Bio-Targeted Polymerized N-Doped Graphene Quantum Dots as an Accurate Tools for Neuroblastoma

You-Sheng Lin(林祐生)[1], Kuen-Song Lin(林錕松)[1,*], U-Ser Jeng(鄭有舜)[2], Chung-Ming Wu(吳浚銘)[2], Yun Chen (陳芸)[3]

[1]Department of Chemical Engineering & Materials Science/Environmental Technology Research Center, Yuan Ze University, Taoyuan, Taiwan, R.O.C.
[2]National Synchrotron Radiation Research Center, 101 Hsin-Ann Road, Hsinchu Science Park, Hsinchu, Taiwan
[3]Department of Surgery, Far Eastern Memorial Hospital, New Taipei City, 220, Taiwan
kslin@saturn.yzu.edu.tw

Abstract

Neuroblastoma is an embryonic tumor in the autonomic nervous system. It is thought to originate from the neural crest tissue and is not exactly the same as the somatic cells in previous studies. It usually occurs in very young children; the mean age of diagnosis was 17 months. Therefore, accurate medical techniques are required to reach the high specific targeted treatment. Graphene quantum dots (GQDs) have been considered as a novel fluorescence gene carrier, basic on the low toxicity and nano scale of particle size. In vitro bio-imaging ability already made GQDs attractive to scientists, then they devoted on combination of gene therapy and application in vitro. However, in vitro images were lack of the concentration of subject organ, chemical supporting limit is also hard to overcome. In here, we presented the polymer modified nitrogen GQDs as fluorescence mark conjugated with gene as treatment of cancer. Polymers offered biocompatibility and activity organic group on the surface. n this study, nitrogen doped GQDs had been synthesized and characterized about the structure (XRD, HR-TEM, DLS, SAXS) and chemical/ physical properties (XPS, UV, PL), which was highly connect with gene delivery. The NSRRC TLS 23A1 and ANSTO Bilby provided novel technique on GQD micelle structure analyzing, which contributed the accurate dispersion status and micelle thermal stability.

Keywords - Graphene Quantum Dots, Gene therapy, Targeted therapy, Neuroblastoma, Bio-imaging.

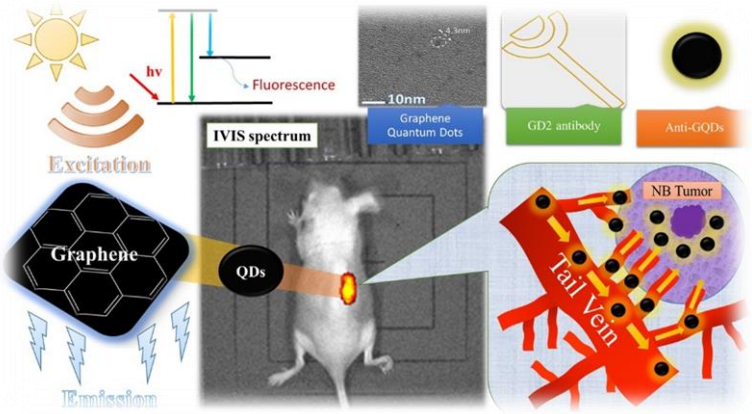


Fig. 1. Research scope of Bio-Targeted Polymerized N-Doped Graphene Quantum Dots as Tools for Neuroblastoma.

Introduction

Neuroblastoma is an embryonic tumor in the autonomic nervous system. GD2 is one of the specific protein in neuroblastoma cell which can be targeted with antibody. Polymerized GQD complex linked with GD2 antibody in classic process to obtain GD2@gene@PEG: bPEI@N-GQDs as formula in this study. Then, *In Vivo* animal test showed photosensitivity GQD complex performed extreme signal in tumor than other tissues. I In vivo and in vitro test showed GD2@gene@PEG: bPEI@N-GQDs had bio-targeted and cancer inhibition function, this research can provide importance reference for the clinical medication instructions. However, the cross link effect between GQD complexes and target macromolecule has not been confirm with structure and ratios. Small angle scattering can help to solve the uncertain relates of it.

Experimental

Nirtogen graphene quantum dots synthesized by htdrothermal method with citric acid and amounim, products were filitered with 0.22 μm dvice and dialysised with 3,000 MW membrane. The N-GQDs coated with PEG by physical mixing, then transform to telfon cup after PEI adding. The hydrothermal reacted for 12 hours and dialysised with 13,000 MW membrane for 3 days. The specific antibody Anti-GD2 bonded with polymerized GQDs by NHS/EDC method. Final obtained product need to keep in 4ºC refrigerator.

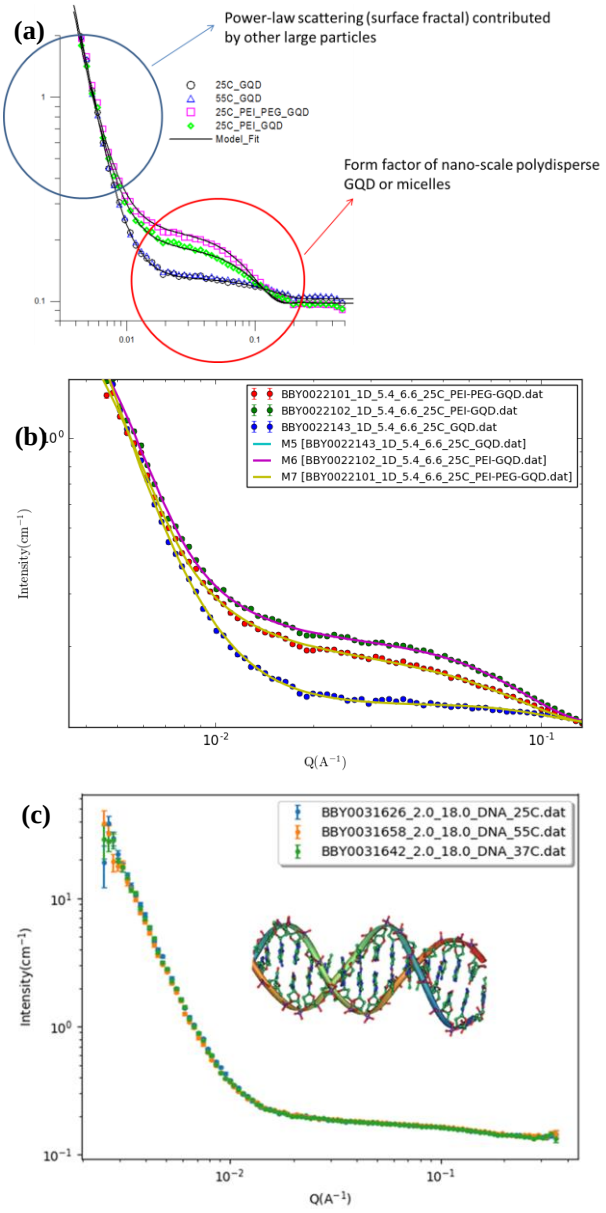
Results

Characterization of PEG: b-PEI@N-GQDs：The N-GQDs analyzed with DLS and HR-TEM to know the quantum size as 2-8 nm diameter. The optical porpeerties provided excotation (360 nm) and emission (550 nm) as well. The small angle scattering (SAXS and SANS) helped to understand the

dispersion and temperature stablity (25-55ºC). *In vitro/ in vivo* of Ab-GD2@PEG: b-PEI@N-GQDs : The *in vitro* Confocal images and *in vivo* animal test provided successful about the tracing and high resolution fluorescence bio-image information afrer Ab-GD2 adding. The composite also analyzed with small angle scattering (SAXS and SANS) as well.

Discussion

The DLS and HR-TEM finished before small angle scattering experiment. The analyze result of SASview provides similare result with prvious ones. The difference between N-GQDs and polymerized N-GQDs can be observed with SANS and SAXS by the size, diameter, scattering intensity, polydispersity et.al. Optical instrument proved avaliable pathway of Ab-GD2@PEG: b-PEI@N-GQDs to neuroblastoma cancer cell (BE2M17). Futheremore, dissected organs explore strong emission in tumor tissue. The result excited the opportunity to know the machnism of polymer N-GQDs combining with Ab-GD2 or other protein. The SAXS and SANS had invegasted the shape transform, density shift, and length/core diameter of micelle structure.



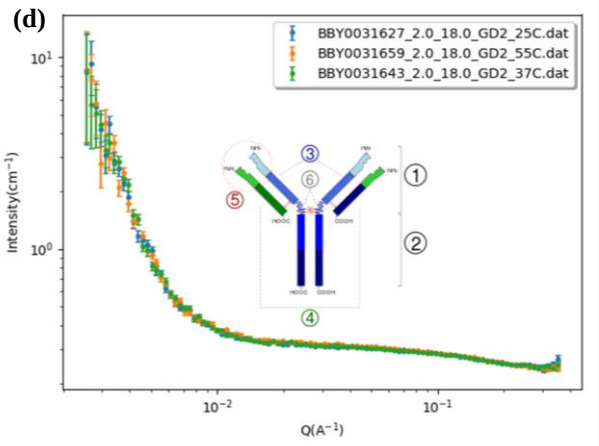


Fig. 2. Small angle scattering of (a)(b) Difference between N doped graphene quantum dots and PEG: b-PEI@N-GQDs. (c),(d) DNA and Ab-GD2 structure transform during temperature increasing.

Acknowledgments

Thanks for NSRRC beamline spokeman U-Ser Jeng about the technique and revise the experiment condition for the research. We also thank for kinding help of beamline manager scientist Chung-Ming Wu for expereriment skill support and data analyze. The appreciation of MOST fund in this study.

References

[1] He, Z., Alexandridis, P. Micellization thermodynamics of Pluronic P123 (EO20PO70EO20) amphiphilic block copolymer in aqueous ethylammonium nitrate (EAN) Solutions. Polymers, 2017; 10(1), 32.

[2] Liu, X., Liu, H.J., Cheng, F. and Chen, Y., 2014. Preparation and characterization of multi stimuli-responsive photoluminescent nanocomposites of graphene quantum dots with hyperbranched polyethylenimine derivatives. Nanoscale, 6(13), 7453-7460.

[3] Mdlovu, N.V., Chen, Y., Lin, K.S., Hsu, M.W., Wang, S.S.S., Wu, C.M., Lin, Y.S., Ohishi, K., 2019. Multifunctional nanocarrier as a potential micro-RNA delivery vehicle for neuroblastoma treatment. J. Taiwan Inst. Chem. E. 2019; 96: 526–537.

[4] Akhtar, S., Non-viral cancer gene therapy: beyond delivery. Nature Publishing Group: 2005.

[5] Vijayanathan, V.; Thomas, T.; Thomas, T., DNA nanoparticles and development of DNA delivery vehicles for gene therapy. Biochemistry 2002, 41 (48), 14085-14094.

[6] Lin, G.; Zhang, H.; Huang, L., Smart polymeric nanoparticles for cancer gene delivery. Molecular pharmaceutics 2015, 12 (2), 314-321.