Although prostate cancer has been known for thousands of years, it is a major public health issue for men even in the 21st century. As the understanding of cancer biology and the signaling pathways advances, it is now well understood that the detection of prostate cancer circulating tumor cells (CTCs) is an invaluable tool for monitoring the progression and finding possible therapies. Driven by this need, in this article, the development of magnetic-nanoparticle-attached theranostic graphene oxide (GO) is reported for targeted capture of prostate CTCs, accurate diagnosis, and combined therapeutic action of prostate cancer. Reported results show that anti-epithelial cell adhesion molecule (EpCAM) antibody and A9-aptamer-attached flower-shaped magnetic nanoparticle-bound 2D graphene can be used for selective capture and label-free multicolor luminescence imaging of LNCaP prostate cancer cell from infected blood. Experimental data demonstrate that indocyanine green (ICG)-bound A9-aptamer-attached theranostic GO is capable of external near IR 785 nm light-driven combined synergistic photothermal and photodynamic treatment of prostate cancer. Possible mechanisms for targeted capture, accurate analysis, and combined therapeutic actions have been discussed. Experimental results show that the theranostic GO can have enormous potential for real-life applications, once optimized properly in clinical settings.

1. Introduction

According to the United States Centers for Disease Control and Prevention,[1] prostate cancer is a major public health issue for US and it is the most common cancer among American men. As per their report, about 230,000 men in the United States will be diagnosed with prostate cancer in one year. In the last 20 years in clinical practice, the level of prostate-specific antigen (PSA) has been used as a predictive biomarker of disease from blood with 4 ng/mL[2] level as the baseline.[1–6] However, several studies indicate that PSA tests can show both false positives and false negatives.[3–8] As a result, the US Preventive Services Task Force (USPSTF) recently recommended against PSA-based screening.[7] This year published results from USC Norris Comprehensive Cancer Center indicates that measuring circulating tumor cells (CTCs) from blood sample may be a better predictor than PSA.[8] Recent studies have shown that the accurate quantitations of CTCs in the bloodstream are very important and it can be used to determine prognosis as well as to find out the effectiveness of cancer therapy.[6–16] As a result, our aim is to find the assay based on simple blood test for finding prostate cancer CTCs, which will allow us to identify whether the cancer has spread to other areas of the body or not. Though CTCs are known since 1857, since in the early stage of cancer, a very few CTCs exist among billions of erythrocytes, clinical doctors need extremely sensitive and specific methods to detect early stage CTCs, which have been hard so far.[14–21] Driven by the need, in this article, we have reported magnetic graphene oxide (GO)-based assay for targeted captured of LNCaP prostate cancer cell from infected blood sample and followed by selective imaging and killing using combined therapy. In this article, we have demonstrated that magnetic-nanoparticle-attached hybrid GO can be used as a “theranostic” platform where capturing, diagnosis, and therapy can be combined within a single multifunctional GO platform. Since membrane
glycoprotein like epithelial cell adhesion molecule (EpCAM) is responsible for cell migration and proliferation. United States Food and Drug Administration (US-FDA) has approved a CTC analysis assay, which utilized ferrofluids conjugated with anti-EpCAM antibodies. However, recently reported data show that due to tumor heterogeneity, subpopulations of metastatic tumor cells often do not express enough EpCAM to detect CTCs. To overcome this, we have designed a versatile therapeutic platform using anti-EpCAM antibody and A9 aptamer, which has the capability to target clinically relevant LNCaP prostate cancer, which is known to overexpress prostate-specific membrane antigen (PSMA) biomarkers. A9 aptamer is known to recognize and bind to PSMA in intracellular or cytoplasmic epitope.

Once graphene was produced in the laboratory by Novoselov et al. in 2004, due to the remarkable electronic and structural properties, it has revolutionized the scientific community in the last 10 years. However, due to the presence of zero optical bandgap, graphene is not suitable to be used as a fluorescence imaging material. On the other hand, recently, we and other groups have reported that GO, a 2D material derived from graphene exhibits remarkable tunable fluorescence properties. By modifying various oxygen-containing groups in GO sheets, we have designed magnetic-nanoparticles-attached hybrid GO, which exhibits interesting optical and magnetic properties. Here, we have used the magnetic property of theranostic hybrid GO for selective capture of CTCs from infected blood sample, as shown in Scheme 1A. Similarly, tunable optical properties of theranostic hybrid GO has been used for multicolor imaging of tumor cells as shown in Scheme 1B. Due to the strong near-infrared (NIR) absorbance, recently several articles have demonstrated that GO nanoparticle can be used for targeted imaging and photothermal therapy (PTT) of cancer. Since we now understood that a single therapeutic agent can have limited efficiencies in clinical environment due to the drug-resistance profiles, in current manuscript for targeted therapy, we have used NIR-driven PTT and photodynamic therapy (PDT) together, as shown in Scheme 1C. We have utilized GO’s intrinsic NIR optical absorbance, for photodynamic killing of prostate cancer. Since indocyanine green (ICG) is a US-FDA approved photosensitizer, we have used ICG-bound A9 aptamer and a magnetic-nanoparticle-attached theranostic GO platform for combined synergistic therapy of prostate cancer.

2. Results and Discussions

Theranostic hybrid GO was developed by attaching cystamine-functionalized flower-shaped magnetic nanoparticle with 2D GO and prostate-cancer-specific antibody. Details of the synthesis procedure have been discussed in experimental section. In brief, initially flower-shaped magnetic nanoparticle was synthesized from FeCl₃ using ethylene glycol, anhydrous sodium acetate, and 1,6-hexadiamine. The Hitachi 5500 Ultra high-resolution scanning electron microscope (SEM) and JEM-2100F transmission electron microscope (TEM) were used to characterize the final product. SEM and TEM images as shown Figure 1A,B clearly show that we have produced flower-shaped magnetic nanoparticle. As shown in Figure 1C, the X-ray diffraction (XRD) data show clear crystal planes 220, 311, 222, 400, 422, 511, and 440, which correspond to the formation of Fe₃O₄ nanocrystals. The XRD data indicate that iron oxide nanoparticles are most likely mixtures of maghemite (γ-Fe₂O₃), hematite (α-Fe₂O₃), The
Figure 1. A) High-resolution TEM image shows the morphology of anti-EpCAM antibody-attached magnetic nanoflowers. Inserted high-resolution picture confirms the flower shape of the nanoparticle. B) High-resolution SEM picture confirms the shape of anti-EpCAM antibody-attached magnetic nanoparticle to be of flower shape. C) XRD patterns of the freshly prepared iron oxide nanoparticles. The lattice patterns are associated with iron oxide. The patterns are comparable with standard XRD for the Fe₂O₃ (JCPDS data: PDF number 39–1346). D) TEM image shows the morphology of freshly prepared anti-EpCAM antibody and A9 aptamer-bound magnetic nanoparticle-attached hybrid graphene oxide (GO). E) SEM image shows the morphology of freshly prepared aptamer and antibody-bound magnetic-nanoparticle-attached hybrid GO. Inserted figure shows EDX mapping clearly indicates the presence of iron nanoparticle in 2D hybrid GO. F) FT-IR spectrum from theranostic GO verifies existence of —C=O, —OH, —CN, —SH, and —NH groups. G) Raman spectrum from theranostic GO verifies existence of D and G bands of GO. H) Photograph shows that aptamer and antibody-boundflower-shapedmagnetic-nanoparticle-attached hybrid GO is highly magnetic and as a result, we can separate them by using a bar magnet.
superconducting quantum interference device (SQID) magnetometer measurement indicates that flower-shaped magnetic nanoparticle exhibits superparamagnetic behavior with specific saturation magnetization 52.8 emu g$^{-1}$. For understanding the advantages of using flower-shaped magnetic nanoparticle with respect to spherical magnetic nanoparticle, we have also prepared spherical shape iron oxide magnetic nanoparticle. After that, for targeted capture, imaging, and combined therapy, amino-functionalized flower-shaped magnetic nanoparticles were attached with anti-EpCAM antibody using a glutaraldehyde spacer method, as we and others have reported before.[11,14,16–18] To find the number of anti-EpCAM antibody molecules in each magnetic nanoparticle, we have used Cy3-labeled anti-EpCAM antibody. The amount of Cy3-labeled EpCAM antibody was measured by fluorescence. By dividing the total number of Cy3-labeled antibody by the total number of magnetic nanoparticles, we estimated that there were about 600–700 EpCAM antibody per flower-shaped magnetic nanoparticle. This experiment was performed 5–6 times, and average values are reported here.

In the next step, GO was produced by graphite exfoliation using the modified Hummers method as we and others have reported recently.[8,9,17] After that, to attach the amino-functionalized magnetic nanoparticles to 2D GO sheets, acid chloride-functionalized GO was produced. For this purpose, COOH-functionalized GO was treated with thiouyl chloride. Next, the acid chloride groups were used as chemical anchors for the attachment of the amino-functionalized magnetic nanoparticles and NH-modified A9 aptamer.

The FT-IR spectrum of the 2D theranostic GO in Figure 1F shows a strong vibrational peak at $\approx 1680$ cm$^{-1}$ corresponding to the vibrational mode of the carbonyl (C=O) groups from the aptamer and GO. Also, peaks were observed at $\approx 1725$ cm$^{-1}$ for the carbonyl (C=O) stretch of carboxylic acid. It also shows the clear characteristic peaks of antibodies at 2524 cm$^{-1}$ and 1088 cm$^{-1}$ and these are SH and CS stretching vibrations, respectively. Additionally, a vibrational peak was observed for NCO vibrations at $\approx 2270$ cm$^{-1}$ due to antibody. Similarly, two bands at 3250 and $\approx 3360$ cm$^{-1}$ are due to NH group in amine-functionalized nanoparticle, aptamer, and antibody. Raman spectrum of hybrid GO displays a D-band at 1340 cm$^{-1}$ and a G-band at 1612 cm$^{-1}$, as shown in Figure 1G. The strong D band in hybrid 2D GO spectra clearly indicates that the degree of oxidation is high. The ultra high-resolution SEM and TEM images of the hybrid 2D microstructure as shown in Figure 1D,E clearly show the presence of the flower-shaped magnetic nanoparticles at the edges and on the surface of the modified GO. The inserted energy-dispersive X-ray (EDX) spectroscopy map in the lower left-hand corner of Figure 1D clearly shows the presence of Fe nanoparticle in the hybrid 2D GO material. The photograph shown in Figure 1E indicates that the magnetic-nanoflower-attached hybrid GO is highly magnetic and as a result, the separation is possible using a small bar magnet. Measurements using a SQID magnetometer indicate that the hybrid GO exhibits superparamagnetic behavior with specific saturation magnetization of 31.2 emu g$^{-1}$.

Next to find out how the cell capture efficiency varies when GO is attached with only anti-EpCAM antibody or A9 aptamer. Figure 2B clearly shows that the capture efficiency is maximum when GO is attached with both anti-EpCAM antibody and A9 aptamer. The experimental data indicate that the capture efficiency is only 58% in the presence of anti-EpCAM antibody and similarly the capture efficiency is about 80% in the presence of A9 aptamer. On the other hand, the capture efficiency is only 97% in the presence of both. The reported above experimental data clearly show that hybrid GO binds with anti-EpCAM antibody and A9 aptamer exhibits the best performance. After that, to understand whether our theranostic GO can be used to capture cancer cell from the mixture of cancer cells and noncancerous cells, we have performed the same experiment for the mixture of 4 $\times$ 10$^3$ LNCaP cancer cells with 6 $\times$ 10$^3$ human skin HaCaT keratinocytes cells. Figure 2C clearly shows 98% cell capture efficiency using anti-EpCAM antibody and A9-aptamer-attached theranostic GO and on the other hand, about 0% capture efficiency with GO without anti-EpCAM antibody and A9 aptamer conjugation. As we have discussed before, the selective separation of CTCs from the infected site is due to the binding of CTCs to the anti-EpCAM antibody and LNCaP-specific A9 aptamer presence in 2D theranostic GO space. As shown in Figure 2A, our ELISA experimental results clearly show that the CTC separation efficiency is zero when we have not attached anti-EpCAM antibody and A9 aptamer with theranostic hybrid GO. On the other hand, ELISA results also show that around 97% CTC were separated in the presence of anti-EpCAM antibody and A9-aptamer-attached theranostic GO. Our experimental ELISA results clearly show that the separation and enrichment efficiency using anti-EpCAM antibody and A9-aptamer-attached theranostic GO can be around 97%.

Next, to find out the amount of aptamer, we have used Cy3-attached—NH-modified A9 aptamer. After incubation for several hours, we have separated the unbound aptamer by centrifugation. From the fluorescence analysis of unbound aptamer, we have estimated that around 20 000 aptamer in per hybrid graphene material.

To demonstrate that the theranostic GO developed by us can be used for CTC analysis close to clinical settings, 4 $\times$ 10$^3$ LNCaP cancer cells were spiked into 10 mL suspensions of citrated whole rabbit blood. After 90 min of gentle shaking, 450 μL theranostic hybrid GO was added to infected blood sample. After 30 min of incubation at room temperature under gentle shaking, we have used a bar magnet to separate LNCaP cells-attached theranostic GO. After that, we have used enzyme-linked immunosorbent assay (ELISA), TEM, and fluorescence imaging to characterize supernatant and suspension of LNCaP cells-attached theranostic GO, as shown in Figure 2A.

As a negative control experiment, we have also developed magnetic-nanoparticle-attached GO without anti-EpCAM antibody and A9 aptamer conjugation. As we have discussed before, the selective separation of CTCs from the infected site is due to the binding of CTCs to the anti-EpCAM antibody and LNCaP-specific A9 aptamer presence in 2D theranostic GO space. As shown in Figure 2A, our ELISA experimental results clearly show that the CTC separation efficiency is zero when we have not attached anti-EpCAM antibody and A9 aptamer with theranostic hybrid GO. On the other hand, ELISA results also show that around 97% CTC were separated in the presence of anti-EpCAM antibody and A9-aptamer-attached theranostic GO. Our experimental ELISA results clearly show that the separation and enrichment efficiency using anti-EpCAM antibody and A9-aptamer-attached theranostic GO can be around 97%.
separated by theranostic GO. Similarly, as shown in Figure 2D, the TEM images also show that LNCaP cells were captured by the theranostic GO. Figure 2E,F clearly show that the luminescence of GO can be tuned just by varying the excitation energy without changing its chemical composition and size, as we and others have reported before. All the above experimental results clearly show that the theranostic GO is capable of separating CTCs from infected blood sample.

Next, to understand whether the theranostic GO can be used for NIR-light-driven therapy. Therapy experiments were

Figure 2. A) ELISA results show LNCaP cancer cell capture efficiency of our theranostic GO from infected blood. Plots also show that separation efficiency is zero in the absence of anti-EpCAM antibody and A9 aptamer. B) ELISA results show how LNCaP cancer cell capture efficiency varies for anti-EpCAM antibody-attachedGO,A9-aptamer-attachedGO, and theranostic graphene attached with both, anti-EpCAM antibody, and A9 aptamer. C) ELISA results show cancer cell capture efficiency of our theranostic GO from cell mixture of LNCaP cell and normal skin HaCaT cell. Plots also show that cancer cell separation efficiency is zero in the absence of anti-EpCAM antibody and A9 aptamer. D) TEM image shows that LNCaP cells are captured by theranostic GO. E–F) Fluorescence image shows multicolor imaging of LNCaP cells after capture by anti-EpCAM antibody and A9-aptamer-attached hybrid GO. We have used 410 and 510 nm light as excitation sources. G) Bright-field image of the same LNCaP Cells.
performed using 785 nm excitation light. Before NIR-light-driven therapy experiment, we have performed the cytotoxicity experiment for the theranostic GO in the absence of NIR light. For this purpose, theranostic GO was incubated with LNCaP cells for 12 h in the absence of NIR light. After that, we have used an MTT assay to determine the cell viability. MTT test is a colorimetric assay, which can be used to find the number of viable cells present after incubation. As shown in Figure 3A, the MTT assay data clearly show that no cell death was observed even after 12 h of incubation. The above test clearly shows that anti-EpCAM antibody and LNCaP-specific A9-aptamer-attached theranostic GO developed by us are not cytotoxic in the absence of external NIR light. Next, we have performed 785 nm light-driven PTT experiment using LNCaP cells-attached theranostic GO. We have used 785 nm light at 1 W cm⁻² power for 20 min for PTT experiment. Using the MTT test, we found out that only 21% LNCaP cells were dead due to the PTT as shown in Figure 3A. Since GO absorption cross-section is very low at 785 nm, the observed hypothermia effect is also low. Akhavan et al.[46] have reported complete destruction of the cancer cells under irradiation of an 808 nm laser source with power density of 7.5 W cm⁻². Recently, they have also reported[45] that magneto-PTT with 7.5 W cm⁻² NIR irradiation and 2 gray (Gy) gamma radiation radiotherapy can be used to kill most of the cancer cells within 2 min. So PTT efficiency may be improved by increasing the laser power density or by combining with other therapy. [43–45]

To improve the 785 nm light driving LNCaP cancel cell-killing efficiency, we have used ICG-bound A9-aptamer-attached theranostic GO as a combined therapy material. After that, to find out the LNCaP cell-killing efficiency of PTT, PDT, and combined therapy, we have performed several different experiments. At first, to find out the possible cytotoxicity of ICG-attached theranostic GO, we have incubated ICG-bound theranostic GO with LNCaP cell for 12 h in the absence of NIR light. After that, we have performed the MTT test to find the cell viability. Our experimental data as shown in Figure 3A indicate no LNCaP cancer cell death even after 12 h of incubation. The above-reported cytotoxicity results shows that ICG-bound theranostic GO is not cytotoxic in the absence of external 785 nm NIR light. However, as shown in Figure 3A, 785 nm NIR-light-induced experimental data indicate that about 90% of LNCaP cancer cells were dead when ICG-bound theranostic GO-attached LNCaP cells were irradiated with 785 nm light at 1 W cm⁻² power for 15 min. We have also performed trypan blue assay experiment to determine the amount of cell death due to the combined therapy effect. For this purpose, we have added trypan blue after combined therapy experiment.

The cell death during combined therapy process could be due to numerous factors including reactive oxygen species (ROS)-induced cancer cell death due to the presence of ICG and thermal disintegration by GO. Next, to find out amount to cell death for PDT only, we have mixed only ICG with LNCaP cells and incubated for 30 min. After that, we have exposed the mixture to 785 nm light for 20 min. We have kept the ICG concentration and the laser power the same for all the experiments. As shown in Figure 3A, our experimental data indicate that of 50% LNCaP cancer cells are dead when only ICG was used for PDT. All the above results clearly indicate that ICG-bound theranostic GO-based combined therapy efficiency is much higher than individual therapy.
To understand the synergistic killing mechanism in the case of combined therapy, we have performed several experiments and these are as follows: 1) We have measured the temperature change during therapy process using a MikroShot Camera via thermal imaging at 1-min interval. Our experimental data indicate that the temperature increased to about 40 °C when ICG-bound theranostic GO-attached LNCaP cells were exposed to 785 nm laser light at 1 W cm\(^{-2}\) power for 20 min. Our experimental data also indicate that the temperature increased to only 29 °C for LNCaP prostate cancer cells in the absence of theranostic GO under the same light exposure condition. Now during the therapy process using ICG-bound theranostic GO-attached LNCaP cells, ICG can generate ROS in the presence of 785 nm light to kill cancer cells. So, the efficiency of photodynamic killing should be dependent on the formation of ROS capability. To understand the mechanism clearly, we have also determined the cellular ROS formation during PDT and combined therapy process using singlet oxygen sensor green reagent (SOSG; Sigma). For this purpose, we have measured the fluorescence intensity at 528 nm using the microplate reader with the excitation wavelength at 485 nm. As reported in Figure 3C, we found out elevated ROS formation in the presence of ICG-bound theranostic GO, which shows that the photothermal effect of GO was able to enhance the formation of singlet oxygen. As a result, we have noted improved photodynamic cancer cell-killing efficiency during the observed synergistic therapeutic effect.

3. Conclusions

In this article, we have reported the development of anti-EpCAM antibody and A9-aptamer-attached theranostic GO, which has capability to simultaneously deliver separation, enrichment, and label-free accurate diagnosis of LNCaP prostate cancer cells from infected blood sample. Our results also demonstrate that ICG-bound theranostic GO is capable of multimodal therapeutic functions to kill LNCaP prostate cancer cells. Experimental data show that theranostic GO can be used for enabling detection and combined treatment of LNCaP cancer cells in a single procedure. Reported data prove that the theranostic graphene developed by us can be used not only for separation from infected blood, but can also be used for label-free luminescence imaging as well as for combined PTT and PDT therapy. Our results also show that the combined therapy can dramatically enhance the possibility of destroying LNCaP cancer cells in vitro due to the synergistic therapeutic effect. Even though the theranostic GO have shown promising results for LNCaP prostate cancer isolation and imaging from the whole blood sample, as well as for combined therapy capability, we need to admit that we are at a relatively early stage of development. After the optimization of different parameters, theranostic GO-based assay could have enormous potential for applications in CTC detection, analysis, and killing from the clinical sample.

4. Experimental Section

Materials: We have purchased graphite, KMnO\(_4\), nitric acid, ethylene glycol, FeCl\(_3\), sodium borohydride, sodium acetate, and 1,6-hexadimine, antibody from Fisher Scientific and Sigma–Aldrich. LNCaP human prostate cancer cell lines, growth media to grow cells, were purchased from the American Type Culture Collection (ATCC, Rockville, MD).

Synthesis of Theranostic Graphene Oxide: Theranostic hybrid GO was developed by attaching cystamine-functionalized flower-shaped magnetic nanoparticle with 2D GO and prostate-cancer-specific aptamer/antibody. Flower-shaped magnetic nanoparticles were synthesized by dissolving 1.5 g FeCl\(_3\) in ethylene glycol. After that we have added 2.0 g anhydrous sodium acetate. In the next step, 4.0 g of 1,6-hexadimine was added and stirred vigorously to acquire a transparent solution. Then the mixture was sealed in a teflon-lined stainless steel autoclave and was heated at 230 °C for 8 h. After that the product was washed with hot water and ethanol. We then kept the product under ultrasonic condition to remove the solvent and unbound 1,6-hexadimine effectively. At the end, we have dried at 50 °C to get the black powder. We have used Hitachi 5500 Ultra high-resolution SEM and JEM-2100F TEM to characterized them, as shown in Figure 1A.

In the next step, we produced GO using the modified Hummers reported method, where graphite exfoliation has been performed using strong oxidizing agents, as we and others have reported before.[29–31] For this purpose, we have used 1 g of graphite powder and it was treated with 1 g of Na\(_2\)O\(_2\) in 45 mL of H\(_2\)SO\(_4\) and 3 g of KMnO\(_4\), without changing the temperature for 30 min. After that, we have attached amino-functionalized magnetic nanoparticles with 2D GO sheets. For this purpose at first, acid-chloride-functionalized GO was produced from —COOH-functionalized GO by treating with thionyl chloride, as we have reported before.[29,32] At the end, the acid chloride groups were used as chemical anchors for the attachment of the amino-functionalized magnetic nanoparticles and —NH-modified A9 aptamer. The Ultra high-resolution SEM and TEM image was used to characterize theranostic 2D microstructure, as shown in Figure 1C,D. EDX map in the lower left-hand corner of Figure 1D clearly shows the presence of Fe nanoparticle in the hybrid 2D GO material.

Magnetic Property Measurement: We have used Quantum Design MPMS–XL, USA SQUID magnetometer for the measurement of the magnetic properties of theranostic GO.

Cancer Sample Preparation and Incubation with Theranostic Graphene Oxide: LNCaP prostate cancer cells were grown according to the ATCC procedure in a 5% CO\(_2\) incubator at 37 °C using ATCC medium supplemented with 10% premium fetal bovine serum (FBS) and antibiotics (10 IU mL\(^{-1}\) penicillin G and streptomycin) in 75-cm\(^2\) tissue culture flasks. After it grows to 10\(^5\) Cells mL\(^{-1}\), different concentrations of LNCaP cancer cells were spiked into 10 mL suspensions of citrated whole rabbit blood. After 90 min of gentle shaking, 450 µL of theranostic hybrid GO was added to infected blood sample. After 30 min of incubation, a bar magnet has been sued to separate LNCaP cells-attached theranostic GO.

Fluorescence Analysis: After selective capture of LNCaP can cells from infected blood sample using a bar magnet, we have used an Olympus IX71 inverted confocal fluorescence microscope fitted with a SPOT Insight digital camera for fluorescence imaging.

PTT, PDT, Combined Killing, and Find Out the Killing Efficiency: For NIR-light-tiggered therapy experiment, we have used a portable continuous wavelength OEM laser operating at 670 nm, with 1–2 W cm\(^{-2}\) power as an excitation light source at different time interval. All different light-driven therapy experiment have been performed at exactly same wavelength, same power. After that we have used the MTT assay and trypan blue assay to determine the cell viability.

Finding ROS Generation: We used SOSG reagent (Sigma), according to manufacturer’s protocol, to find amount of ROS formation. For this purpose, we used a microplate reader to measure the fluorescence intensity at 528 nm. We used 485 nm excitation wavelength for this study.

Acknowledgements

Dr. Ray and Dr. Sardar is grateful for NSF-PREM grant # DMR-1205194, NSF-PREM grant # DMR-0934218 for their generous funding.

Received: June 30, 2014
Revised: July 19, 2014
Published online: