair[10]. When measuring the size of a nanocage with FRET calculation, people can detect the diameter of a nanocage in a short time without cryo-TEM imaging.

1.2. Studying surface chemistry of silver nanodots

1.2.1. Properties of silver nanodots

In general, materials on a solid surface are more difficult to be characterized with respect to their unique properties compared to that in a solution due to their natural complexity[11]. Especially in the field of nanoscale, not only their surrounding environment but also their extremely small size influence their properties differently with larger metal particles[12 13].

When silver nanoparticles (AgNPs) are irradiated by a specific wavelength of the light source, the conduction electron on the metal surface simultaneously oscillates and this collective oscillation of electron clouds is called plasmon resonance (SPR)[14]. These silver nanoparticles can absorb the light efficiently due to SPR but cannot emit bright fluorescence. In contrast, nanodots not only can absorb the light but also emit bright fluorescence unlike silver nanoparticles[15].

Like previously introduced applications, usually silver nanodots have been studied and characterized in solution[16]. However, their properties on the

surface have not been studied yet enough. Therefore, in this research, we focused on studying the surface properties of the silver nanodots, especially to know if the predominantly generated emitter species and their emission spectra are same as those in solution or not.

1.2.2. DNA attachment for studying silver nanodot surface properties

Luminescent silver nanodot stability and brightness can be enhanced by various protection groups, such as peptide, polymer, or ssDNA[17 18], and finally ssDNA was chosen for the protection group for silver nanodots in this research. One of the chemical properties of ssDNA protected silver nanodot is that the spectrally pure emitter excited at a certain wavelength can be generated[19]. For example, when the silver nanodot is protected by 5'-CCCCCCCCCCCCCCCCCCCC-3' (C_{20}) sequence, it can generates green emitters at 523 nm, with a 467 nm excitation[20]. These emitters can be generated spectrally pure in solution.

In solution, most of the materials can be purified and relatively well separated

from interruption of external factors from outside of system such as second contamination. However, on the surface condition, all materials are kept on the bare environment and influenced by various factors. Even silver nanodots were protected by the same species of ssDNA as in solution, their chemical or photophysical properties can be changed due to their different surrounding conditions. Thus the experiment about generation of emitter species and their emission spectra affected by environmental changing was performed.

In this research, different types of ssDNA was tested for detecting emission spectra on the glass surface, but the C_{20} was mainly discussed hereafter. Various types of DNA can be used to generalize results in specific experiment condition, but only two to three additional types of DNA sequence was used for experiment, because we wanted to first examine the results of experiments in different environments using a single DNA sequence.

1.3. DNA attachment on the surface

Before detecting the emission spectra of silver nanodot transferred to DNA, firstly DNA should be successfully attached on the surface. To optimize DNA

printing on the surface, two different methods with a focus on either physical or chemical bonding were considered.

1.3.1. Microcontact printing

DNA can be fixed to glass surface with different ways such as lithography[21 22 23 24 25], inkjet printing[26 27 28 29], and microcontact printing[30 31 32 33], which are proven methods that can uniformly attach the target materials onto the surface. However, for the lithography and inkjet printing, they have the disadvantage that the process of attaching target materials onto the surface is not so simple. To attach the materials by using these methods, especially for inkjet printing, the device to uniformly attach the materials on the surface is needed.

However, the microcontact printing method is one of the method that can print target materials on the surface efficiently by easy way, using elastic stamp generally made of polydimethylsiloxane (PDMS)[31 32 34]. This PDMS stamp can be easily prepared by using source materials such as SU-8 with low cost compared with using inkjet device.

Advantage of microcontact printing method is the simplicity of protocol[30]. The

printing protocol is generally performed in order of incubating the printing materials onto PDMS stamp, rinsing and drying of printing materials, and printing these materials from PDMS stamp to target surface. This process of transferring the material to the target surface is very simple, so if only the PDMS stamp and the printing material are well prepared, a large amount of samples under various conditions can be prepared in one time preparation. In addition, well made PDMS stamp can be anytime used after first time preparation, a cost-effective method.

1.3.2. Covalent bonding

Covalent bonding is the binding interaction between two atoms which sharing the electron pairs. When attach the DNA to another materials by using covalent bonding, the crosslinker should be used between the DNA and its target materials[35 36]. Usually the functional groups on the glass surface are hydroxyl group (–OH) from the oxidized silica surface[37 38], so the silane crosslinker mostly had a specific functional groups such as methoxy (–OMe) or ethoxy (–OEt) group that can react with –OH on the glass surface. On the other side of

crosslinker, the materials that can connected with DNA such as NHS ester groups are positioned.

The advantage of this method is that if the covalent bond between the DNA and the surface is successful, it indicates that DNA exists exactly at that point. Unlike printing methods in which only physical contact exists between materials, covalent bonds can fix the target materials to surfaces much more stably due to crosslinking between molecules. Thus, if silver is transferred to the point where DNA is exactly connected on the surface, the emission detected at that point is exactly an emission from DNA.

1.3.3. Microcontact printing for DNA attachment

People tried different types of method to attach DNA on the surface condition, such as chemical covalent bonding method by using different chemicals and microcontact printing method. Each method has its own advantage and disadvantage compared with others.

If DNA attached to surface by covalent bonding, the interaction between surface and DNA become stronger and more stable. Even after transferring the

silver solution, they will not be detached from surface easily due to their strong interaction. In this case DNA on the surface can be detected much more efficiently. However, several steps of experiment preparation are required to induce stable chemical bonding between DNA and surface, and also processing time is much longer than microcontact printing method.

Microcontact printing methods can also attach the DNA to the surface, but binding force between DNA and surface is much weaker and DNA stability on the surface is worse compared with covalent bonding methods. However, once PDMS stamp and DNA solutions are made for transferring, they can be easily reused for several times of experiment. In addition, their printing process is very simple and DNA printing under various conditions can be attempted due to a relatively short experimental time. The disadvantage of weak binding force between DNA and the surface can be overcome by mixing DNA with high viscosity glycerol and spraying silver solution.

The purpose of this experiment is to detect the surface properties of silver nanodot on the surface condition and for this purpose, the transfer of DNA to the surface must be efficiently performed and a clear distinction is needed whether the emission generated from surface is from DNA or not. Therefore, a

printing protocol capable of confirming a large amount of samples under various surface conditions is needed. Even if the binding interaction between surface and DNA is much weaker, checking various conditions at once is much more efficient and also printing DNA with a clear shape and size is much easier way to distinguish if this emission is from DNA or not. For these reasons, the microcontact printing method was finally used for our sample preparation.

1.4. Factors affecting the printing efficiency and emission spectra

In this research, the several parameters were compared to optimize how can attach DNA on the glass surface more efficiently. First of all, hydrophilicity of the glass coverslip was tested if it can influence the DNA printing efficiency. Coverslips were modified with methyl group or primary amine group attached silane. Simply washed coverslips without modification were also tested. Not only glass coverslip surface, also stamp surface hydrophilicity was tested to transfer DNA from coverslip to stamp efficiently. Then the concentration of DNA and glycerol also examined if they can be physically attached onto the glass

surface stable or not.

To identify if the silver nanodots surface properties were changed on the surface condition or not, emission spectra were detected by a microscope. Samples were prepared in several different conditions after successful DNA printing. Because silver and DNA cannot move and interact with each other on the surface as flexibly as in solution, the reaction system was differently prepared in humid and dry condition. The modifying materials of coverslip that decide the surface functional groups were prepared also differently.

Ⅱ. Experimental section

2.1. Materials

(3-aminopropyl)triethoxysilane (APTES), silver nitrate, polyacrylic acid (PAA), isobutyl(trimethoxy)silane (IBTMS), sodium hydroxide, sodium borohydride, 3-(2-aminoethylamino)propyldimethoxymethylsilane, anhydrous methanol, ethanol, toluene, glycerol, polyvinyl alcohol (PVA) were purchased from Sigma Aldrich and used as received. Acetone, hydrochloric acid, hydrogen peroxide were purchased from Daejung Chemicals&Metals. Ammonia solution was purchased from Samchun Chemicals. SYLGARD™ 184 Silicone Elastomer Kit for making PDMS stamp was purchased by Dow Chemical Company. All ssDNA sequences were purchased from Integrated DNA Technologies.

2.2. Instruments

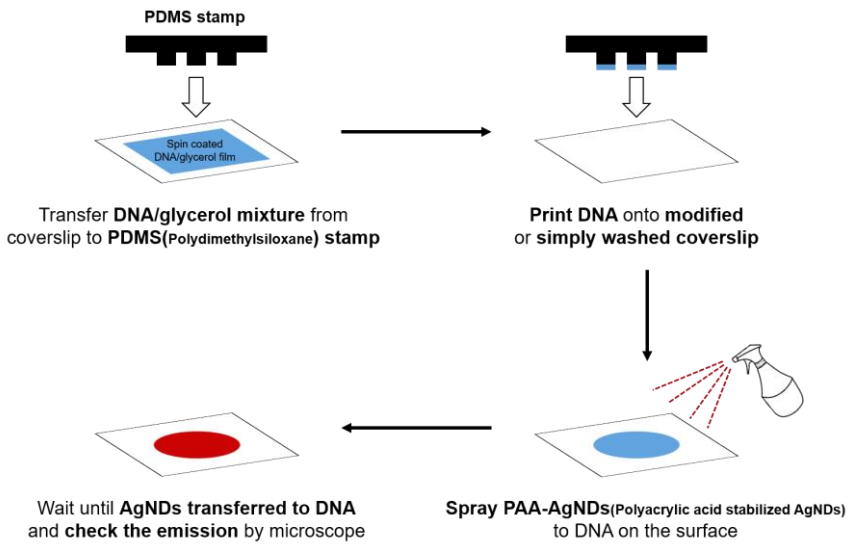
Surface printed pattern of silver nanodots were detected by an Olympus IX81 microscope, with an Andor LucaEM S 658M camera. DNA and glycerol mixture

was spin coated by Rhabdos SC-100RPM spin coater. PDMS stamp was baked by Drying oven FO-450M. All samples were prepared in deionized water (DIW) purified by a Millipore Direct-Q3 ultrapure water system with a resistivity of 18.2 MΩ cm. Solution pH was examined by Eutech SG/pH510 pH meter.

2.3. Preparation of glass coverslips

Glass coverslips were prepared in three different surface condition. Simply washed with detergent and rinsed by DIW, and modified to hydrophilic and hydrophobic. By hydrophilic modification, coverslips were immersed in acidic, basic solution to make hydroxyl group on the surface, then modified with APTES[39]. Hydrophobic modification was performed as same protocol with hydrophilic modification, only type of silane was changed from APTES to IBTMS. To prepare polymer coated coverslips, PVA and PAA solution of same concentration (5 mg/mL) were spin coated by Rhabdos spin coater.

2.4. Preparation of samples



Scheme 1. Protocol of sample preparation. DNA-glycerol mixture was transferred from acetate/APTES modified coverslip to PDMS stamp, and printed on the coverslips of different condition. After DNA printing, polyacrylic acid stabilized silver nanodots (PAA-AgNDs) were sprayed and silver clusters were transferred from PAA to surface DNA.

APTES modified coverslips were prepared in two types; one is for making a thin DNA-glycerol film to transfer DNA mixture from coverslip to PDMS stamp;

another is for transferring DNA pattern from PDMS stamp to the coverslip surface. All APTES modified coverslips were spin coated with an acetate buffer (50 mM, pH 4.67) to protonate surface primary amine group. A DNA-glycerol solution was spin-coated on the acetate/APTES modified coverslip. DNA spin-coated film was contacted to hydrophilic modified PDMS stamp and dried by nitrogen gas. The stamp was then firmly pressed to another acetate/APTES modified coverslip. After DNA totally dried on the coverslip, PAA-AgNDs (0.243 mM, prepared in DIW) was sprayed to DNA pattern, and subsequently silver nanodots in PAA were transferred to ssDNA on the glass surface and became ssDNA encapsulated silver nanodots.

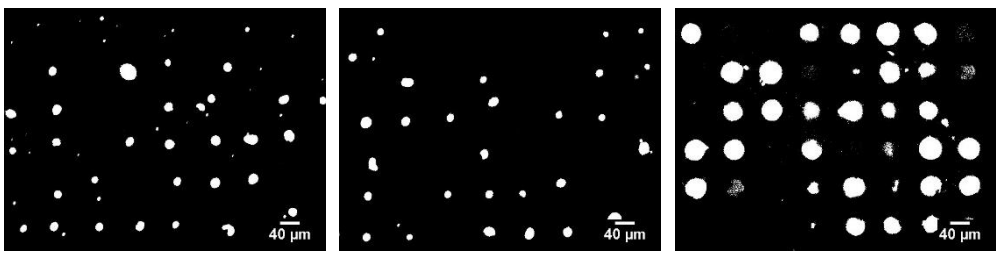
Ⅲ. Results and Discussion

3.1. Optimizing DNA printing on the surface

Printing DNA in standard size and shape on the surface is very important process of this research. During detecting DNA and silver nanodots on the coverslip surface, it is necessary to verify whether the observed emission is definitely from DNA or other materials, because even only PAA-AgNDs were sprayed on the surface without DNA, also bright emissions can be detected by a microscope.

However, when DNA was simply attached on the surface without any specific patterns, the emission from surface DNA which silver nanodots were transferred and the emission from PAA-AgNDs were hard to be distinguished. The reason why DNA was clearly patterned on the surface, not simply attached, was to make sure that detected emission is from a which material. Therefore, to optimize the DNA printing with clear shape on the surface, various of parameters were tested among the printing protocol.

3.1.1. Hydrophilicity of glass coverslip and PDMS stamp



(A) (B) (C)

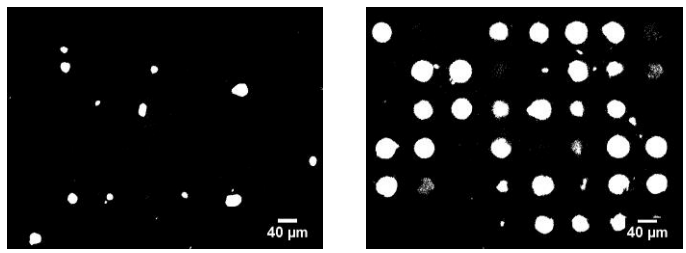
Figure 1. DNA (2 mM) mixed with glycerol (20 %) patterns on the differently modified coverslip, printed by APTES modified PDMS stamp. Coverslips were detergent and DIW washed (A), IBTMS modified (B), APTES modified (C). All patterns were detected by microscope imaging; 420-460 nm excitation filter and 545-580 nm emission filter were used.

DNA patterns were all successfully printed in each of three different coverslip, but the amount of each patterns were differently printed depends on the surface hydrophilicity.

In simply washed coverslip, the amount of DNA patterns were very less, size of pattern was also smaller than regular size, 40 μm (**Figure 1A**). It seems DNA did not transferred enough from PDMS stamp to glass coverslip due to weak

binding interaction between DNA and unmodified glass surface. Similar results were also shown in hydrophobic condition (**Figure 1B**). Like unmodified coverslip, DNA patterns were regularly printed but size of each pattern was smaller than 40 μm and also the number of pattern dots was slightly less than unmodified condition. It seems due to methyl group attached IBTMS, by which the binding interaction between hydrophobic coverslip surface and hydrophilic DNA become weaker.

Only on the hydrophilic silane modified glass surface, the regular size of DNA pattern was successfully generated (**Figure 1C**). In this condition, coverslip surface was modified with APTES that contains amine groups in the carbon chain. DNA could have much stronger interaction with a protonated amine group modified surface compared with other hydrophobic or simply washed surface.

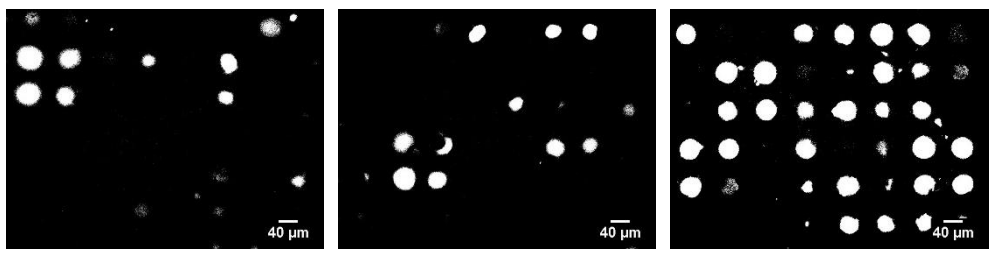


(A) (B)

Figure 2. DNA (2 mM) mixed with glycerol (20 %) patterns on APTES modified coverslip, printed by differently modified PDMS stamp. PDMS stamps were unmodified (A) or modified with APTES (B). All patterns were detected by microscope imaging; 420-460 nm excitation filter and 545-580 nm emission filter were used.

The hydrophilicity of PDMS stamp was also very important for successfully transferring the DNA from DNA-glycerol film to PDMS stamp, and then from PDMS stamp to coverslip to be patterned. DNA patterns were not successfully printed on the surface when using PDMS stamp without any modification (**Figure 2A**). However, the clear DNA patterns appeared when using APTES modified PDMS stamp (**Figure 2B**). Similar as coverslip hydrophilicity, DNA did not attached efficiently to unmodified PDMS stamp due to their low hydrophilicity.

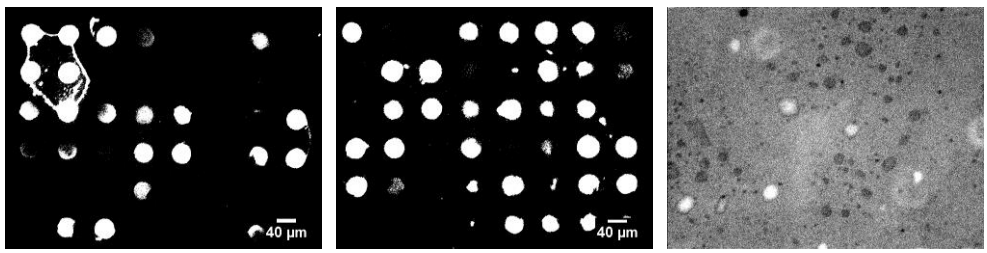
3.1.2. Concentration of DNA and glycerol



(A) (B) (C)

Figure 3. Different concentration of DNA mixed with glycerol (20 %) patterns on the APTES modified coverslip, printed by APTES modified PDMS stamp. Concentration of DNA was 0.5 mM (A), 1.0 mM (B), 2.0 mM (C). All patterns were detected by microscope imaging; 420-460 nm excitation filter and 545-580 nm emission filter were used.

Concentration of DNA also has affected to print the DNA on the surface. Until the concentration of DNA reached to 2 mM, the number of patterns was very less, 0.5 and 1.0 mM (**Figure 3A, 3B**). DNA patterns on the surface were successfully printed when their concentration reached to at least 2 mM (**Figure 3C**) and most of them appeared with standard size in a clear circle shape.



(A) (B) (C)

Figure 4. DNA (2 mM) mixed with different concentration of glycerol patterns on APTES modified coverslip, printed by APTES modified PDMS stamp. Concentration of glycerol was 10 % (A), 20 % (B), 40 % (C). All patterns were detected by microscope imaging; 420-460 nm excitation filter and 545-580 nm emission filter were used.

During printing protocol, silver nanodot was transferred from sprayed PAA-AgNDs solution to DNA patterns on the glass surface. Because water-based solutions were sprayed on the surface, DNA was required to be viscous to keep the clear patterns. So different concentration of viscous glycerol was mixed together with DNA. When the concentration of glycerol was between 10 to 20% (**Figure 4A, 4B**), DNA was successfully fixed on the surface. It seems when the concentration of mixed glycerol increased, fixing efficiency of DNA also increased.

However, the printing efficiency of DNA was decreased in too high concentration of glycerol (**Figure 4C**). When the concentration of glycerol reached to 40%, almost no bright DNA emitters were detected on the surface. It seemed most of DNA molecules were buried in the glycerol and their emission was also blocked.

3.2. Different emission spectra of ssDNA protected silver nanodots depending on the surface condition

After successfully print the DNA on the glass surface, we detected emission spectra of silver nanodots on the surface with different wavelength, 480-520 nm (green range), 545-580 nm (yellow range), 585-625 nm (red range), 665-700 nm (near-IR range), and all samples were excited at 420-460 nm. DNA protected silver nanodots have specific emission spectra under different excitation source when silver nanodots were transferred to solution condition, which means DNA-silver compound can generate pure emitters. However, on the surface condition, emission spectra of DNA-silver compound were generated differently depends on their environment even using same sequence

of ssDNA. To study spectral properties of silver nanodot on the surface, their emission spectra were monitored and compared in different condition. The emission intensity can be treated as equal to the amount of the emitters generated.

3.2.1. Influence of system humidity

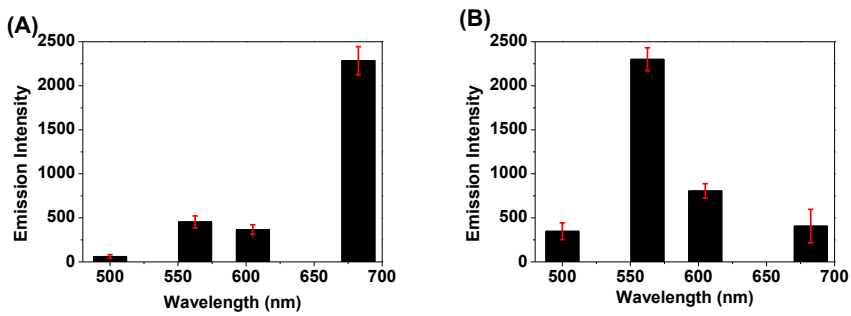


Figure 5. Emission spectra of silver nanodots transferred to C_{20} DNA on the coverslip surface. Each samples were kept in the opened system (A) and sealed system (B) after silver solutions were sprayed to surface DNA.

Silver solutions were sprayed to DNA printed pattern on the glass surface and dried for 1 hr. Samples were kept in different Petri dish. First sample was kept in an open Petri dish without any sealing, second sample was kept in a

sealed Petri dish. The second Petri dish was covered with a lid and the small gap between Petri dish and the lid was completely sealed with parafilm paper, therefore the moisture in the system was saturated during DNA-silver solution reaction.

In opened system (**Figure 5A**), the emission intensity of green to red range were much lower than that in the near-IR range, which means most of the emitters were generated in the near-IR range. Except the near-IR emitters, emission intensity of yellow emitter was highest and of green and red emitter was lower than yellow, indicating not only near-IR emitters were generated, even less amount of yellow emitters were also generated.

However in sealed system (**Figure 5B**), the emission intensity of near-IR range was significantly decreased and of yellow range was highly increased compared with the opened system, which means yellow emitters were predominantly generated. The opening and closing of each system might induced the ambient humidity of the silver solution differently and also affected the reaction time and environment at which silver was transferred to DNA.

In opened system, solutions were evaporated in a short period and the transferring time from silver to DNA was affected by silver solution's

evaporation speed. Due to short evaporation time in opened system, silver might not be fully transferred to DNA as in a solution. Then the generation of emitter species might be influenced by this insufficient interaction between silver and DNA. However, some of the silver might be transferred to DNA successfully even in a short period, so even small amount of yellow emitters were also generated, not only near-IR emitters.

By contrast in sealed system, sprayed silver solution might be remained on the surface for much longer time than as in opened system. The reaction time between silver and DNA might similar as in solution due to humid environment in the Petri dish. Even DNA molecules were fixed with glycerol on the surface, they can more freely interact with silver clusters in the moisture saturated system. During surface DNA was covered with the droplet of silver solution, it can be interacted with silver cluster as much similar condition as in solution until solution droplet evaporated. However, due to perfect sealing of Petri dish, system might be remained in a saturated state and the interaction between silver and DNA can be last for a sufficient time.

In opened system, dry condition, the interaction between silver and DNA was insufficient due to quick evaporation of silver solution. However, in sealed

system, humid condition, during the surface DNA were contacted with silver droplet for a sufficient time, they can be redissolved into silver solution and reacted with silver more efficiently as in solution. Thus, the generation of emitters on the surface can be affected by the system humidity which can influenced the interaction time between silver and DNA.

3.2.2. Influence of surface modifying material

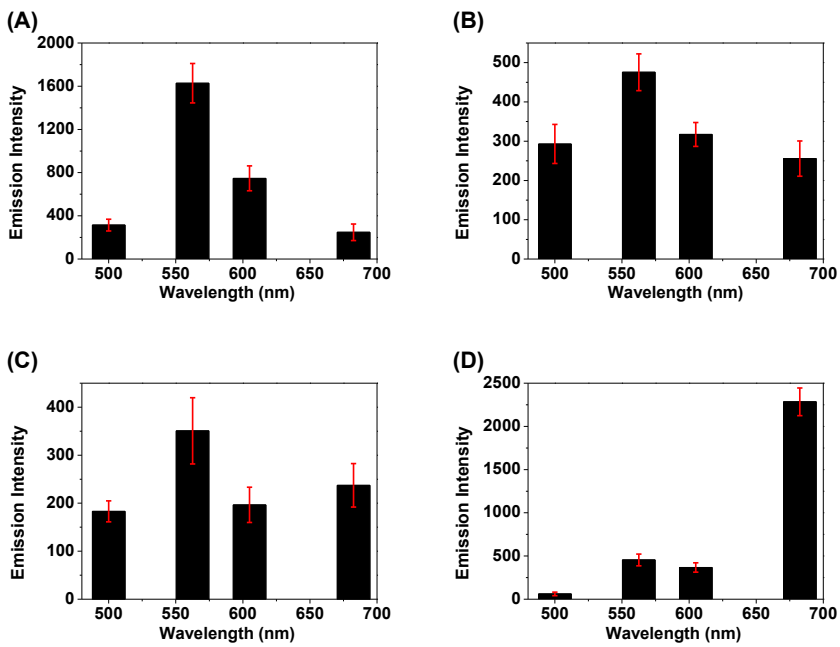


Figure 6. Emission spectra of silver nanodots transferred to C_{20} DNA depends on the coverslip modifying materials. Each coverslips were spin coated with

PVA (A), modified with IBTMS, hydrophobic silane (B), simply washed (C), modified with APTES, hydrophilic silane (D). All samples were prepared in opened system, without any Petri dish sealing.

Emission spectra of C_{20} protected silver nanodots was also shown differently depends on their surface modifying material. Surface modifying materials are important factors for attaching DNA to the glass surface because their binding force might be decided by the affinity with DNA. The binding force depending on the surface modifying material is important factor of rearrangement between silver cluster encapsulated in PAA and surface DNA. Because systems were opened in every condition, the silver solution sprayed on the surface DNA might be evaporated in a short period under dry environment, and unlike humid condition, might not be able to reacted with surface DNA in sufficient time. So unlike in solution, how flexibly DNA can react with silver clusters in a surface environment where it cannot move freely, can be decided by the binding force between the surface modifying material and DNA.

Coverslips were modified with different materials and different functional groups were covered on the each coverslip surface. When compared with C_{20}

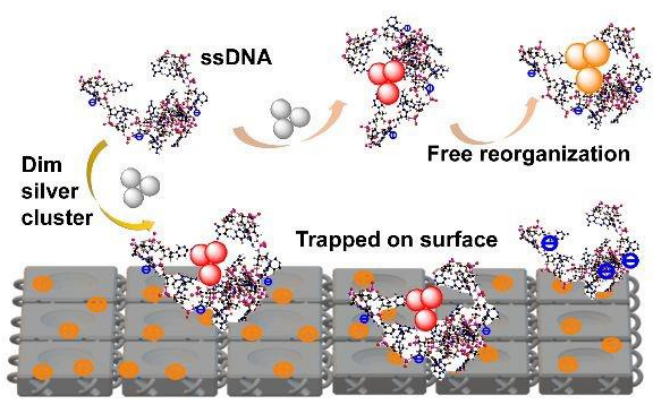
emitters in solution, the most similar emission spectra were detected in the PVA, polymer coated surface, and the most different emission spectra was detected in the APTES, hydrophilic silane modified surface.

In PVA coated condition (**Figure 6A**), similar as in humid condition, yellow emitters were generated the most and near-IR emitters were the less. Similar as in PVA, in IBTMS modified hydrophobic condition (**Figure 6B**), the number of yellow emitters was highest, and near-IR emitters was lowest; similar emission spectra was appeared. However, the relative amount of yellow emitters compared with near-IR was decreased than PVA condition. In unmodified coverslip condition (**Figure 6C**), the yellow and near-IR emitter were generated almost the same amount, but yellow was slightly higher. In APTES hydrophilic modified condition (**Figure 6D**), near-IR emitters were predominantly generated compared with other emitters.

Depends on the surface modifying material and condition, the emission spectra was differently generated even with the same opened system and the same DNA sequence used. Therefore, this result was influenced by how flexibly the DNA can move on the each of surface condition.

DNA was physically attached with coverslip surface after transferred from

PDMS stamp and their binding force with coverslip surface was different in each of surface condition. As tested in printing step, APTES modified surface had stronger affinity with DNA due to their hydrophilicity than IBTMS modified surface, which means DNA can be flexibly moved in IBTMS condition than APTES condition. The same principle might be applied between unmodified and APTES modified condition, DNA can more flexibly move in unmodified condition. In PVA coated surface, because all area of coverslip surface was physically blocked by concentrated PVA, the affinity between surface and DNA might be decreased compared with other condition.



Scheme 2. Kinetic trapping of silver nanodots and DNA on the surface. DNA and silver clusters were hard be reorganized efficiently due to strong interaction between surface and materials, the low flexibility of DNA.

Depends on the surface flexibility of DNA and silver, different emitters can be generated. When there was strong affinity between surface and DNA, DNA can be kinetically trapped on the surface and cannot move freely and flexibly. In this condition, even if silver solution was successfully transferred to surface DNA, they cannot be reorganized efficiently because DNA is strongly fixed and trapped on the surface. Due to their inefficient reorganization, the species of emitters also differently generated. The near-IR emitters were predominantly generated when DNA was strongly fixed on the surface such as APTES hydrophilic modified condition.

By contrast, when the affinity between the surface and DNA was less, DNA can move more flexibly even on the surface condition and the reorganization and reconstruction between silver clusters and DNA can be more efficiently occurred. In this condition, more similar emitters can be generated as in solution. Mostly yellow emitters were generated when DNA and surface affinity was less such as PVA coated or IBTMS hydrophobic modified condition. The same principle can be applied to the generation of different emitters depends on system humidity because DNA can more flexibly move in humid condition than dry condition.

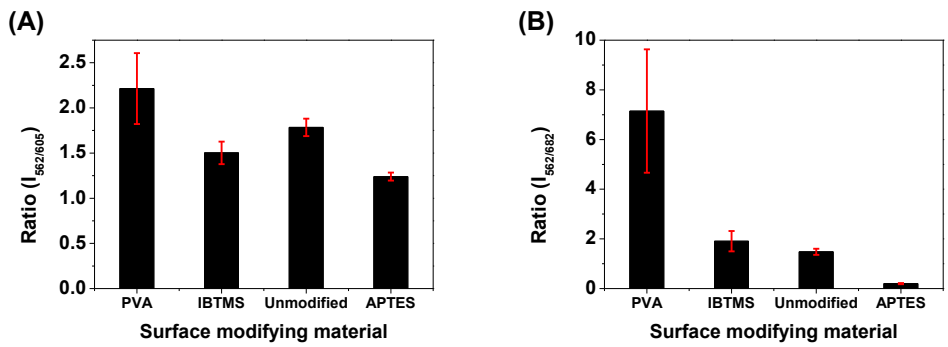


Figure 7. The ratio of C_{20} emission intensity depends on different surface modifying materials between two emitters, the yellow and red emitters (A), yellow and near-IR emitters (B).

The emission spectra depend on the surface modifying materials was differently appeared in each of condition. Experiment result indicated that the DNA flexibility on the surface had an important role in the generation of emitters species.

Basically C_{20} emitters can emit at 523 nm, therefore the red emitters cannot be predominantly generated in any conditions, especially the yellow and red emitters were generated in almost similar ratio in every condition (**Figure 7A**). The amount of red emitter was considered that not significantly affected by different surface modifying materials. It seemed that only the near-IR emitters

were affected by different surface conditions (**Figure 7B**), depending on how strong the interaction between the surface and the material is.

3.2.3. Influence of surface pH

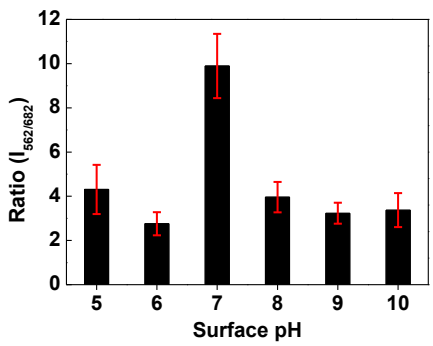


Figure 8. The ratio of C_{20} emission intensity between the yellow and near-IR emitters depends on surface pH. DNA was printed on the simply washed coverslip spin coated by PAA with different pH (5, 6, 7, 8, 9 and 10).

Generation of emitters was also affected by different surface pH (**Figure 8**). The ratio between yellow and near-IR was detected almost similarly in all range of pH, between 2 to 4. Because every value was over the 1, this result indicated that the yellow emitters were generated more than near-IR emitters in every pH.

All samples were prepared in simply washed coverslip and the near-IR emitters were always less generated than yellow emitters in this unmodified condition (**Figure 6C, 7B**), so this result seemed reasonable. The ratio between yellow and near-IR emitter was slightly increased compared with previous unmodified condition, the reason might be due to polymer spin coating on the coverslip surface (**Figure 6A, 7B**).

However in pH 7, value was highly increased compared with other pH condition. The value was near 10 in pH 7, while the average values of other pH condition were nearby 2 to 4. Result might be influenced by electrostatic force between surface and DNA. In lower surface pH, surface was charged more positively and electrostatic interaction between negatively charged DNA was increased. Therefore, more near-IR emitters were generated due to increasing of kinetic trapping between surface and DNA. However, in neutral charged pH 7, which has a lowest electrostatic interaction with negatively charged DNA, the amount of DNA trapped on the surface was much less that other pH condition, thus less number of near-IR emitters was generated. Finally, the ratio between yellow and near-IR emitters were highest in pH 7.

3.2.4. Influence of DNA sequence

The generation of near-IR emitters were not only influenced by different surface condition, also by the sequence of DNA used for silver transfer[19 40]. Even C_{20} was mostly used for the experiment, different sequence of DNA sequences were also compared. All different DNA sequence condition was prepared in APTES modified coverslip due to printing efficiency and pattern recognition.

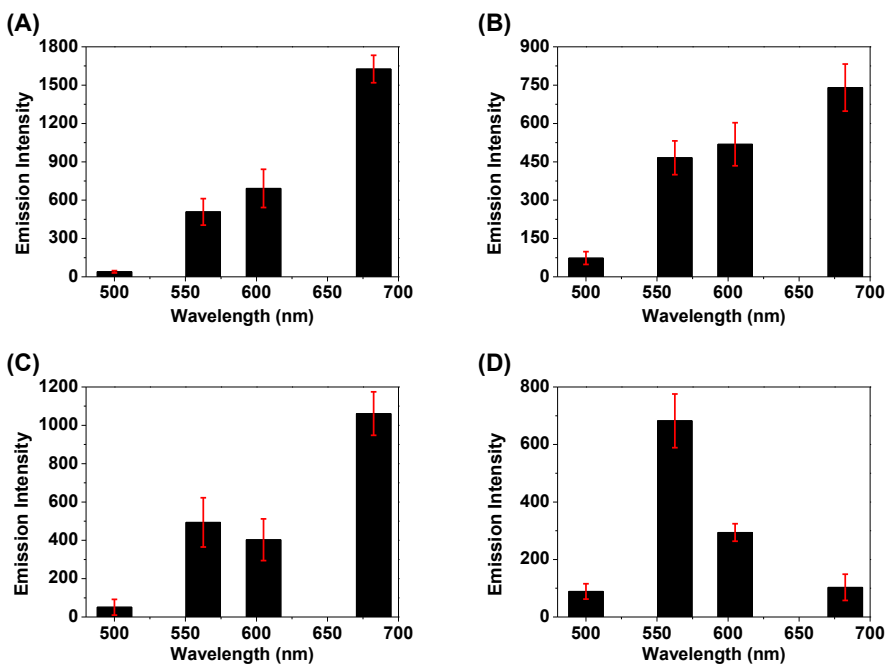


Figure 9. Emission spectra of silver nanodot transferred to different sequence of DNA. 5'-TTCCCACCCACCCCGGCCC-3' DNA used sample was kept in the opened system (A) and sealed system (B), 5'-TTAACCCCCCCCCCCCTTAA-3' ($TTAAC_{12}$) DNA used sample was also kept in the opened system (C) and sealed system (D) after silver solutions were sprayed to surface DNA.

Emission spectra was also influenced by different DNA sequence. When silver was transferred to the 5'-TTCCCACCCCCCCCGCCC-3' DNA in dry condition (**Figure 9A**), the near-IR emitters were predominantly generated, and the amount of the remaining emitters were increased sequentially from the lower to higher wavelength. Tendency of emission spectra was also similar in humid condition (**Figure 9B**), the amount of green emitter was lowest, near-IR emitter was highest. Even the amount of near-IR emitter was decreased compared with dry condition, near-IR amount was still highest unlike C_{20} which near-IR emitter amount became less than other emitters.

However, in $TTAAC_{12}$ condition, their emission spectra were generated similar as C_{20}. Near-IR emitter was predominantly generated but yellow also slightly generated in dry condition (**Figure 9C**), and the amount of near-IR

emitter was significantly decreased and yellow highly increased in humid condition (**Figure 9B**). The emission spectral tendency of $TTAAC_{12}$ in both dry and humid condition almost similarly appeared as C_{20} (**Figure 5**), even the amount difference between yellow and near-IR was less.

Though only two additional DNA sequences were used for experiment, their emission spectra difference depends on humidity differently appeared in each sequence. In addition, the generation of near-IR emitters was always influenced by the surface flexibility of DNA and silver nanodots regardless of DNA sequence.

Ⅳ. Conclusions

DNA printing efficiency was mostly influenced by the concentration of materials and the hydrophilicity of PDMS stamp and surface. Through the DNA printed in a clear shape on the surface, it was possible to be certain that detected emission was from the silver nanodots transferred to DNA.

The emission spectra of silver nanodots which transferred to the DNA on the surface condition differently appeared as in solution. On the surface, emitters were not generated the same as in solution and the large amount of near-IR emitters were additionally generated. The generation of the emitters on the surface condition was influenced by various factors such as system humidity, surface modifying materials, surface pH, or DNA sequence and related to how the DNA and silver nanodots were kinetically trapped on the surface. The near-IR emitters were easily generated when DNA and silver nanodots could not be reorganized efficiently while they were strongly trapped on the surface and cannot be moved flexibly unlike solution. In contrast, when DNA and silver nanodots could move more flexibly on the surface, emitters were generated more similarly as in solution.

These result indicated that emitters can be selectively generated on the surface even using same DNA sequence by controlling the surface environment.

Ⅴ. References

(1) Díez, I.; Ras, R. H. A. Fluorescent silver nanoclusters. *Nanoscale* 2011, *3* (5), 1963-1970, 10.1039/C1NR00006C. DOI: 10.1039/C1NR00006C.

(2) Choi, S.; Dickson, R. M.; Yu, J. Developing luminescent silver nanodots for biological applications. *Chemical Society Reviews* 2012, *41* (5), 1867-1891, 10.1039/C1CS15226B. DOI: 10.1039/C1CS15226B.

(3) Xie, Y.-P.; Shen, Y.-L.; Duan, G.-X.; Han, J.; Zhang, L.-P.; Lu, X. Silver nanoclusters: synthesis, structures and photoluminescence. *Materials Chemistry Frontiers* 2020, *4* (8), 2205-2222, 10.1039/D0QM00117A. DOI: 10.1039/D0QM00117A.

(4) Ritchie, C. M.; Johnsen, K. R.; Kiser, J. R.; Antoku, Y.; Dickson, R. M.; Petty, J. T. Ag Nanocluster Formation Using a Cytosine Oligonucleotide Template. *The Journal of Physical Chemistry C* 2007, *111* (1), 175-181. DOI: 10.1021/jp0648487.

(5) Yuan, X.; Luo, Z.; Yu, Y.; Yao, Q.; Xie, J. Luminescent Noble Metal Nanoclusters as an Emerging Optical Probe for Sensor Development. *Chemistry - An Asian Journal* 2013, *8* (5), 858-871. DOI: https://doi.org/10.1002/asia.201201236.

(6) Guisbiers, G.; Mejía-Rosales, S.; Leonard Deepak, F. Nanomaterial Properties: Size and Shape Dependencies. *Journal of Nanomaterials* 2012, *2012*, 180976. DOI:

10.1155/2012/180976.

(7) Choi, S.; Yu, J. Understanding Interactions between Cellular Matrices and Metal Complexes: Methods To Improve Silver Nanodot-Specific Staining. *Chemistry – A European Journal* 2016, *22* (36), 12660-12664. DOI: https://doi.org/10.1002/chem.201602489.

(8) Yeh, H.-C.; Sharma, J.; Han, J. J.; Martinez, J. S.; Werner, J. H. A DNA—Silver Nanocluster Probe That Fluoresces upon Hybridization. *Nano Letters* 2010, *10* (8), 3106-3110. DOI: 10.1021/nl101773c.

(9) Frangioni, J. V. In vivo near-infrared fluorescence imaging. *Curr Opin Chem Biol* 2003, *7* (5), 626-634. DOI: 10.1016/j.cbpa.2003.08.007 From NLM.

(10) Zhao, Y.; Choi, S.; Yu, J. In Situ Generated Silver Nanodot Förster Resonance Energy Transfer Pair Reveals Nanocage Sizes. *The Journal of Physical Chemistry Letters* 2020, *11* (16), 6867-6872. DOI: 10.1021/acs.jpclett.0c01950.

(11) Biener, J.; Wittstock, A.; Baumann, T. F.; Weissmüller, J.; Bäumer, M.; Hamza, A. V. Surface Chemistry in Nanoscale Materials. *Materials* 2009, *2* (4), 2404-2428. DOI: 10.3390/ma2042404 PMC.

(12) Petty, J. T.; Zheng, J.; Hud, N. V.; Dickson, R. M. DNA-Templated Ag Nanocluster Formation. *Journal of the American Chemical Society* 2004, *126* (16), 5207-5212. DOI: 10.1021/ja031931o.

(13) Zheng, J.; Nicovich, P. R.; Dickson, R. M. Highly Fluorescent Noble-Metal

Quantum Dots. *Annual Review of Physical Chemistry* 2007, *58* (1), 409–431. DOI: 10.1146/annurev.physchem.58.032806.104546.

(14) Kelly, K. L.; Coronado, E.; Zhao, L. L.; Schatz, G. C. The Optical Properties of Metal Nanoparticles: The Influence of Size, Shape, and Dielectric Environment. *The Journal of Physical Chemistry B* 2003, *107* (3), 668–677. DOI: 10.1021/jp026731y.

(15) Yu, J. From Coinage Metal to Luminescent Nanodots: The Impact of Size on Silver's Optical Properties. *Journal of Chemical Education* 2014, *91* (5), 701–704. DOI: 10.1021/ed400416b.

(16) Zheng, J.; Dickson, R. M. Individual Water-Soluble Dendrimer-Encapsulated Silver Nanodot Fluorescence. *Journal of the American Chemical Society* 2002, *124* (47), 13982–13983. DOI: 10.1021/ja028282l.

(17) Choi, S.; Yu, J. Recent development in deciphering the structure of luminescent silver nanodots. *APL Materials* 2017, *5* (5), 053401. DOI: 10.1063/1.4974515.

(18) Jeon, S. M.; Choi, S.; Lee, K.; Jung, H.-S.; Yu, J. Significantly improved stability of silver nanodots via nanoparticles encapsulation. *Journal of Photochemistry and Photobiology A: Chemistry* 2018, *355*, 479–486. DOI: https://doi.org/10.1016/j.jphotochem.2017.05.045.

(19) Guo, W.; Yuan, J.; Dong, Q.; Wang, E. Highly Sequence-Dependent Formation of Fluorescent Silver Nanoclusters in Hybridized DNA Duplexes for

Single Nucleotide Mutation Identification. *Journal of the American Chemical Society* 2010, *132* (3), 932-934. DOI: 10.1021/ja907075s.

(20) Choi, S.; Yu, J.; Patel, S. A.; Tzeng, Y.-L.; Dickson, R. M. Tailoring silver nanodots for intracellular staining. *Photochemical & Photobiological Sciences* 2011, *10* (1), 109-115, 10.1039/C0PP00263A. DOI: 10.1039/C0PP00263A.

(21) Kim, S. H.; Liu, Y.; Hoelzel, C.; Zhang, X.; Lee, T.-H. Super-Resolution Optical Lithography with DNA. *Nano Letters* 2019, *19* (9), 6035-6042. DOI: 10.1021/acs.nanolett.9b01873.

(22) Lum, W.; Gautam, D.; Chen, J.; Sagle, L. B. Single molecule protein patterning using hole mask colloidal lithography. *Nanoscale* 2019, *11* (35), 16228-16234, 10.1039/C9NR05630K. DOI: 10.1039/C9NR05630K.

(23) Seo, J. H.; Shin, D. S.; Mukundan, P.; Revzin, A. Attachment of hydrogel microstructures and proteins to glass via thiol-terminated silanes. *Colloids Surf B Biointerfaces* 2012, *98*, 1-6. DOI: 10.1016/j.colsurfb.2012.03.025 From NLM.

(24) Kershner, R. J.; Bozano, L. D.; Micheel, C. M.; Hung, A. M.; Fornof, A. R.; Cha, J. N.; Rettner, C. T.; Bersani, M.; Frommer, J.; Rothemund, P. W. K.; et al. Placement and orientation of individual DNA shapes on lithographically patterned surfaces. *Nature Nanotechnology* 2009, *4* (9), 557-561. DOI: 10.1038/nnano.2009.220.

(25) Hung, A. M.; Noh, H.; Cha, J. N. Recent advances in DNA-based directed assembly on surfaces. *Nanoscale* 2010, *2* (12), 2530-2537, 10.1039/C0NR00430H.

DOI: 10.1039/C0NR00430H.

(26) Xuan, T.; Shi, S.; Wang, L.; Kuo, H.-C.; Xie, R.-J. Inkjet-Printed Quantum Dot Color Conversion Films for High-Resolution and Full-Color Micro Light-Emitting Diode Displays. *The Journal of Physical Chemistry Letters* 2020, *11* (13), 5184-5191. DOI: 10.1021/acs.jpclett.0c01451.

(27) Li, Y.; Li, P. C. H.; Parameswaran, M.; Yu, H.-Z. Inkjet Printed Electrode Arrays for Potential Modulation of DNA Self-Assembled Monolayers on Gold. *Analytical Chemistry* 2008, *80* (22), 8814-8821. DOI: 10.1021/ac801420h.

(28) Chu, Y.; Qian, C.; Chahal, P.; Cao, C. Printed Diodes: Materials Processing, Fabrication, and Applications. *Advanced Science* 2019, *6* (6), 1801653. DOI: https://doi.org/10.1002/advs.201801653.

(29) Kim, B. H.; Onses, M. S.; Lim, J. B.; Nam, S.; Oh, N.; Kim, H.; Yu, K. J.; Lee, J. W.; Kim, J.-H.; Kang, S.-K.; et al. High-Resolution Patterns of Quantum Dots Formed by Electrohydrodynamic Jet Printing for Light-Emitting Diodes. *Nano Letters* 2015, *15* (2), 969-973. DOI: 10.1021/nl503779e.

(30) Nakao, H.; Gad, M.; Sugiyama, S.; Otobe, K.; Ohtani, T. Transfer-Printing of Highly Aligned DNA Nanowires. *Journal of the American Chemical Society* 2003, *125* (24), 7162-7163. DOI: 10.1021/ja034185w.

(31) Lange, S. A.; Benes, V.; Kern, D. P.; Hörber, J. K. H.; Bernard, A. Microcontact Printing of DNA Molecules. *Analytical Chemistry* 2004, *76* (6), 1641-1647. DOI: 10.1021/ac035127w.

(32) Xu, C.; Taylor, P.; Ersoz, M.; Fletcher, P. D. I.; Paunov, V. N. Microcontact printing of DNA-surfactant arrays on solid substrates. *Journal of Materials Chemistry* 2003, *13* (12), 3044-3048, 10.1039/B307788H. DOI: 10.1039/B307788H.

(33) Yu, A. A.; Savas, T.; Cabrini, S.; diFabrizio, E.; Smith, H. I.; Stellacci, F. High Resolution Printing of DNA Feature on Poly(methyl methacrylate) Substrates Using Supramolecular Nano-Stamping. *Journal of the American Chemical Society* 2005, *127* (48), 16774-16775. DOI: 10.1021/ja055762e.

(34) Geissler, M.; Roy, E.; Deneault, J.-S.; Arbour, M.; Diaz-Quijada, G. A.; Nantel, A.; Veres, T. Stretching the Stamp: A Flexible Approach to the Fabrication of Miniaturized DNA Arrays. *Small* 2009, *5* (22), 2514-2518. DOI: https://doi.org/10.1002/smll.200900862.

(35) Jaekel, A.; Stegemann, P.; Saccà, B. Manipulating Enzymes Properties with DNA Nanostructures. *Molecules* 2019, *24* (20), 3694.

(36) Zheng, Y.; Lalander, C. H.; Thai, T.; Dhuey, S.; Cabrini, S.; Bach, U. Gutenberg-Style Printing of Self-Assembled Nanoparticle Arrays: Electrostatic Nanoparticle Immobilization and DNA-Mediated Transfer. *Angewandte Chemie International Edition* 2011, *50* (19), 4398-4402. DOI: https://doi.org/10.1002/anie.201006991.

(37) Wu, P.; Hogrebe, P.; Grainger, D. W. DNA and protein microarray printing on silicon nitride waveguide surfaces. *Biosensors and Bioelectronics* 2006, *21* (7), 1252-1263. DOI: https://doi.org/10.1016/j.bios.2005.05.010.

(38) Schlingman, D. J.; Mack, A. H.; Mochrie, S. G. J.; Regan, L. A new method for the covalent attachment of DNA to a surface for single-molecule studies. *Colloids and Surfaces B: Biointerfaces* 2011, *83* (1), 91-95. DOI: https://doi.org/10.1016/j.colsurfb.2010.11.002.

(39) Qin, M.; Hou, S.; Wang, L.; Feng, X.; Wang, R.; Yang, Y.; Wang, C.; Yu, L.; Shao, B.; Qiao, M. Two methods for glass surface modification and their application in protein immobilization. *Colloids and Surfaces B: Biointerfaces* 2007, *60* (2), 243-249. DOI: https://doi.org/10.1016/j.colsurfb.2007.06.018.

(40) Latorre, A.; Somoza, Á. DNA-Mediated Silver Nanoclusters: Synthesis, Properties and Applications. *ChemBioChem* 2012, *13* (7), 951-958. DOI: https://doi.org/10.1002/cbic.201200053.

국문 초록

다양한 은 나노닷 특성은 용액 상태에서 연구되어 왔고 특성화되어 왔다. 하지만 표면에서 그들의 특성에 관한 연구는 아직 충분히 연구되지 않았다. 본 연구는 유리 커버슬립 표면에 단일 가닥 DNA(ssDNA)를 프린트하고 그 위에 폴리아크릴산으로 안정화된 은 나노닷(PAA-AgNDs)을 이동시켜, 그 표면에서 ssDNA로 둘러싸인 은 나노닷의 발광 스펙트럼을 조사하였다. 용액에서와 같은 ssDNA와 PAA-AgNDs를 사용하더라도, 유리 표면에서는 시스템의 습도, 표면 개질 물질, 표면 pH와 같은 표면 조건에 따라 다른 파장을 지닌 발광체가 우세하게 생성되었다. 표면과 DNA가 강한 친화력을 가질 때 DNA는 표면에 동역학적으로 갇힐 수 있고, 용액에서와 같이 유연하게 은 나노닷으로 재구성될 수 없다. 같은 DNA와 은 나노닷을 사용하더라도 표면 조건을 조절함으로써 다양한 발광체를 생성할 수 있다.

주요어: 은 나노닷, 표면 특성, DNA 프린팅, 동역학적 갇힘, 표면 DNA-은 재구성

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