Nanoparticles as Biomarkers and Biosensors

Anchal Srivastava*, R K Shukla, Nishant Kumar and Anu Katiyar

Department of Physics, University of Lucknow, India

Submission: September 04, 2017; Published: September 25, 2017

*Corresponding author: Anchal Srivastava, Department of Physics, University of Lucknow, India, Email: asrivastava.lu@gmail.com

Abstract

Biomarker, a measurable indicator of some biological state or condition, has journeyed for better from organic fluorescent substances to chalcogenide quantum dots to biocompatible metal oxide semiconductor nanoparticles. Single-band up-conversion nanoparticles have been realized doing away with any kind of spectral interference with the living cell auto fluorescencense. Zinc oxide based thin films and nanostructures have shown striking performance due to its high isoelectric point and other multifunctional characteristics besides non toxic nature to in vivo applications. Rare earth doped ZnO and ZnAl\(_2\)O\(_4\) nanoparticles can be used as biomarkers. Also, nanomaterials such as nanoparticles, nanorods, nanowires, etc. offer large surface to volume ratio and therefore they can offer high sensitivity. Added with the advanced nanofabrication techniques ZnO based portable biosensors will not remain a dream but still a lot of diligence is required to achieve the target.

Introduction

A biomarker generally refers to a measurable indicator of some biological state or condition, for example presence of life in an organism or disease in the organism. A biomarker is a detectable or traceable substance which can be already present or can be introduced into an organism to examine certain organ function. Biomarkers indicate a change in the state of a protein that correlates with the risk or progression of a disease, or with the effect of given medicinal treatment. Detection of biomarkers requires biosensors bioanalytical devices which take shape on integration of disciplines like engineering, medical, physical and biological sciences. Thus the efficacy of biosensor lies in the capability to deliver an unambiguous measurable output signal response for either diagnostic or therapeutic purpose when a biomarker interacts with the transducing surface of the sensor. Nanomaterials such as nanoparticles, nanorods, nanowires, etc. effectively improve the performance characteristics of a biosensor [1-3]. Nanotextured surfaces intensively impact the sensor performance offering unique features besides increased surface area for biomolecular binding [4,5].

Metal oxide based nanoparticles as biomarkers

Though organic fluorescent biomarkers are widely used in biology and medicine [6,7], they fall short of good resolution of marker’s emission from cell’s auto fluorescence during in vivo studies due to their quite wide emission and excitation bands. Moreover, marker excitability reduces upon excitation resulting in fall of the signal. To this handicap, a solution can be found in semiconductor based nanoparticles. Initially chalcogenides viz. CdTe, CdS and CdSe nanoparticles (NPs)/quantum dots (QDs) were taken with their surface capped using relevant material to obtain selectivity [8,9].

The signal intensity thus obtained is much higher than the auto fluorescence of the living tissues and also than that obtained from in use fluorescent dyes but the debilitating parts are

i. fluctuations of emission energies as a function of size of QDs [10],

ii. fluorescence intermittency [11-14] and

iii. the toxicity of Cd which has a lot of chances to be freed from its compound due to photolysis and/or due to slow dissolution effected by the living cells and bodily fluids.

In order to overcome these shortcomings, biocompatible metal oxide semiconductor nanoparticles ZnO, ZnAl\(_2\)O\(_4\), and ZrO\(_2\) came in as biomarkers. Their luminescence is activated using rare earth ion dopants [15] so that the emission spectra be a function of RE properties and not that of the size of nanoparticles. For this reason size of the NPs is kept much larger than the QDs. A control on their shape, size, morphology and crystallographic phase can be had by choosing appropriate method of synthesis and/or optimization of various process parameters/chemicals [16,17]. Wet chemical methods hydrothermal, microwave assisted hydrothermal method [18], sol gel method [19,20], and pulsed laser ablation in liquid medium [21] may be used for synthesis of
NPs. Photo excitation of RE doped oxide nano biomarkers using visible light avoiding ultraviolet radiation which is highly toxic for living cells is explored [18]. Along with biocompatibility, biodistribution and elimination of the marker from the human body is also important. Emission from such biomarkers and that from cells have unambiguously different decay times and therefore a time resolved PL can separately detect them. The 4f-4f excitation of RE intra shell transitions leads to weak emission whereas 4f5d or charge transfer excitations [22] limit the choice of hosts. However, use of \(Y_2O_3\) in RE doped oxide nanoparticles is a way out. \(ZnO\), nanoparticles doped with \(Pr, Tb\) or \(Eu\) and stabilized by \(Y_2O_3\) make the system very attractive for biological and medical applications [17,22].

RE doped \(ZnO\) NPs exhibit strong defect related wide PL bands [23] but AI codoping of \(ZnO\): Eu nanoparticles improves the situation [18]. Intense RE emission was obtained for Pr doped \(ZrO_2\) NPs [24] involving two photon infrared (IR) excitation process thereby completely getting rid of cell’s auto fluorescence. These NPs were tested on adult mice and results were encouraging regarding quick uptake, biodistribution and elimination from the body [25,26].

Detection of biomarkers - Biosensors

Preparation of ssingle-band up-conversion nanoparticles with different colors have been reported [27] which could achieve the multiplexed simultaneous in situ biodetection of biomarkers in breast cancer cells and tissue specimens. Better simultaneous quantification of proteins as compared to classical immunohistochemistry (IHC) technology was obtained. Sensitive, selective and multiplexed molecular detection is needed for gene and protein profiling, drug screening and clinical diagnostics [28-30]. For cancer diagnosis, the identification of potential diagnostic biomarkers and target molecules among the plethora of tumor onco-proteins is required.

An unfailing but simple technology is required for quantitative analysis of biomarkers existing simultaneously/dynamically in tumour cells and tissues [31-35]. Diagnosis as well as prognosis of tumours is based on classical immunohistochemical (IHC) technology was obtained. Sensitive, selective and multiplexed molecular detection is needed for gene and protein profiling, drug screening and clinical diagnostics [28-30]. For cancer diagnosis, the identification of potential diagnostic biomarkers and target molecules among the plethora of tumor onco-proteins is required.

An unfailing but simple technology is required for quantitative analysis of biomarkers existing simultaneously/dynamically in tumour cells and tissues [31-35]. Diagnosis as well as prognosis of tumours is based on classical immunohistochemical (IHC) technology was obtained. Sensitive, selective and multiplexed molecular detection is needed for gene and protein profiling, drug screening and clinical diagnostics [28-30]. For cancer diagnosis, the identification of potential diagnostic biomarkers and target molecules among the plethora of tumor onco-proteins is required.

For such situations fluorescence imaging with optical microscopy is a better alternative offering higher detection sensitivity and direct relationships for biomarker quantification and prediction of therapeutic response. Though the results obtained using fluorescence dyes regarding protein biomarkers are closely related with the clinical finding but the photo bleaching occurring in organic dyes during multicolor fluorescence measurement poses severe limitations [36-38]. Moreover, separate excitation wavelengths or sources are required. These limitations can be overcome by quantum dots (QD). QDs are few nanometers sized semiconductor particles with their optical and electronic properties remarkably different from those of larger particles. Many QDs show electro or photo luminescence and the emission frequencies can be precisely tuned by changing the dots’ size, shape and material, giving rise to potential applications viz. medical imaging besides transistors, solar cells, LEDs, diode lasers, second harmonic generation and quantum computing. Being more photo stable and excitable in the same spectral range, QDs are advantageous as compared to fluorescent dyes provided any spectral interference with the intrinsic fluorescence of biological tissues is minimized, which can be effected by prolonging the illumination duration of QD stained specimens [39,40]. Spectral deconvolution of the data is also required and such techniques slow down the screening rate and sensitivity.

Another alternative is the use of rare earth up conversion nanoparticles (UCNPs), which can be excited by the wavelengths in the infrared region which are not absorbed by the tissue. Due to up conversion, photoluminescence emissions will be in the visible range. Rare earth ions with +3 oxidation states have much narrow emission line widths as compared to those from QDs thus reducing any inter emission overlap and thereby facilitating multiplexed detections of biomarkers [37,38,41]. Red and near infrared [42,43] single band up conversion emissions have been realized. A simple and brilliant method of achieving single band up conversion emission with different colors in the blue, green and red regions is reported [39] by coating the up conversion nanocrystals with a screen layer containing an organic dye with a high molar absorption coefficient as nanofilters to remove the unwanted emission bands.

The biomarker expression levels in breast cancer cell specimens determined using these single band UCNPs, standard western blotting (WB) and immunohistochemical technologies (IHC) exhibit excellent correlation among these three methods; however, the WB and IHC methods can probe only one biomarker at a time. Significantly, the application of antibody conjugated single band UCNP molecular profiling technology can achieve the multiplexed simultaneous in situ biodetection of biomarkers in breast cancer cells and tissue specimens and produces more accurate results for the simultaneous quantification of proteins present at low levels compared with IHC [39].

The single band UCNPs were prepared by b NaGdF\(_4\),20\% Yb, 2% Er@NaGdF\(_4\) (core nanocrystal), pure silica layer (spacer) and amino reactive organic dyes doped silica layer (selective nanofilter capping of the nanocrystals). The choice of the dyes is such that they absorb all the emissions except one and thus the layer acts as a filter.

\(ZnO\) as biosensor

Zinc oxide based thin films and nanostructures have shown unparalleled and promising performance due to high isoelectric point and other multifunctional characteristics. Further being
biocompatible, ZnO has extensively been studied as a material for biosensor development. The fascinating properties of ZnO help retain biological activity of the immobilized biomolecule and help in achieving enhanced sensing performance. And the technological advancements have transformed the diagnostic biosensors to a handheld portable one. ZnO can form anisotropic nanostructures such as nanoparticles, nanorods, nanowires etc. and therefore has capability to recognize biomolecules, deliver drug particularly in the treatment of malignancy and to be used for novel bioelectronics devices [44,45].

Due to its wide band gap of 3.37 eV and fast electron transfer kinetics, ZnO is an appropriate material for designing electrical or electrochemical sensors. ZnO due to inherent inhomogeneities such as thiol, carboxyl and phosphonic acid groups [46-48]. Biomarkers indicative of pathophysiological conditions, which include proteins, enzymes or metabolites, are released into bloodstream when cardiac muscles experience stress due to ischemia [49]. Measurement of these biomarkers helps in diagnosis and prognosis of diseases. For the purpose, ZnO biosensors are promising as they meet the clinical requirements but still a long journey is required for making them a regular diagnostic tool.

Conclusion

Biomarkers and biosensors take shape with the integration of engineering, medical, physical and biological sciences. Organic fluorescence biomarkers which are widely used in biology and medicine do not offer good resolution between marker’s emission and cell’s auto fluorescence during in vivo studies due to their quite wide emission and excitation bands. Capped CdTe, CdS and CdSe quantum dots give good signal intensity but pose a severe problem of toxicity. Rare earth doped nanomaterials such as ZnO nanoparticles, nanorods, nanowires etc. offer large surface to volume ratio besides being biocompatible and therefore they are used for biosensing with high sensitivity. Added with the advanced nanofabrication techniques ZnO based portable biosensors will not remain a dream but still a lot of diligence is required to achieve the target.

References

1. Agah A, Hassibi A, Plummer JD, Griffin PB (2005) Design requirements for integrated biosensor arrays. European Conference on Biomedical Optics, Munich, Germany.
2. Patolsky F, Zheng G, Lieber CM (2006) Nanowire sensors for medicine and the life sciences. Nanomedicine 1(1): 51-65.
3. Carrara S, Ghoreishizadeh S, Olivo J, Baj Rossi C, et al. (2012) Fully integrated biochip platforms for advanced healthcare. Sensors Basel 12(8): 11013-11060.
4. Gooding JJ, Lai LM, Goon JY (2009) Nanostructured electrodes with unique properties for biological and other applications. Chemically Modified Electodes 11: 1-56.
5. Solanki PR, Kaushik A, Agrawal VV, Malhotra BD (2011) Nanostructured metal oxide based biosensors. NPG Asia Materials 3(1): 17-24.
6. Nida DL, Rahman MS, Carlson KD, Kortum RR, Follen M, et al. (2005) Fluorescent nanocrystals for use in early cervical cancer detection. Gynecol Oncol 99(1-3): S89-94.
7. Karleya D, Gupta D, Tiwari A (2011) Biomarker for Cancer: A Great Promise for Future. World J Oncol 12(4): 151-157.
8. Yang FF, Yu JS, Xie Y (2008) Synthesis and Characterization of Water-soluble CdSe/CdS/ZnS Core-shell Quantum Dots. Chin J Inorg Chem 24 (7): 1142-1147.
9. Rieger S, Kulkarni RP, Darcy D, Fraser SE, Koster RW, et al. (2005) Quantum dots are powerful multipurpose vital labeling agents in zebra fish embryos. Dev Dyn 234: 670-681.
10. Rakshit S, Vasudevan S (2008) Resonance Energy Transfer from β-Cyclodextrin-Capped ZnO:MgO Nanocrystals to Included Nile Red Guest Molecules in Aqueous Media. ACS Nano 2: 1473-1479.
11. Kuno M, Fromm DP, Hamann HF, Gallagher A, Nesbit DJ, et al. (2000) No exponential “blinking” kinetics of single CdSe quantum dots: A universal power law behavior. J Chem Phys 112: 3117-3120.
12. Schuster J, Cichos F, Borczyskowski CV (2005) Blinking of Single Molecules in Various Environments. Opt Spectrosc 98(5): 712-717.
13. Frantsuzov P, Kuno M, Janko B, Marcus RA (2008) Universal emission intermittency in quantum dots, nanorods and nanowires. Nat Phys 4: 519-522.
14. Dickson RM, Cubitt AB, Tsien RY, Moerner WE (1997) On/off blinking and switching behavior of single molecules of green fluorescent protein. Nature 388: 355-358.
15. Qin X, Liu X, Huang W, Bettinelli M, Liu M, et al. (2017) Lanthanide-Activated Phosphors Based on 4f-5d Optical Transitions: Theoretical and Experimental Aspects. Chem Rev 117(5): 4488-4527.
16. Yoshimura M, Suda H (1994) Hydrothermal Processing of Hydroxyapatite. Past, Present, and Future. Hydroxyapatite and Related Materials CRC Press Inc. pp. 45-72.
17. Wolska E, Sibera D, Wiktorowski BS, Yatsunenko S (2011) Photoluminescence and Chromaticity Properties of ZnO Nanopowders Made by a Microwave Hydrothermal Method. Acta Phys Pol A 120: 908-910.
18. Wolska E, Kaszewski J, Kiełbik P, Gryn J, Godlewski MM, et al. (2014) Rare earth activated ZnO nanoparticles as biosensors. Optical Materials 36(10): 1655-1659.
19. Srivastava A, Kumar N, Misra KP, Khare S (2014) Blue-light luminescence enhancement and increased band gap from calcium-doped zinc oxide nanoparticle films. Mater Sci Semicond Process 26: 2629-266.
20. Srivastava A, Kumar N, Khare S (2014) Enhancement in UV emission and band gap by Fe doping in ZnO thin films. Opto-Electron. Rev 22(1): 68-76.
21. Amans D, Malaterre C, Diouf M, Mancini C, Chaput F, et al. (2011) Synthesis of Oxide Nanoparticles by Pulsed Laser Ablation in Liquids Containing a Complexing Molecule: Impact on Size Distributions and Prepared Phases. J Phys Chem C 115(12): 5131-5139.
22. Godlewskia M, Yatsunenko S, Nadolska A, Opalska A, Lojkowski W, et al. (2009) Nanoparticles doped with TM and RE ions for applications in optoelectronics. Opt Mater 31(3): 490-495.
23. Sharma DK, Sharma KK, Kumar V, Sharma A (2016) Effect of Ce doping on the structural, optical and magnetic properties of ZnO nanoparticles. J Mater Sci: Mater Electron 27(10): 10330-10335.
24. Fiedelus JD, Yatsunenko S, Godlewski M, Paszkowicz W, Werner Malento E, et al. (2009) Relation between structural properties of Pr3+-doped yttria-stabilized zirconia nanopowders and their luminescence efficiency. Scripta Mater 61(4): 415-418.
25. Baek M, Choi SJ, Choy JH, Chung HE, Jeong J, et al. (2012) How to cite this article: Anchal Srivastava, R K Shukla, Nishant Kumar , Anu Katiyar. Nanoparticles as Biomarkers and Biosensors. Curr Trends Biomed Eng & Biosci. 2017; 9(3): 555762. DOI: 10.1098/CTBEB.2017.09.555762.
Pharmacokinetics, tissue distribution, and excretion of zinc oxide nanoparticles. Int J Nanomedicine 7: 3081-3097.

26. Cho WS, Cheol BK, Lee JK, Jayoung Jeong, Che JH, et al. (2013) Comparative absorption, distribution, and excretion of titanium dioxide and zinc oxide nanoparticles after repeated oral administration. Part Fibre Toxicol 10: 1-9.

27. Zhou L, Wang R, Yao C, Li X, Wang C, et al. (2015) Single-band up conversion nanoprobe for multiplexed simultaneous in situ molecular mapping of cancer biomarkers. Nature communication 6: 6938.

28. Braun GB, Friman T, Pang HB, Pallaoro A, Mendoza THD, et al. (2014) Etchable plasmonic nanoparticle probes to image and quantify cellular internalization. Nat Mater 13(9): 904-911.

29. Qing Q, Jiang Z, Xu L, Gao R, Mai L, et al. (2014) Free-standing kinked nanowire transistor probes for targeted intracellular recording in three dimensions. Nat Nanotechnol 9(2): 142-147.

30. Shen Y, Zhou J, Liu T, Tao Y, Jiang R, et al. (2013) Plasmonic gold mushroom arrays with refractive index sensing figures of merit approaching the theoretical limit. Nat Commun 4: 2381.

31. Tian B, Cohen Karni T, Qing Q, Duan X, Xie P, et al. (2010) Three dimensional, flexible nanoscale field-effect transistors as localized bioprobes. Science 329(5993): 830-834.

32. Hanash S (2004) Integrated global profiling of cancer. Nat Rev Cancer 4(8): 638-644.

33. Zheng GF, Patolsky F, Cui Y, Wang WU, Lieber CM, et al. (2005) Multiplexed electrical detection of cancer markers with nanowire sensor arrays. Nat Biotechnol123: 1294-1301.

34. Duan X, Gao R, Xie P, Cohen Karni T, Qing Q, et al. (2012) Intracellular recordings of action potentials by an extracellular nanoscale field-effect transistor. Nat Nanotechnol17: 174-179.

35. Zrazhevskiy P, Gao X (2013) Quantum dot imaging platform for single-cell molecular profiling. Nat Commun 4: 1619.

36. Perry P, Wolff S (1974) New Giemsa method for the differential staining of sister chromatids. Nature 251: 156-158.

37. Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos AP, et al. (1998) Semiconductor nanocrystals as fluorescent biological labels. Science 281: 2013-2016.

38. Han MY, Gao XH, Su JZ, Nie S (2001) Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules. Nat Biotechnol 19: 631-635.

39. Zhou L, Wang R, Yao C, Li X, Wang C, et al. (2015) Single-band up conversion nanoprobe for multiplexed simultaneous in situ molecular mapping of cancer biomarkers. Nature Communications 6: 6938.

40. Yezhelyev MV, Al Hajj A, Morris AI, Marcus AL, Liu T, et al. (2007) In situ molecular profiling of breast cancer biomarkers with multicolor dots. Adv Mater 19: 3146-3151.

41. Dejnsek M, Streitsov A, Pal S, Frutos AG, Powell CL, et al. (2003) Rare earth-doped glass microbarcodes. Proc Natl Acad Sci USA, 100(2): 389-393.

42. Tian G, Gu Z, Zhou L, Yin W, Liu X, et al. (2012) Mn2+ dopant-controlled synthesis of NaF: Yb/Er up conversion nanoparticles for in vivo imaging and drug delivery. Adv Mater 24(9): 1226-1231.

43. Wang J, Wang F, Wang C, Liu Z, Liu X (2011) Single-band up conversion emission in lanthanide-doped KMnF3 nanocrystals. Angew Chem Int Ed Engl 50(44): 10369-10372.

44. Özgür Ü, Alivov YI, Liu C, Tekeb A, Reshchikov MA, et al. (2005) A comprehensive review of ZnO materials and devices. J Appl Phys 98(4): 041301.

45. Zhao Z, Lei W, Zhang X, Wang B, Jiang H, et al. (2010) ZnO-based amperometric enzyme biosensors. Sensors Basel 10(2): 1216-1231.

46. Chen J, Ruther RE, Tan Y, Bishop LM, Hamers RJ, et al. (2012) Molecular adsorption on ZnO (2010) single-crystal surfaces: morphology and charge transfer. Langmuir 28(28): 10437-10445.

47. Hotchkiss PJ, Malicki M, Giordano AJ, Armstrong NR, Marder SR, et al. (2011) Characterization of phosphonic acid binding to zinc oxide. J Mater Chem 21(9): 3107-3112.

48. Moreira NH, Garcia A, Rosa AL, Frauenheim T, Dejneka MJ, Streltsov A, Pal S, Frutos AG (2012) Molecular adsorption on ZnO surfaces with organic molecules. Presented at: SPIE, San Francisco, USA.

49. Aldous SJ (2013) Cardiac biomarkers in acute myocardial infarction. Int J Cardiol 164(3): 282-294.