

그래핀 기반의 광학적 바이오 센서 연구 동향

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1. 서론

- ▶ 광학적 바이오센서는 빛을 이용해 인지하는 센서로 빛을 감지할 수 있는 광소자, 발색을 일으킬 수 있는 광물질, 이러한 빛의 변화를 외부의 간섭 없이 측정할 수 있는 외부기구 등으로 되어 있으며 복잡하고 고가의 분석기기가 필요 없다는 점이 큰 장점이다. 센서 종류로는 혈당측정 바이오센서, DNA 검출, 헤모글로빈, 단백질 검출, 효소나 생리 활성 물질 검출 등 진단학적으로 중요한 센서로 일부 상용화되었다.¹
- ▶ 광학적 바이오센서에서 형광을 이용한 센서는 형광 자체의 높은 감응도로 인한 장점을 지니고 있으나 형광 물질의 농도, 형광 물질 주위의 pH, 극성, 온도 등의 변화, 그리고 형광 물질 자체의 광 불안정성 등과 같은 요인에 크게 영향을 받고 있어 이러한 요인들을 최소화하기 위한 노력 중 하나로서, excitation 혹은 emission 의 형광 강도의 비의 변화를 관측하는 방법으로 fluorescence resonance energy transfer(FRET)이다.²
- ▶ FRET 원리를 이용한 광학적 바이오 센서는 생체 및 환경 분야에 이미 널리 활용되고 있고, 무한한 잠재적 가능성으로 인해 활발히 연구되고 있으며 지금까지 개발 동향을 크게 유기 형광 물질, 양자점, 그리고 나노입자에 대해서 보고되고 있다.
- ▶ 본 정보는 그래핀 기반의 광학적 바이오 센서에 대한 최근 연구 동향에 대해 기술하고자 한다.

2. Graphene–quantum dots composites을 이용한 electrogenerated chemiluminescence immunosensing

➤ Fabrication of graphene–quantum dots composites for sensitive electrogenerated chemiluminescence immunosensing ³

-Poly(diallyldimethylammonium chloride) (PDDA)-protected graphene–CdSe (P-GR-CdSe) 복합필름을 이용하여 human IgG (HIgG)를 검출하기 위한 electrogenerated chemiluminescence (ECL) immunosensor 연구에 대한 보고임.

-P-GR-CdSe 복합필름은 높은 ECL intensity, 훌륭한 전기적 전도도, 빠른 응답, 충분한 안정성, 고 민감도를 측정함으로써 ECL biosensors로의 타당성을 증명함.

-P-GR-CdSe 복합 필름 위에 PDDA 와 gold nanoparticles (GNPs)의 공유결합은 ECL intensity를 향상시켜주었고, antibody (Ab), goat anti–human IgG은 P-GR-CdSe/PDDA/GNP 필름 위에 연결한 후 bovine serum albumin (BSA)를 blocking 함으로써 human IgG를 검출하기 위한 ECL immunosensor의 개발을 시도함.

-ECL immunosensor는 0.005 pg mL^{-1} 의 검출한계, $0.02\text{--}2000 \text{ pg mL}^{-1}$ 의 linear range를 가지는 HIgG의 고 민감도를 보임으로써 이 센서는 높은 specificity, 훌륭한 재현성, 오래 시간의 안정성을 보였으며 단백질 센싱을 위한 기대되는 센서임을 보고함.

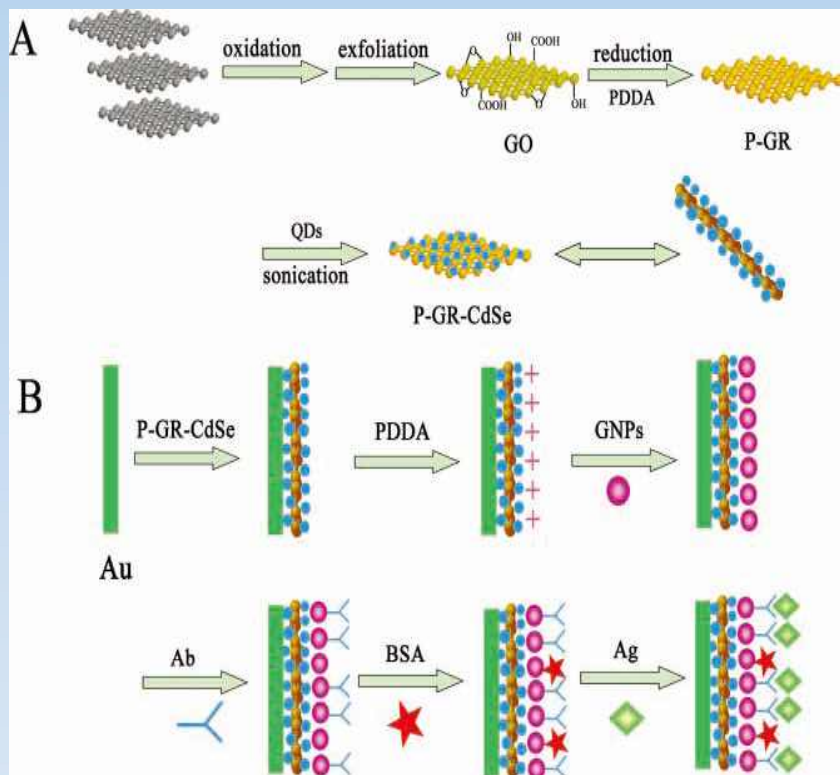


Figure 1. (A) Schematic representation of preparation procedure of P-GR-CdSe composites, including the oxidation of graphite (gray blocks) to graphite oxide (GO) with abundant oxygen functionalities, the in situ reduction of GO in the presence of PDDA to obtain positively charged PDDA protected graphene (P-GR) colloids, and the preparation of P-GR-CdSe composites via electrostatic interactions under sonication. (B) Schematic illustration of the stepwise immunosensor fabrication process, including the formation of P-GR-CdSe composite film on the Au electrode, the linkage of PDDA to the film, the conjugation of GNPs to PDDA, the immobilization of antibody (Ab) on the electrode via GNPs, and the specific immunoreaction.

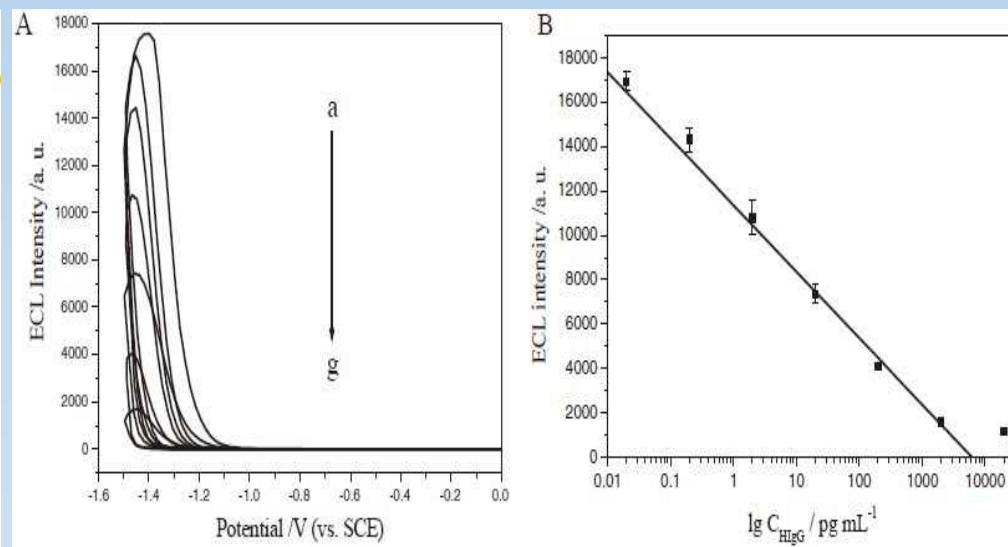


Figure 2. (A) ECL – potential curves of the immunosensor in the absence (a) and presence (b–g) of different concentrations of HIgG (pg mL^{-1}): a) 0, b) 0.02, c) 0.2, d) 2, e) 20, f) 200, g) 2 000, scanning from 0 V to -1.5 V with a scan rate of 200 mV s^{-1} . The voltage of the PMT was set at 600 V. (B) Calibration curve for HIgG determination. Error bars were calculated from triple parallel experiments.

Serum samples	1	2	3	4
Immunosensor [pg mL^{-1}] ^{a)}	0.96	2.03	28.1	314.4
ELISA [pg mL^{-1}]	0.90	1.94	26.7	300.0
Relative deviation [%]	6.7	4.6	5.2	4.8

^{a)} Average value from three successive determinations.

Table 1. Comparison of serum HIgG levels determined using two methods.

3. Peptide-functionalized colloidal graphene의 enzyme 광학적 검출

➤ Peptide-functionalized colloidal graphene via interdigitated bilayer coating and fluorescence turn-on detection of enzyme⁴

-그래핀 기반 광학적 센싱에 의한 검출방법에서 중요한것 중의 하나는 높은 solubility와 colloidal 안정성을 가지는 기능화된 그래핀의 준비와 fluorophore와 그래핀의 표면 사이의 거리가 5nm이하로 최적화해야하므로 본 연구는 이러한 문제를 해결하기 위하여 interdigitated bilayer type coating을 통한 3nm이하의 두께, 높은 colloidal 안정성, graphene-fluorophore substrate의 빠른 광학적 응답등의 결과를 보고함.

-peptide-functionalized colloidal graphene를 준비 단계:

- (1) 그래핀 산화물 소수성으로 만들기 위하여 정전기적 결합에 의한 didodecyldimethyl ammonium bromide (DDAB)으로 코팅.
- (2) 수용성 폴리머(양쪽 친매성 폴리머: poly(maleic anhydride-1-octadecene)는 interdigitated bilayer structure로 부착됨.
- (3)아미노기를 가지고 있는 diamine는 폴리머의 anhydride groups에 반응 시켜 amine 기를 유도함. 그 다음 Hydrazine 처리에 의한 그래핀 산화물을 그래핀으로 전환시키고 화학적 공유결합에 의하여 peptide 결합을 시켜 준비함.

-Peptide-functionalized colloidal graphene은 enzyme의 광학적 검출을 위하여 준비하였으며 광학적 센싱을 위한 현광 염색은 peptide에 공유결합에 의해 연결됨. 현광 염색은 그래핀에 의하여 quenching되나 nanomolar 영역에서 chymotrypsin의 농도에 따라 enzyme 검출 민감도를 보임. 이 연구결과는 in vitro enzyme assay의 가능성을 증명함.

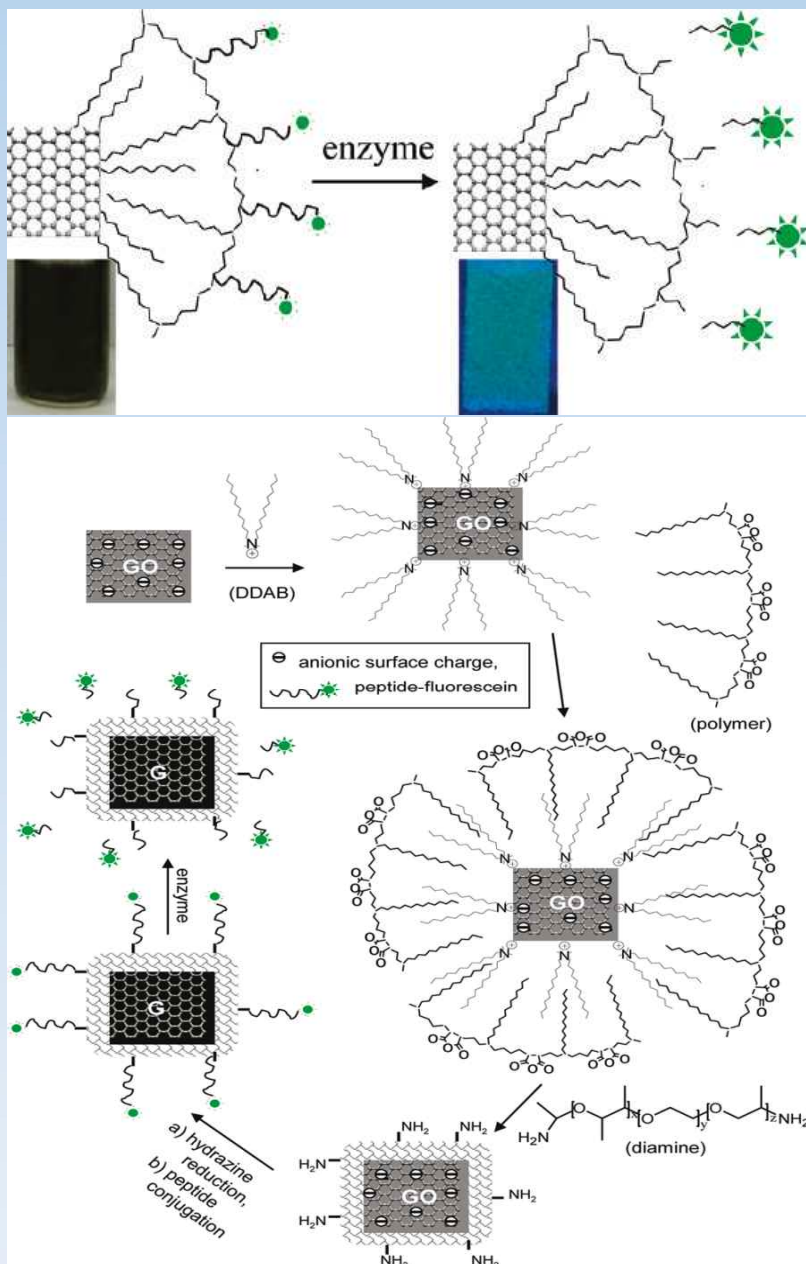


Figure 3. Coating and Functionalization Strategy of Colloidal Graphene Oxide (GO) and Graphene (G)

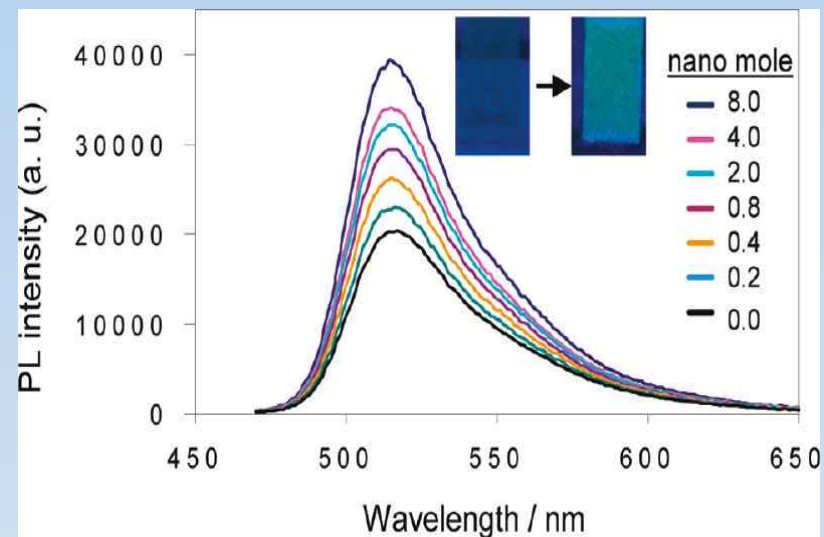


Figure 4. Enzyme assay using functional graphene. Different amounts of R-chymotrypsin are mixed with colloidal solution of peptide-functionalized graphene and fluorescence spectra were measured after 5 h. Inset shows the visible change after 5 h as observed under UV light for 8 nano mole enzyme.

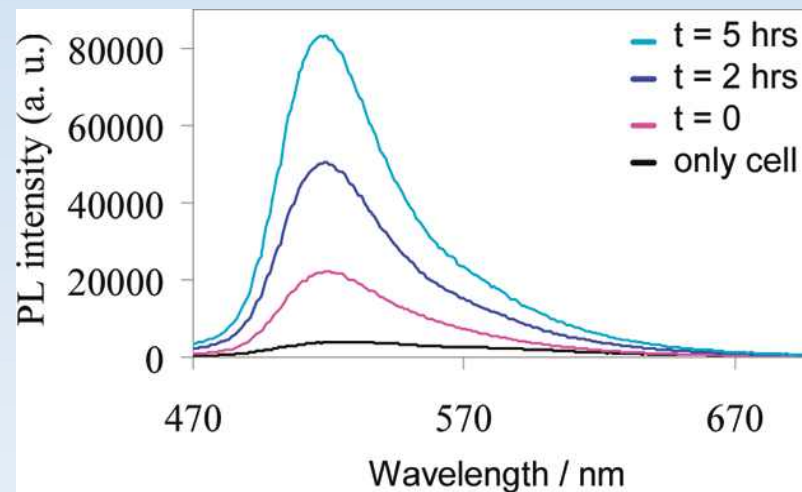


Figure 5. Cellular enzyme assay using functional graphene. H9C2 cells along with culture media were mixed with peptide-functionalized graphene solution, incubated for different time and then fluorescence was recorded. Increased fluorescence is due to the enzyme action.

4. Intracellular protease sensor로써 graphene oxide-peptide conjugate

➤ Graphene oxide-peptide conjugate as an intracellular protease sensor for caspase-3 activation imaging in live cells⁵

-살아 있는 세포 (live-cell)에서 세포사멸 (apoptosis)와 관련있는 caspase-3 activation를 센싱하기 위하여 fluorophore를 라벨화한 graphene oxide (GO)-peptide conjugate를 기반으로 intracellular protease sensor를 개발하고 그 원리를 증명하고자함.

-Caspase-3 activation에 대한 intracellular protease sensor 개발은 dysregulated apoptosis에 대한 약물 검사에 필요할 뿐만 아니라 생리학적, 병리학적으로 protease의 역할을 이해하는것이 매우 중요하기 때문에 필요함.

-GO-peptide conjugate가 다량의 caspase-3를 검출하기 위한 간단하면서 고 민감성, 선택성 있는 센서임을 증명하였으며 또한 세포 안으로 잘 인도되어 caspase-3 activation의 high contrast imaging하기 위한 intracellular protease sensor임을 증명함.

-다중 방식 in vitro assays나 multicolor fluorophore tags를 붙인 다른 종류의 peptide 결합에 의한 multiple proteases의 live-cell imaging등으로 메디컬 연구나 임상진단 등 다양하게 응용할 수 있음을 보고한 논문임.

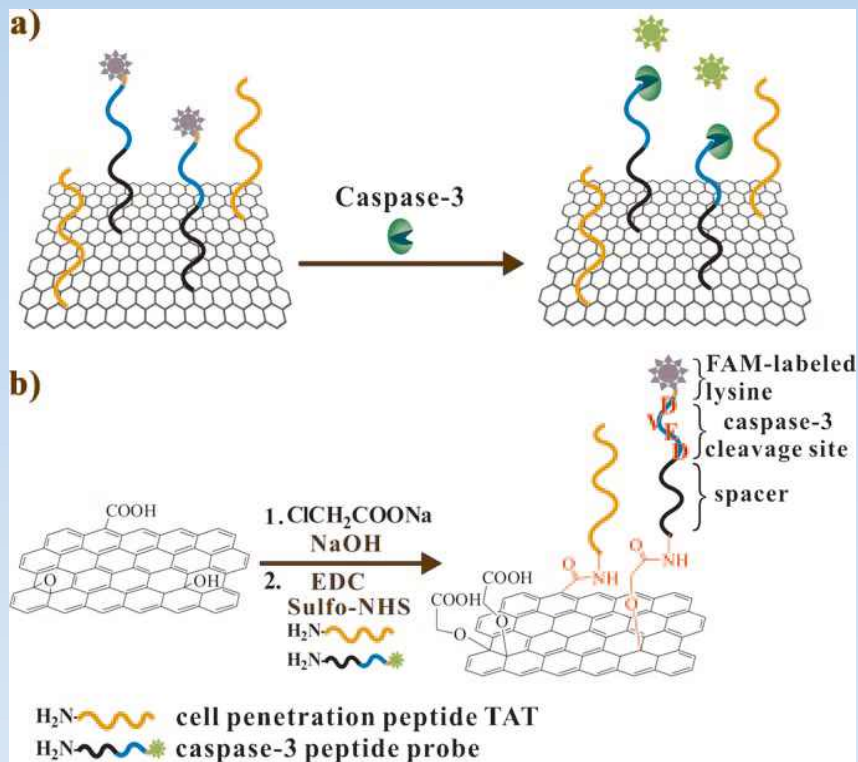


Figure 6. (a) Caspase-3 detection using GO-peptide conjugate. (b) Construction of GO-peptide conjugate. EDC=N-ethyl-N'-[3-(dimethylamino) propyl]carbodiimide hydrochloride, Sulfo-NHS=N-hydroxysulfosuccinimide.

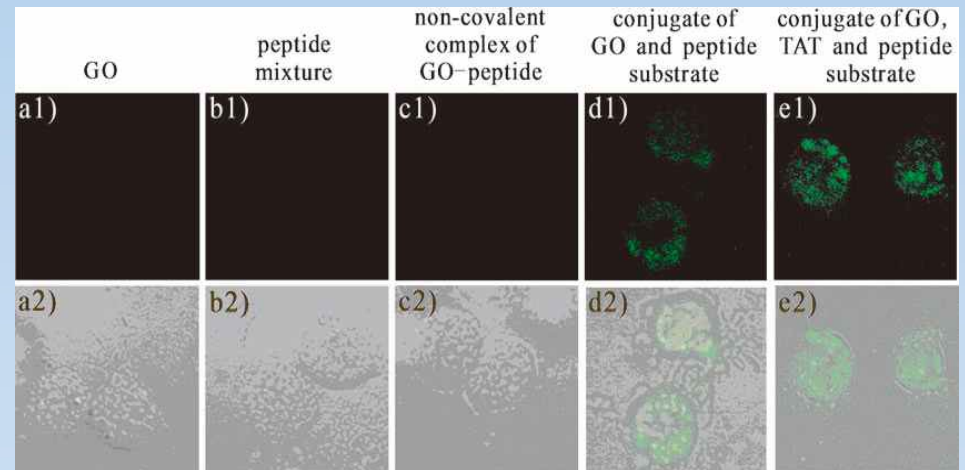


Figure 7. Confocal fluorescence microscopy images of HeLa cells treated with 4 mM STS for 4 h after incubating with (a) GO, (b) 1.5 mM mixture of fluorescein-labeled caspase-3-specific substrate peptide and cell penetration peptide TAT, (c) noncovalent complex of GO-peptide, (d) conjugate of GO and substrate peptide, and (e) conjugate of GO, TAT, and substrate peptide, for 8 h at 37°C in cell growth medium. Concentration of GO, complex, and conjugates: 70 mgmL⁻¹. (1) Fluorescence images; (2) overlap of fluorescence and bright-field

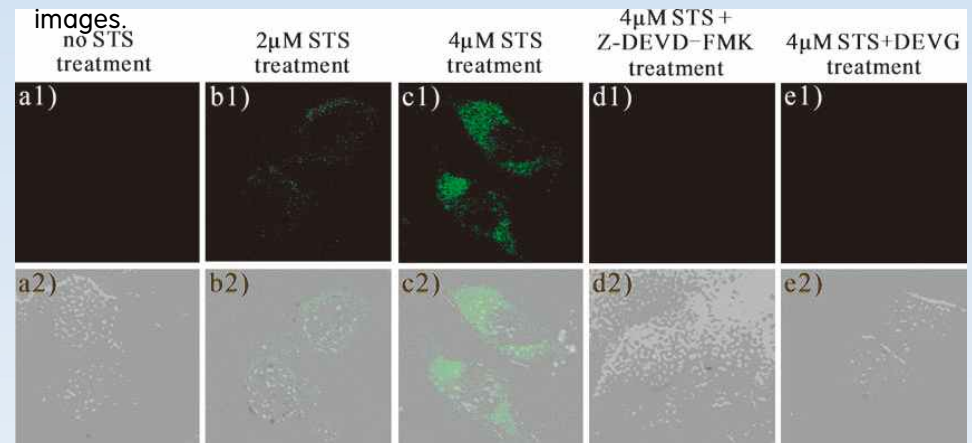


Figure 8. Confocal fluorescence microscopy images of HeLa cells treated with (a) no STS, (b) 2 mM STS, (c) 4 mM STS, (d) 4 mM STS and 100 μM inhibitor Z-DEVD-FMK for 4 h after incubating with conjugate of GO and caspase-3-specific peptide, and (e) treated with 4 mM STS for 4 h after incubating with conjugate of GO and nonspecific peptide substrate DEVD. (1) Fluorescence images; (2) overlap of fluorescence and bright-field images.

5. Thrombin을 검출하기 위한 fluorescence resonance energy transfer (FRET) aptasensor

➤ Graphene fluorescence resonance energy transfer aptasensor for the thrombin detection⁶

-Thrombin을 검출하기 위한 고 민감도, 고선택성을 가진 fluorescence resonance energy transfer (FRET) aptasensor 개발은 dye가 라벨된 압타머를 assemble한 그래핀 기반으로 준비하였음.

-FRET의 원리에 의하여 dye가 라벨된 압타머의 fluorescence가 그래핀과의 비 공유 결합에 의하여 fluorescence quenching됨.

-Thrombin의 결합은 quadruplex-thrombin complexes의 형태로 압타머와 결합하면서 그래핀의 표면으로부터 dye가 멀어지는 원리에 의하여 quenching되었던 fluorescence가 회복됨을 보임.

-그래핀의 높은 fluorescence quenching효과, 특유의 구조, 전기적 성질때문에 그래핀 FRET aptasensor는 buffer와 blood serum사이에서 고 민감도, 고 선택성을 보였으며, 31.3 pM의 낮은 검출 한계를 보임.

-그래핀 FRET aptasensor는 cancer cell recognition, 적절한 압타머를 이용한 cocaine sensors등의 응용성을 기대할 수 있는 센서임.

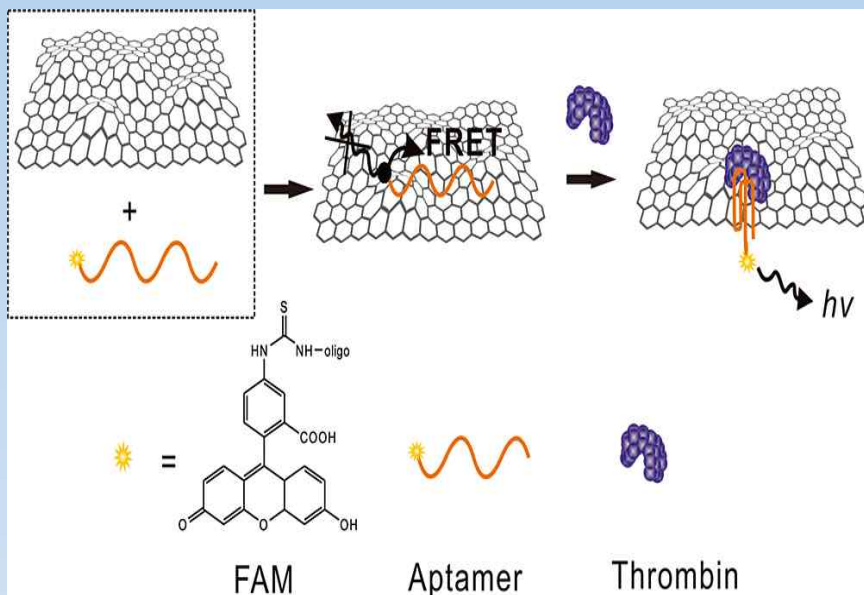


Figure 9. Schematic Demonstration of Graphene FRET Aptasensor and the Detection Mechanism for Thrombin^a
^a Fluorescence of dye labeled aptamer is quenched when aptamer binds to graphene due to FRET between dyes and graphene. The fluorescence recovers while thrombin combines with aptamers to form quadruplex-thrombin complexes which have much less affinity to graphene, causing FAM far away from the graphene surface.

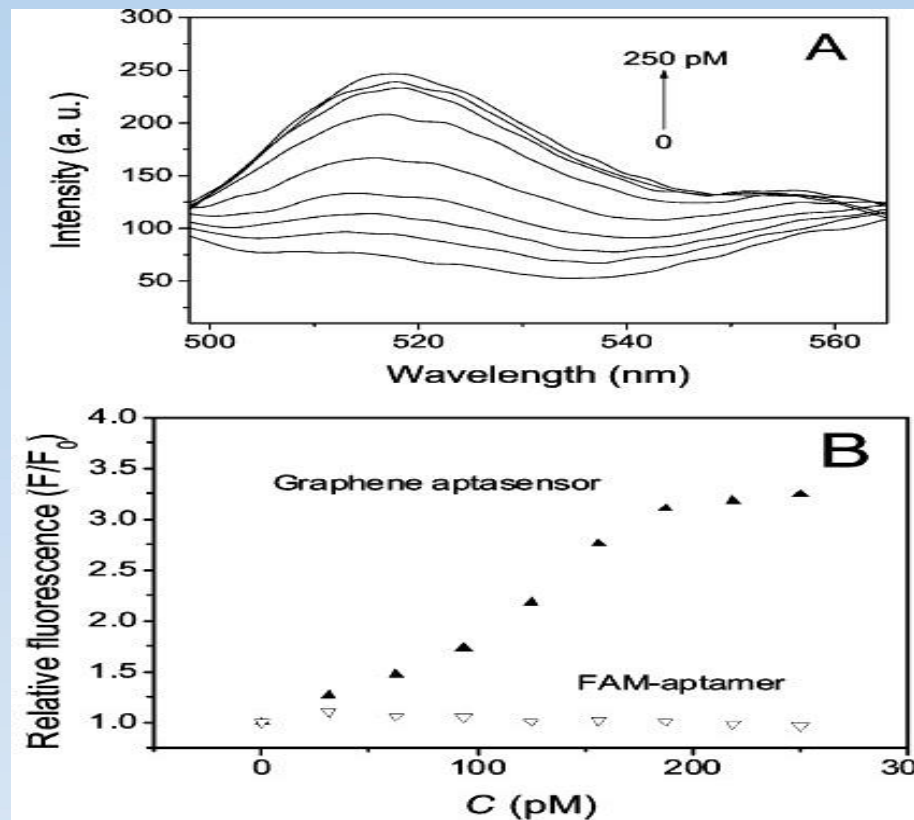
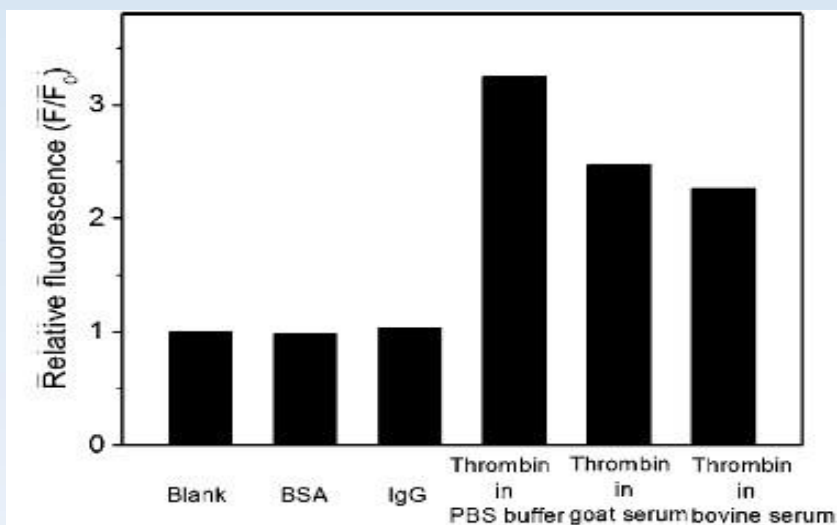


Figure 10. (A) Fluorescence spectra of the graphene aptasensor via different concentrations of thrombin (0, 31.3, 62.5, 93.8, 125, 156.3, 187.5, 218.8, and 250 pM) in 20 mM PBS buffer (pH 7.0). (B) Relative fluorescence changes of FAM-aptamer and the graphene aptasensor via thrombin concentrations. Relative fluorescence changes are calculated by F/F_0 , where F_0 and F are the fluorescence intensity without and with thrombin, respectively. FAM-aptamer concentration: 20 nM. Excitation wavelength: 470 nm.

Figure 11. Relative fluorescence intensity of the graphene aptasensor incubated in blank PBS buffer; 1 μ M BSA in PBS; 1 μ M IgG in PBS; 250 pM thrombin in PBS, goat serum, and bovine serum, respectively. Relative fluorescence intensity (F/F_0) is calculated by F/F_0 , where F_0 and F are the fluorescence intensity without and with the presence of BSA, IgG, and thrombin, respectively. FAM-aptamer concentration: 20 nM. Excitation wavelength: 470 nm. Emission wavelength monitored: 517 nm.

6. In vitro and in vivo imaging probe로 이용하기 위한 Multi-functional graphene

➤ Multi-functional graphene as an in vitro and in vivo imaging probe⁷

-이 연구는 in vitro와 in vivo imaging을 위한 biomedical 응용하고자 water-dispersibility (~2.5 g/l), fluorescence property (emission maximum at 526 nm), 자기적 성질을 가지는 multi-functional graphene (MFG)를 microwave와 sonication 방법에 의하여 개발함.

-MGF는 in vitro와 in vivo imaging에서 낮은 세포독성을 보였으며 이는 뛰어난 fluorescent marker임을 증명함.

-HeLa cells에서 MFG의 in vitro cellular imaging는 cytoplasmic region에 잘 위치하고 있었으며 lysotracker staining은 lysosomal region에서 MFG degradation의 가능성을 보여줌.

-Zebrafish에서 MFG의 in vivo whole-animal imaging은 머리부터 꼬리까지 잘 분포됨을 나타냈으며 독성 또한 나타나지 않았음.

-결과적으로 biocompatible MFG는 in vitro, in vivo imaging probe임을 증명하였고 biomedical diagnostics, magnetically guided drug/gene delivery, photothermal/photodynamic therapies등의 다양한 biomedical field의 응용성이 기대됨.

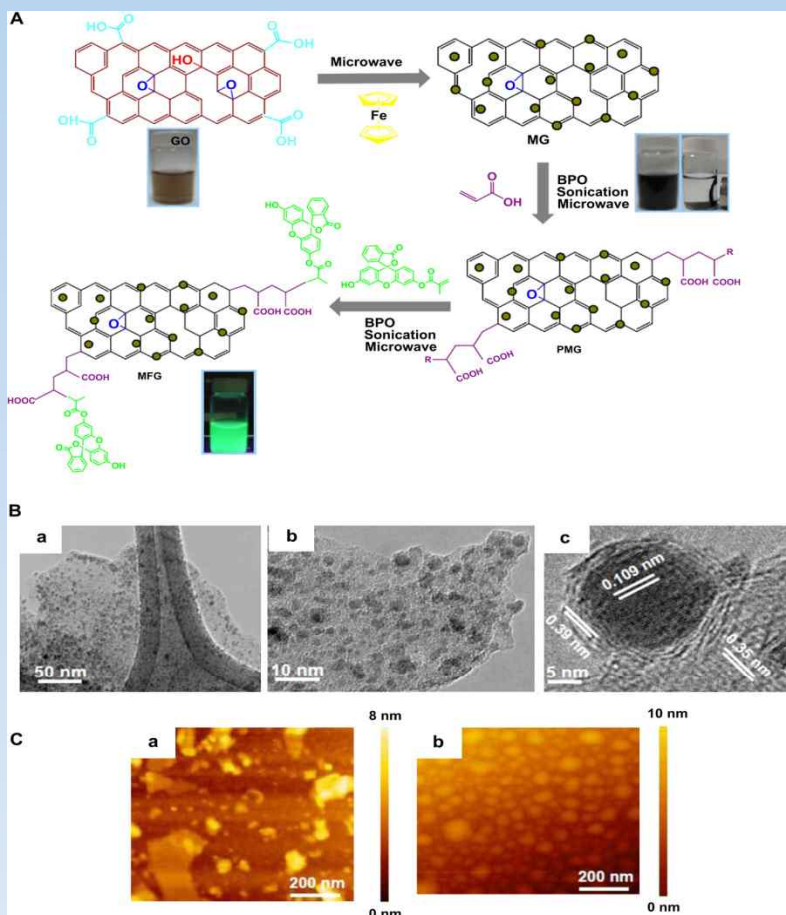


Figure 12. (A) Schematic representation for the synthesis of MFG using microwave-heated sonication-assisted process and surface functionalization with AA and FMA. (B) TEM analyses of MG (a) low, and (b) high magnification TEM images of MG, (c) TEM image of MG showing Fe filled NPs embedded within graphene sheet surrounded with the graphene layers, and (C) AFM images of (a) GO, and (b) MFG.

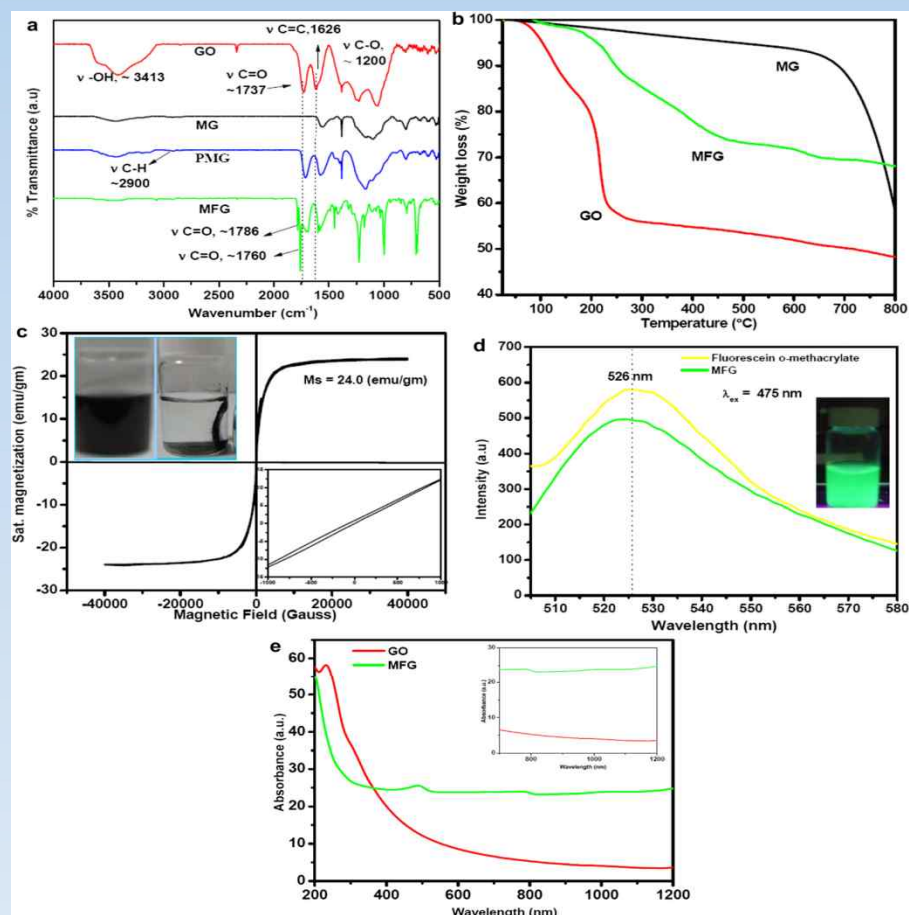


Figure 13. Representative (a) FT-IR spectra, (b) TGA thermograms of GO, MG, and MFG, (c) Magnetization curve of MG (top inset showing the photograph of suspended MG in black aqueous solution, bottom inset showing the close view of the hysteresis loop), and (d) Fluorescence spectra ($\lambda_{ex} = 475$ nm) of MFG (green line) and fluorescein o-methacrylate alone as a reference (red line). Inset representing the green fluorescence image of MFG aqueous solution upon exposure to UV light. (e) UV-Vis-NIR of GO and MFG. Inset represents the extended NIR region. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

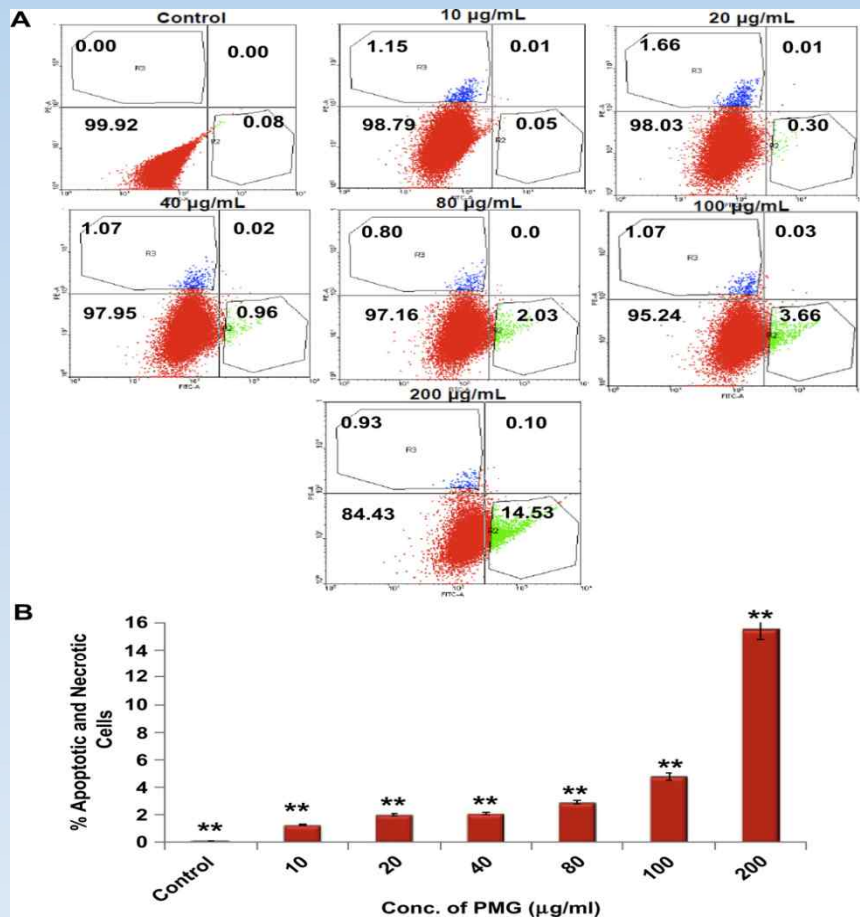


Figure 14. Apoptosis detection by Annexin-V assay of HeLa cells treated with PMG for 24 h, respectively. (A) Flow cytometry dot plot (x-axis FITC and y-axis is PE). The number of dots in each quadrant represents the number of the specific cells. The lower left-hand quadrant indicates normal cells, the lower right-hand indicates early apoptotic cells, the upper right hand indicates middle phase apoptotic cells, and the upper left-hand indicates late phase apoptotic cells or necrotic cells. (B) The bar graph representation of the % apoptotic and necrotic cells from the dot plot at different concentrations of PMG. The results were obtained from 4 separate experiments and the bar represents mean S.E. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

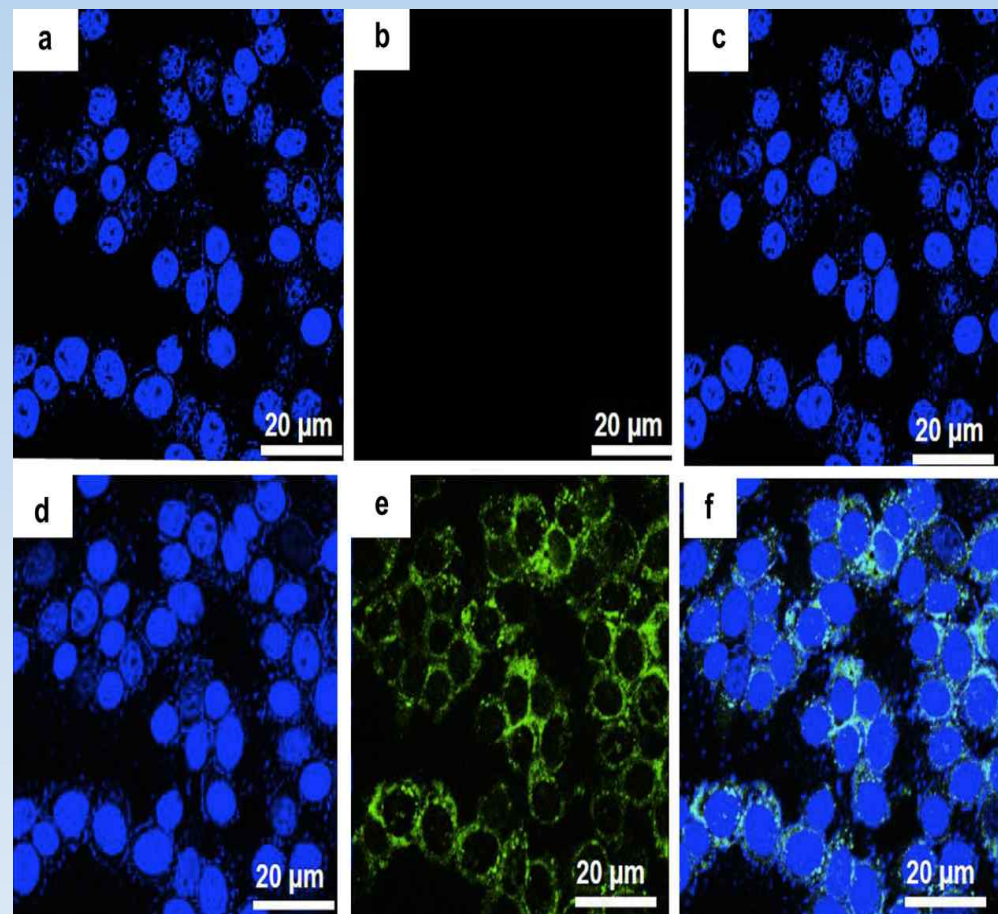
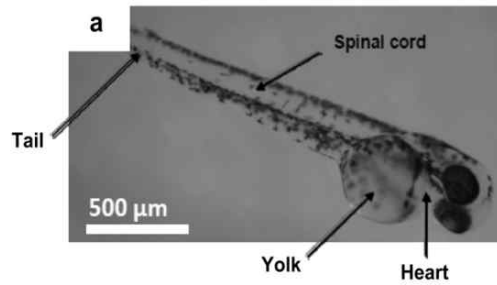
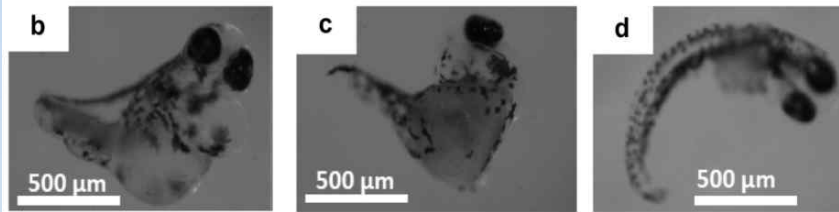


Figure 15. CLSM images of control (upper row) and treated (lower row, incubated with 20 μ g/ml of PMG for 24 h) HeLa cells. In all images the nuclei of HeLa cells were stained with DAPI blue dye. (a) and (d) The nucleus images were recorded with a DAPI filter. (b) and (e) The green fluorescence from PMG was recorded with a FITC filter. (c) and (f) The image is an overlap of respective stained nucleus and the fluorescence from PMG. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A No Microinjection – Normal development



After GO injection - Abnormalities



After MFG injection - Abnormalities

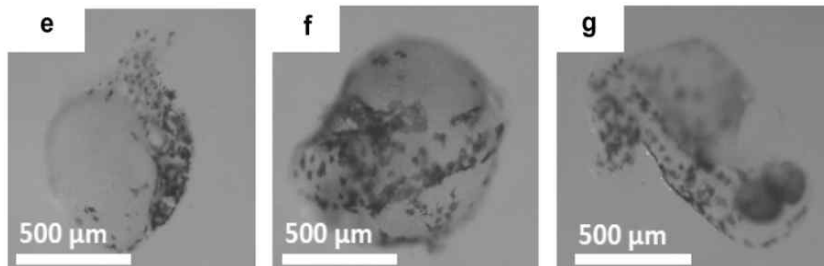


Figure 16. (A) In vivo cytotoxicity of zebrafish at different concentrations of MFG from 0 to 72 hpf (hours-post fertilization). (a) Normal development (control), (b and e) cardiac malfunction and heart expansion, (c, d and g) tail and spinal cord flexure, (f) yolk sac edema, after injecting the GO and MFG (0.05 and 0.1 ng/nl). (B and C) Effect of zebrafish survival rate monitored at an interval of 24 h, without and with microinjection of DI water as a control and after microinjection of GO and MFG (0.05 and 0.1 ng/nl) as experimental set.

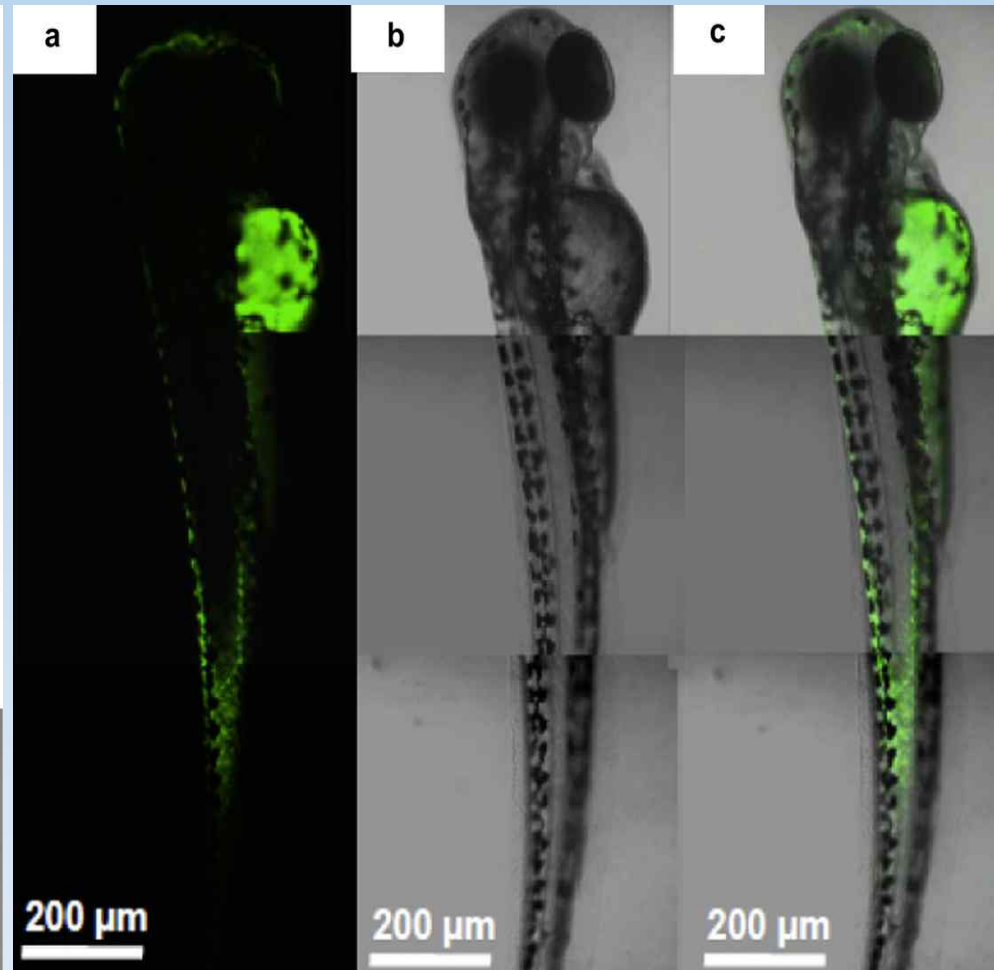


Figure 17. Distribution of MFG inside a fully developed (72 hpf) zebrafish using CLSM (a) fluorescence image of MFG with a FITC filter, (b) DIC image, and (c) Overlay image.

4. 결론

- ▶ 바이오 기질과의 상호작용에 의한 색깔 변화, 형광억제 현상 (quenching), FRET 원리를 이용한 그래핀 기반 광학적 바이오 센서에 대한 생체 및 환경 분야 응용성에 대하여 활발히 연구되고 있다.
- ▶ 그래핀 대비, 그래핀 산화물(GO)이 heterogeneous electronic structure를 가지고 있어 넓은 영역의 파장대에서 현광특성을 보이고 있으므로 그래핀 산화물을 이용한 물리적, 생물학적 응용뿐만 아니라 광학적 성질의 최근 연구동향들이 보고되고 있다.⁸
- ▶ 그래핀 기반 광학적 바이오센서의 개발은 그래핀의 전기적, 현광적 특성을 이용하여 세포 이미징, DNA 검출, 단백질 검출, in vitro 및 in vivo imaging probe 등의 높은 검출 감도, 실시간 측정 및 진단 도구로서 biomedical diagnostics 등의 다양한 응용성을 제시하고 있다.

5. 참고문헌

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