Published in final edited form as:

Small. 2013 January 28; 9(2): 215–221. doi:10.1002/sml.201202090.

Bacteriophage Bionanowire as a Carrier for Both Cancer-Targeting Peptides and Photosensitizers and its use in Selective Cancer Cell Killing by Photodynamic Therapy

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Photodynamic therapy (PDT) has received greater attention in the recent years although the first photodynamic drug, acridine, was invented about 100 years ago.^[1] This is due in great part to the advent of understanding therapeutic light properties and the development of fiber optic lasers. PDT is a treatment that involves the combination of endogeneous oxygen, light and photo-active compounds (commonly termed photosensitizers) to produce singlet oxygen ($^1\text{O}_2$), which can induce cell death and destroy tumors.^[2] Currently, some photosensitizers have been approved by the United States Food and Drug Administration while many remain in clinical trials.^[3] These photosensitizers are efficiently being used to treat several types of cancers and a variety of other diseases.^[4]

The preferential accumulation of a photosensitizer in a malignant tumor followed by irradiation with an appropriate wavelength of light can generate cytotoxic $^1\text{O}_2$, resulting in the death of malignant cells or tissues via apoptosis and/or necrosis.^[4] However, the extent of photodamage in PDT is multifactorial, depending on the type, absorption maxima, and subcellular localization of photosensitizer, the light exposure time and fluence rate, sensitizer dose, and oxygen availability.^[5] Currently, PDT is limited by the available

photosensitizer concentration at the target site, which in turn limits the photodynamic efficiency. Thus, the greatest challenge in front of PDT researchers is to find new avenues for improving photodynamic efficiency by selectively targeting and eradicating the tumor.

Filamentous bacteriophage (also known as phage), such as M13 or fd-tet, is a class of viruses that specifically infect gram negative bacteria. The filamentous phage can be pictured as a biological nanowire (~900 nm long and ~7 nm wide) where DNA comprising the core is enclosed by coat proteins (Scheme 1C). There are five genetically modifiable coat proteins.^[6] The major coat proteins (called pVIII, ~3900 copies for fd-tet phage) compose the side wall of the phage while four other minor coat proteins (called pIII, pVI, pVII, pIX, about 5 copies each) constitute the two distal ends.^[7] Since the coat proteins are encoded by the DNA within the phage, the surface of the phage can be genetically modified by fusing a foreign peptide to the N-terminal end (the solvent exposed end) of the coat proteins, leading to the development of the phage display technique.^[8] Phage display has been used in multiple applications ranging from templating materials synthesis, biological sensing, promoting stem cell differentiation, and to therapeutic delivery.^[9] Previously, phage display technology has been successfully used to obtain a target specific phage with cancer cell-targeting or tumor-homing peptides displayed on the surface,^[10] however, its application in PDT is not shown to the best of our knowledge. Here, we report a new strategy of combining phage display technology with photochemistry (the use of photosensitizers) to fulfill current gaps in PDT (Scheme 1). Towards this direction we present the conjugation of photosensitizer to phage (specific to breast cancer cell lines) to form phage-photosensitizer complex and evaluated its in vitro photodynamic therapy efficiency. We chose our recently selected SKBR-3 breast cancer cell-targeting phage with a unique SKBR-3 cell-targeting peptide (VSSTQDFP) fused to each of the ~3900 copies of pVIII on its side wall (Scheme 1C).^[11] The breast cancer cell targeting phages were screened using a random major coat displayed phage library called landscape phage library.^[12] In this work, the phage nanowire is used as a targeting vehicle to deliver photosensitizer to the desired site for PDT applications (Scheme 1C). In order to retain the targeting specificity of the phage nanowire, we partially modified the N-terminus of the phage surface with 9-ethenyl-14-ethyl-4,6,13,18-tetramethyl-20-oxo-3-phorbinepropanoic acid, known as pyropheophorbide-a (PPa, a well-established photosensitizer),^[13] using 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC) as a coupling reagent (Schemes 1, S1 and S2).^[14] Thus, the resulting phage is expected to be partially modified with PPa, retaining the target binding capacity at the free N terminus of the pVIII not conjugated to PPa. These phage enabled photosensitizers broaden the available clinical repertoire of target specific sensitizers used in PDT.

To determine the optimum PPa-phage complex for targeted PDT, we prepared PPa-phage conjugates (Figure 1 and S1) with different nominal conjugation percentages (10–100%), which are defined as the ratio between nominal PPa molecules conjugated to the major coat protein of each phage and the total number of major coat proteins (3900) of each phage. The PPa-phage conjugates were synthesized by conjugating –COOH group of the PPa to the N-terminal end of pVIII of breast cancer specific phage through EDC/NHS (NHS: *N*-hydroxysulfosuccinimide) conjugation chemistry (Scheme 1A,B and S1,S2). The extent of functionalization was controlled by adjusting the molar ratio of phage and PPa.^[15] Figures 1A and S1A show that the absorption maxima of PPa-phage at 405 and 668 nm increase with the increase in the percentage conjugation of PPa. The fluorescence spectra at lower wavelength (Figure S1B) clearly show that the increase in the percentage conjugation of PPa gradually decreases the tryptophan fluorescence due to fluorescence resonance energy transfer (FRET) between tryptophan of the major coat protein of phage and PPa, which indicates the successful conjugation of PPa on major coat protein. The fluorescence spectra at higher wavelength (Figure 1b) clearly show that the increase in the percentage

conjugation of PPa decreases the fluorescence due to the energy hopping between highly ordered PPa molecules on phage. All of these results, along with FT-IR spectrum of the PPa-phage complex (Figure S2), suggest the successful conjugation of PPa to the coat protein of phage.

We termed the resulting conjugates based on the percent of conjugation. The 100% conjugated PPa-phage complex, where PPa is conjugated to 100% of the 3900 copies of pVIII per phage particle, is fully modified and thus termed f-PPPa (Scheme 1A). The 50% conjugated PPa-phage complex is partially modified and thus termed p-PPPa (Scheme 1B). Among various complexes synthesized, our later experiments were primarily focused on 10%, 50% and 100% PPa-phage complex (Figure 1) to find the suitable complex for optimal activity. The photophysical properties of both half and fully modified complexes were observed using UV-Vis and fluorescence spectroscopy (Figure 1). Interestingly, we found 50% conjugation, where PPa is conjugated to 50% of the 3900 copies of pVIII per phage particle, is the ideal candidate for targeted PDT owing to its favorable photophysical properties (Figure 1) as well as the exposure of ~1900 copies of targeting peptides per phage particle in the complex. Our results clearly show that there is no absorption shift in UV-Vis spectra of PPa-phage complex in dimethyl sulphoxide (DMSO) (Figure 1a), but we do observe the significant change in fluorescence with increase in percentage of PPa-phage conjugation (Figure 1b). Fluorescence maxima of PPa was increased linearly up to 50% conjugation then started decreasing and finally reached a constant fluorescence intensity at 100% conjugation (Figure 1b). This is likely due to the energy hopping between the PPa molecules when they are close enough to transfer the energy between the highly ordered PPa molecules on the phage. We can also observe the decrease in fluorescence intensity of phage at 328 nm with increase in percentage conjugation of PPa (Figure 1b, inset), which is likely due to the energy transfer between tryptophan of phage and PPa after the successful conjugation. From UV-Vis spectra (Figure 1a), we estimate that the f-PPPa is ~3800 molecules and the p-PPPa is ~1800 molecules of PPa per phage from the absorption maxima of PPa at 420 nm before and after conjugation. This result is consistent with the expected conjugation percentage.

In order to understand the morphology of phages before and after photosensitizer modification, we used electron microscopy (Figure 2). Briefly, unmodified phages were ~900 nm in length and ~7 nm in diameter with a very flexible structure (Figure 2A). However, upon phage modification with fully and partially coated sensitizers, we observed significant changes in phage morphology. In the case of f-PPPa, we found a complete morphological change where the phage was straightened and rigid (Figure 2B) rather than flexible, as in the case of the unmodified phage (Figure 2A). This major morphological change could be due to the strong hydrophobic interactions between the PPa macrocycles. In contrast, we did not observe such drastic morphological changes in the case of p-PPPa (Figure 2C). These results further confirm the successful conjugation of PPa onto the phage nanowires.

The minimum triplet energy required for the generation of $^1\text{O}_2$ is 22.5 kcal. It is possible that the large protein-based structures, such as phage, can change the ground and excited electronic states of PPa. The photophysical properties of PPa-phage complexes (Figure 1A,B) indicate that p-PPPa was a suitable photosensitizer for targeted PDT due to its less energy hopping compared to f-PPPa. The p-PPPa complex was thus tested to confirm $^1\text{O}_2$ production to determine its efficiency for use in PDT by both time-resolved (Figure 1C) and steady state (Figure S3 and Scheme S3) methods by comparing the Quantum Yield (Φ_{Δ}) with PPa in DMSO with known Φ_{Δ} of 0.52.^[16] Time-resolved spectroscopy (Figure 1C) clearly shows the phosphorescence of singlet oxygen and confirmed the generation of singlet oxygen from PPa-phage complex after the irradiation of light. We estimated the

relative Φ_{Δ} by comparing the $^1\text{O}_2$ phosphorescence intensity from p-PPPa to that of a reference sensitizer PPa via the time resolved method using a liquid nitrogen cooled Ge-Diode detector and Nd-YAG laser at 532 nm as previously reported.^[17] For p-PPPa DMSO solution, the relative Φ_{Δ} was determined to be 0.37 ± 0.002 and the $^1\text{O}_2$ lifetime (τ_{Δ}) was measured to be 7.6 μs which is similar to the τ_{Δ} previously reported in DMSO.^[18] The $^1\text{O}_2$ production was also confirmed in the steady state method using 1,3-diphenylisobenzofuran (DPBF), a well-known $^1\text{O}_2$ trap,^[19] in DMSO at 658 ± 10 nm of continuous laser (Figure S3). The absorption of PPa and p-PPPa were fixed to 0.1 at 658 nm. A decrease in DPBF absorption at 410 nm confirmed the formation of DPBFO₂ by its reaction with $^1\text{O}_2$ produced from photolysis (Figure S3B and Scheme S3). We estimated the amount of $^1\text{O}_2$ molecules produced in both PPa and p-PPPa by calculating the concentration of DPBFO₂ formed (Figure 3B). Kinetic calculation confirmed that the concentration of DPBF was sufficient to intercept more than 95% of the $^1\text{O}_2$ produced in the solution. In both the time resolved (Figure 1C) and steady state (Figure S3) methods, the $^1\text{O}_2$ quantum yield of p-PPPa was high compared to f-PPPa. The less $^1\text{O}_2$ production for f-PPPa (Φ_{Δ} is 0.21 ± 0.002) arose from the very high excitonic coupling between the PPa molecules. From the Φ_{Δ} studies, we have found that the p-PPPa is a good candidate for PDT. In addition, ~1900 copies of SKBR-3 breast cancer targeting peptides are exposed to the environment for targeting the SKBR-3 cancer cells per phage nanowire in the p-PPPa complex.

In order to confirm the p-PPPa complex is stable in serum, we performed serum stability studies (Figure S4). As shown in the Figure S4, characteristic peak (~670 nm) from PPa is observed even after 6 days of incubation, suggesting the presence of the p-PPPa complex in serum after long time exposure to serum and thus a reasonably good stability of the p-PPPa complex in serum. Furthermore, the binding of p-PPPa to breast cancer cells was examined by immunofluorescence using anti-phage antibody labeled with Alexa fluor 488. Figure 3 clearly demonstrates the binding of photosensitizer conjugated phage to SKBR-3 breast cancer cells. To further assess the quantitative viability of p-PPPa complex on SKBR-3 and MCF-7, we performed MTT cell cytotoxicity assay in the absence (dark toxicity) and presence of light (photodynamic effect) (Figure 4). From the dark toxicity studies as shown in Figure 4A, p-PPPa showed slight toxicity (not statistically significant) to the SKBR-3 cells which could be probably because the exposure of light inside the plate reader produced $^1\text{O}_2$ which resulted in some of the cell death. However, the cell viability studies in Figure 4B indicates that most of SKBR-3 cells were dead after exposure with external 658 nm laser due to the photodynamic action, whereas in the case of MCF-7 cells 65% of cells were viable. The small percentage of cell death in MCF-7 cells might be due to the nonspecific binding of p-PPPa. Additionally, the time course cell viability studies in Figure 4C reveal that the PDT is successful in killing SKBR-3 cells.

Furthermore, we conducted in vitro PDT in SKBR-3 and control MCF-7 cancer cell lines to verify the feasibility of using our complex to selectively kill target cancer cells. Figure 5 shows the microscope images of control MCF-7 (Figure 5A–C) and target SKBR-3 cells (Figure 5D–F). All the images were captured at 10 $\times$ objectives. Figures 5A,D are light microscope images of MCF7 and SKBR3 cells, respectively. Figures 5B, C, E, and F are fluorescence images of laser treated SKBR3 and MCF-7. The extent of live and dead cells upon laser exposure is determined by using Invitrogen live/dead cell (Calsein AM & Ethidium homodimer) viability kit, in which live cells fluoresce green and dead cells fluoresce red. All cells were treated with the same power and exposure time of laser at 658 nm.

As shown in Figure 5E,F, SKBR-3 cells treated with PPa alone (Figure 5E) and p-PPPa complex (Figure 5F) were found dead after laser irradiation as expected. However, MCF-7 cells treated with PPa were found dead (Figure 5B) while the same cells treated with p-PPPa

remained alive (Figure 5C). These results clearly demonstrate that our novel p-PPPa sensitizer with inherent target specific phage selectively kills SKBR-3 cells upon irradiation with 658 nm laser light. Partially modified phage, having PPa (i.e., p-PPPa), retained their targeting capabilities with SKBR-3 cells and produced cytotoxic $^1\text{O}_2$ upon irradiation with the laser (Figures 1C and 3). However, in the case of MCF-7 cells, p-PPPa did not show any PDT effect due to a lack of phage specificity and binding capabilities which resulted in the drug being completely washed off with phosphate buffered saline (PBS) after 1 h incubation. In the case of PPa, both of the cell types were killed due to the nonspecific localization of the sensitizer and its subsequent photodynamic activity. This clearly indicates that our p-PPPa was highly selective and can efficiently destroy our target SKBR-3 cells alone.

In summary, these novel bacteriophage-enabled photosensitizers open the door for new categories of PDT photosensitizers. By taking advantage of phage display technology, we can develop several types of phage-photosensitizers for various cancers in PDT. These target specific virus photosensitizers can be used in both in vitro and in vivo to selectively abolish a tumor by producing cytotoxic $^1\text{O}_2$ upon illumination with an appropriate wavelength of light at the target site. Currently, we are investigating in vivo selection of tumor-homing phage that can be modified with photosensitizer for in vivo photodynamic therapy.

Experimental Section

Materials

Pyropheophorbide-a (PPa) was purchased from Frontier Scientific. 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), *N*-hydroxysulfosuccinimide (NHS), dimethyl sulfoxide (DMSO), Luria-Bertani (LB), tetracycline and polyethylene glycol (PEG) were purchased from Sigma Aldrich. All the chemicals were used as received without any further purification. Tris was obtained from Fischer. Kanamycin was obtained from Fluka and Sodium chloride was purchased from EMD and used as received. All the other reagents used were analytical grade.

Cell Culture

SKBR-3 and MCF-7 human epithelial breast cancer cells were purchased from ATCC (Manassas, VA). The cells were cultured in Mc.Coy's 5A medium and DMEM medium, respectively, and supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 $\mu\text{g}/\text{mL}$ penicillin and 100 $\mu\text{g}/\text{mL}$ streptomycin) (Sigma, St. Louis, MO). Both the cell lines were grown at 37 °C in 5% CO_2 -humidified atmosphere in 25 cm^2 tissue culture flasks. When the cells reached 90% confluence, they were washed with PBS and harvested with 1 mL of 0.25% trypsin-EDTA solution (Sigma). Cells were then centrifuged and counted in trypan blue to ensure viability and plated at a density of 10 000/well in flat-bottom 12-well plates (Fisher Scientific, Pittsburgh, PA).

Phage Purification

SKBR-3 specific phage displayed with the VSSTQDFP sequence on the N terminus of major coat proteins, were screened using a landscape phage library as described in our earlier work.^[11] Briefly, 1×10^9 transducing units (TU) of a random landscape phage library was used for screening whole SKBR-3 breast cancer cells. Phage were recovered after four rounds of affinity selection and sequenced for inserts. The selected phage was purified by double PEG precipitation as described previously.^[20] Briefly, 40 mL of NZY medium containing 20 $\mu\text{g}/\text{mL}$ tetracycline was inoculated with a fresh single colony of phage-infected cells in a 250 mL flask. The culture was incubated under shaking at 37 °C and 200 rpm overnight. The overnight culture was poured into a 250 mL Beckman centrifuge bottle

and centrifuged at 3000 g for 10 min at 4 °C. The supernatant was poured into a fresh tube and centrifuged at 12 000 g for 10 min at 4 °C. The resultant cleared supernatant was then poured into a fresh tube again, into which 6 mL of PEG/NaCl solution (100 g PEG 8000, 116.9 g NaCl and 475 mL water) was then added. The tube was kept at 4 °C overnight for phage precipitation. The precipitated phage were collected by centrifugation at 31 000 g for 15 min and the supernatant was removed. The pellet was then dissolved in 1 mL Tris buffered saline (TBS) in a micro-centrifuge tube and centrifuged again at 12 000 g for 2 min to remove any nondissolved material. 150 µL PEG/NaCl solution was added to the supernatant and kept on ice overnight. The tube was then centrifuged at 12 000 g at 4 °C for 10 min and the supernatant was removed. The pellet was dissolved in 200 µL of double distilled water by vortexing. The concentration of phage was estimated using a UV-visible spectrophotometer by measuring the absorbance at 269 nm.^[21]

Activation of PPa with EDC

1 mg of PPa was dissolved in 1 mL of DMSO. To this solution 0.4 mg of EDC was added to activate the carboxylic group in PPa (Scheme S1). The reaction mixture was incubated at 37 °C on a roto-shaker for 1 h. The unreacted EDC was quenched by 2-mercaptoethanol (MeSH) to avoid the self-conjugation of phage nanowires in the next step. The reaction mixture was incubated at 37 °C on a roto-shaker for 1 h. The resultant activated PPa was stored at –80 °C for further use.

Conjugation of Cancer Cell-Targeting Bacteriophage and PPa

SKBR-3 specific phage displayed with the VSSTQDFP sequence on the N terminus of major coat proteins, were screened by our lab using a landscape phage library as described elsewhere.^[11] Fully and partially modified PPa-phage complexes were synthesized by changing the molar ratios between PPa and phage as shown in Schemes S1 and S2. They were prepared by dissolving 45 µL and 22.5 µL of EDC activated PPa respectively in 2 mL of phage solution containing 6.4e+12 virions/mL of the genetically engineered VSSTQDFP displayed phage. Both of the samples were dialyzed and preliminarily tested by ninhydrin followed by UV/Visible absorption spectroscopy, infrared spectroscopy and transmission electron microscopy (TEM). Finally, the solution was stored at –20 °C for further studies.

Singlet Oxygen (¹O₂) Measurements

Time resolved and steady state singlet oxygen measurements were performed using Nd-YAG laser and 1,3-diphenylisobenzofuran (DPBF, a molecule that can react with singlet oxygen, Scheme S3 and Figure S3). All the steady state experiments were carried out in an air saturated DMSO solution using picomolar concentrations of DPBF at ambient temperatures of 20–22 °C in completely dark conditions. Samples were photolyzed in a 1 cm quartz cell with a 658 nm diode laser (from laser glow) at 50 mW of power. The disappearance of DPBF was monitored at: 0, 15, 45, 75, 105, 135, and 165 s by its change in absorbance at 410 nm.

Spectroscopy

UV/Vis absorption spectra were acquired on an Implen nanophotometer. Absorption spectra of phage, PPa, p-PPa and f-PPPa were recorded in a 50 µL quartz cell at 23 °C in a mixture of 50% H₂O and 50% DMSO at pH 7.0. Fluorescence spectra of PPa-phage complexes with different ratios of PPa and phage were measured in Shimadzu (RF-5301PC) spectrophotometer. All fluorescence spectra were recorded in a 50 µL quartz cell at 23 °C in DMSO. Fourier transform infrared (FT-IR) spectra were measured in a Nicolet Nexus spectrometer at room temperature (Nicolet Instrument Co., USA). The samples were freeze dried in a vacuum and prepared in a KBr pellet (2%, w/w). The spectra were carried out

between 400 and 4000 cm^{-1} with a total of 32 scans, then collected and processed with the Omnic 5.2a software package.

Transmission Electron Microscopy

Phage and its photosensitizer conjugates were analyzed in a TEM (JEOL-2000FX). A drop of each sample was placed on a dry copper grid (150 mesh) that was previously coated with carbon and stained with 2% uranium acetate. All TEM images were recorded in the same magnification to understand the phage morphology change before and after conjugation with PPa.

In vitro Photodynamic Therapy

We conducted in vitro studies in both the target SKBR-3 and control MCF-7 cancer cell lines to verify the selectivity and efficiency of p-PPPa in PDT. After SKBR-3 and MCF-7 cells had grown for 24 h, a final concentration of 10 μM p-PPPa, prepared in complete McCoy's 5A medium and DMEM medium containing 10% FBS and antibiotics, was added to the cells for an additional 1 h incubation period. After 1 h of incubation, the medium was replaced with fresh complete medium and applied with 7.5 J cm^{-2} of illumination using visible red laser light at 658 nm. The light exposure area covered four wells and was considered as one experimental group exposed simultaneously. The control groups consisted of the following: groups with no treatment, laser light alone, phage alone and PPa alone (absorption at 658 nm is 0.1). For time course incubation studies, the p-PPPa was incubated with SKBR-3 cells and MCF-7 cells at various lengths of time (1 h, 15 h and 24 h). PDT with 7.5 J cm^{-2} of 658 nm light was then delivered at a dose of 50 mW cm^{-2} . During PDT, the total laser exposure time is 2 min with 30 s interval between every 10 s of laser exposure. Following PDT treatment, the cells were returned to the incubator for a few hours and a live/dead cell viability kit was used to evaluate cell death. For quantitative estimation, cell viability study using MTT assay in the presence and absence of laser was performed. Briefly, cells were incubated with only PPa and p-PPPa complex for various time points on a 96-well plate. After incubation, cells were washed and treated with 10–20 μL of MTT reagent (5 mg mL^{-1}) and incubated for 4–6 h. After incubation, the medium was removed and 100 μL of DMSO was added and the absorbance at 490 nm was measured using a plate reader.

Serum Stability Studies

In order to perform serum stability studies, the p-PPPa complex was prepared as stated above and dissolved in serum. Every day, the samples were examined under UV-Visible spectra to detect changes in the absorption profiles over a period of time.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was in part supported by National Institutes of Health (5R21EB009909-02 and 1R21EB015190-01A1). We also would like to thank the financial support from National Science Foundation (DMR-0847758, CBET-0854414 and CBET-0854465), National Institutes of Health (5R01HL092526-02 and 4R03AR056848-03), Oklahoma Center for the Advancement of Science and Technology (HR11-006), Oklahoma Center for Adult Stem Cell Research (434003), and Department of Defense Peer Reviewed Medical Research Program (W81XWH-12-1-0384). YH, LW and CBM would also like to thank Joint Research Fund for Overseas Chinese Young Scholars from National Natural Science Foundation of China (81028010) and Program for Introducing Talents to Universities (B07017) for generous support. We also thank Dr. Paul Cook at the University of Oklahoma for using his instrument and Drs. Ruomei Gao and Xianchun Zhu at Jackson State University for helping with time resolved $^1\text{O}_2$ experiments.

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Figure 1.

Use of spectroscopy to confirm the conjugation of PPa and phage and the production of singlet oxygen from the PPa-phage complex upon light irradiation. (a) UV/Vis absorption spectra of phage-PPa complex at 10%, 50%, and 100% of PPa-phage conjugation. (b) Fluorescence spectra of PPa-phage complex at 10%, 50%, and 100% of PPa-phage conjugation collected around PPa fluorescence maxima at 701 nm and phage fluorescence maxima at 300 nm (inset) to observe the energy hopping and to estimate the percentage conjugation of PPa by tryptophan fluorescence resonance energy transfer (FRET). (c) Time resolved $^1\text{O}_2$ phosphorescence signal of PPa (black), p-PPPa (50%) (red), and f-PPPa (100%) (green) in DMSO at 1270 nm using Nd-YAG laser at 532 nm of excitation wavelength.

Figure 2. High resolution transmission electron microscopy (TEM) images of SKBR-3 specific bacteriophage without any modification (A), with major coat proteins fully modified with PPa (B, i.e., f-PPa) and with major coat proteins partially modified with PPa (C, i.e., p-PPa).

Figure 3. Detection of p-PPPa complex binding affinity to SKBR-3 cells using immunofluorescence microscopy (A–C). (A) SKBR-3 cells treated with p-PPPa complex that were labeled with anti-phage antibody conjugated to a fluorescent green dye, showing fluorescence associated with phage. (B) DAPI-stained SKBR-3 cells treated with p-PPPa complex, showing fluorescence associated with nuclei. (C) is a merged image of (A) and (B).

Figure 4.

Cytotoxicity studies of porphyrin and phage conjugated porphyrin (p-PPPa) on SKBR-3 and MCF-7 cells in the absence (A) and presence of 658 nm laser (B). Time course quantitative estimation of PPa and p-PPPa on SKBR-3 cells viability after PDT (C). (Note: The bars indicated in blue, red, green and orange are corresponding to control (cells without any treatment), phage, PPa and p-PPPa, respectively).

Figure 5. In vitro evaluation of PDT on control MCF-7 cells (A–C) and target SKBR3 cells (D–F). A,D) Light microscope images of MCF-7 and SKBR-3 cells respectively. B,C,E,F) Fluorescent images of MCF-7 and SKBR-3 cells using Live/dead cell viability kit. B,E) upon incubation with only porphyrin and laser irradiation. C,F) Upon incubation with phage-porphyrin complex. (All images were captured at 10× objectives).

Scheme 1.

Schematic diagram of photosensitizer conjugation onto biological nanowire and its targeted photodynamic therapy. (A) Side view of biological nanowire (filamentous phage) with major coat protein (pVIII) fully modified with PPa (no singlet oxygen is produced due to excitonic coupling as a result of close packing of porphyrin residues on the sidewalls). (B) Side view of biological nanowire with major coat protein (pVIII) partially modified with PPa (singlet oxygen is produced due to absence of excitonic coupling) (red arrows) owing to distinct PPa, (C) Illustration of PPa-phage conjugation and *in vitro* photo-dynamic activity in SKBR-3 cells using 658 nm of laser. Green color cells represent the live cells and red represents the dead cells after PDT. In (A) and (B), pink line is the SKBR-3 cell-targeting peptide fused to the N-terminal of pVIII (green).